

Production Diseases In Farm Animals

Edited by
M. Fürll

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Production Diseases in Farm Animals.

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The Other Point Of View:

Production Diseases As Perceived by A Clinician

A Clinician starts his normal working day with a walk around the ward of the Large Animal Clinic for Internal Medicine in Leipzig. This allows the clinician time to discuss with colleagues the cow's diseases such as Abomasal Displacement (DA), puerperal disorders, mastitis, laminitis among others. All these diseases have a fundamental impact on the short economic life of cows. Daily, the clinician asks himself: What causes the disease? When did the illness start and how do the organ disorders relate to each other? Could the disease have been prevented?

What do we know about Production Diseases today? Not every last detail, for example, is known about Abomasal Displacement (DA), but we do have enough knowledge to effectively prevent the disease. 75 per cent of the cows with DA also suffer from puerperal disorders. Could puerperal disorders equally be prevented if a consistent prophylaxis was received? This question becomes a key problem because some cows diagnosed with toxic metritis cannot be cured despite intensive therapy. In cows with laminitis, we require further descriptive and basic research as well as information on possible relations between laminitis and the „metabolic syndrome“. Increased TNF α -concentrations ante partum in cows later diagnosed with laminitis support this hypothesis. The clinical picture of laminitis cannot be explained exclusively with „rumen acidosis“ or „SARA“. Regarding cows with mastitis, the probing question needs to be asked: How can mastitis incidences be reduced by strengthening the natural defence system and abstaining from chemotherapeutic substances?

When the animals are checked out of the hospital, other questions arise: What are the conditions like on return to the farm? Has the food quality changed? Any improvements in cow comfort? Have BVD or paratuberculosis been eliminated or is the return the start of a new cycle of stress, compensation and decompensation? A recent study on milk fever showed that during the dry period cows in a veterinary surgery were overfed with: 90% of Ca, 74% of Na and 88% of K. This example illustrates that well-established knowledge needs to be put into practice more effectively in order to reach the animal.

Solutions to every day problems are given in recent studies, which have been presented at the „International Conference on Production diseases in farm Animals“ since 1968. The conference provides an excellent opportunity to learn in detail about pathophysiologic links between nutritional deficiency and inappropriate keeping of the animals as well as clinical disorders. Scientists from various specialized fields show the path to a more stable health and performance, safer groceries and improved animal welfare. This expert knowledge is extremely useful for the treatment of Production Diseases that are commonly associated with animals kept in modern, agricultural production systems. Hence the series of ICPD conferences was successfully

established in 1968; its fundamental aims are:

- to advance the study and understanding of those diseases and conditions afflicting animals managed for agricultural production.
- to take a broader scope to include many factors influencing metabolism, such as nutrition, endocrinology, physiology, immunophysiology, genetics, and animal behaviour.
- to unite scientists of animal science, veterinary medicine, biochemistry, nutrition, genetics, physiology, immunology, endocrinology, epidemiology and ethology

ICPD conferences have made a beneficial impact for 40 years.

Today, a vibrant international conference is taking place in Leipzig, Germany with participants from 41 countries: Austria, Belgium, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Guinea, Hungary, India, Iran, Iraq, Israel, Italy, Latvia, Lithuania, México, Nigeria, Norway, Poland, Romania, Saudi Arabia, Switzerland, Senegal, Slovakia, Spain, Sweden, Switzerland, Syria, Thailand, The Netherlands, Turkey, United Kingdom, Uruguay, USA and Vietnam. In future, the ICPDs will continue to be high in demand to help solve complex issues in farm animals.

The organisers are committed to this credo.
Thank you to everybody for actively participating.
M. Fürll
Chairperson, 13th ICPD

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1 INTRODUCTION

1.1 THE DAIRY COW: PHYSIOLOGICAL FACTS AND CONCERNS

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Abstract

The production of milk per lactation of dairy cows has been augmented at least tenfold during the last two centuries from less than 1000 kg to more than 10.000 kg in all countries with intensive milk production and further increases can be expected. There is no doubt that this enhanced production of milk is accompanied by a rise in the incidence of a variety of diseases like milk fever, displacement of the abomasum, fat liver, disturbed fertility, mastitis, lameness, retained placenta and endometritis. Most of the diseases occur around parturition or in early lactation and lead to high annual culling rates and economical losses. Hence, when related to the life-span, the productivity of dairy cows has increased only to a small extent or has even remained almost constant. The incidence of the diseases varies and depends on a variety of factors like the feeding regime during the transition period, the management and the comfort of the cows. However, there is a growing body of evidence that the negative energy balance (NEB) in early lactation is closely and negatively related to fertility and predisposes the cows to infectious diseases due to a suppressed immune response. Because the magnitude (daily NEB) and the extension (duration of NEB) appear to be increasing as a consequence of the rapid onset of increasing milk production after parturition and continued high production, the possible risks of NEB for bovine health should become increasingly apparent. Thus, NEB, the concentration of non esterified fatty acids (NEFA) in plasma and the degree of fat liver are correlated. Recent studies both in cows and in non-ruminants support the assumption that NEFA are involved in the pathogenesis of increased production of pro-inflammatory cytokines like TNF- α and IL-6 and can lead to insulin resistance, maybe independently of NEB. Future research should focus on the negative side effects of NEB and NEFA. Breeding strategies should aim not only for an increase in milk production per lactational period, but also for a reduction of NEB with the goal of decreasing health risks for cows.

Key words: dairy cow, milk production, health risks, negative energy balance

Introduction

Ruminants have the unique digestive capability to convert grass and hay into milk. Hence, food resources which cannot be directly used by man¹, are fed to ruminants, which supply man with highly digestible milk and milk products at a reasonably low price. It is not surprising that people were and are interested in an increase of milk production in cows.

Improvements in both animal husbandry and feeding methods to cover both maintenance and milk production have led to a continuous increase in milk production per lactation period. In Germany, milk production two hundred years ago was 900 L per lactation period, increasing over the next 100 years to 2000 L per lactation period. This increase has continued and at the beginning of the 21st century the milk production per lactation (305 days) is 7,492 l (mean of all milk-recorded cows of different breeds; range from 5,181 – 8,150; ADR). It is well documented that milk production in USA, in Israel, in the Netherlands and in the United Kingdom is greater than in Germany, and that milk production is still increasing. Thus, in 2003, the annual rate of increase amounted to 350 l and 129 l in East- (former GDR) and in West-Germany, respectively. In the Netherlands the increase was 727 kg from 2000 to 2006 and in the USA annually 116 kg from 1990 to 1996 (Hansen, 2000). Dairy farms with a mean production of 12.000 l per cow or even more per lactation period are very common in all countries with intensive dairy industry.

This marked increase in milk production in dairy cows has had negative effects on the health of the animals and it has been accompanied by a variety of diseases such as milk fever, displacement of the abomasum, fat liver, disturbed fertility, mastitis, lameness, retained placenta and endometritis (Ingvarsen, 2006; Fleischer et al., 2001). Most of the diseases occur around parturition or in early lactation. High annual culling rates follow, leading to economical losses (Kossaibaiti and Esslemont, 1997). There is now a growing body of evidence that the high milk production and the concomitant increased metabolic demand compromise the health of dairy cows.

It is the intention of this short review to summarize the relevant data (*physiological facts*) concerning nutrient requirement, fermentation in the forestomachs, absorption, liver metabolism and blood flow in dairy cows, to give an impression of the physiological significance of milk production in the cow.

The *concerns* are derived from the gap between energy intake and energy requirement around parturition and in early lactation, which causes a large and long lasting negative energy balance (NEB). The NEB has well documented effects on fertility and immune

¹ The original feeding regime of cows (grass, hay etc.) has been changed due to the high demand of nutrients for milk production and consequently, many components of today's foodstuff of cows like soybean protein and starch can be used by man.

reaction. In addition, there is growing evidence to suggest that the increase of NEFA due to mobilization of fat to cover the energy demand of milk production is linked to an increase in cytokine synthesis and insulin resistance.

Judging from the data in the literature, it appears to this author that it should be possible to formulate not only proposals for further research on the effects of NEB and NEFA on animal health, but also for future breeding strategies for dairy cattle.

The dairy cow: Physiological facts

Milk production requires the intake of nutrients, minerals, trace elements and vitamins according to the amount of milk.

Dry matter intake, SCFA production and absorption: The energy requirement for milk production is well known, and Table 1 summarizes the recommendation for energy intake issued by the German Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie, GfE).

The common milk production of 40 kg/d requires a 4.2 fold increase of energy intake over the maintenance level, which has to be raised by a factor of 5.8 if a milk production of 60 kg/d is observed, as is increasingly the case. Most of the energy intake is starch, which causes a rapid and enhanced fermentation and the release of short chain fatty acids (SCFA) in the rumen, as summarized by Allen (table 2).

Table 1: Energy requirement of lactating cows and the necessary dry matter intake with increasing milk production (body weight 700 kg; recommendation of the GfE)

Milk Production Kg/d (4 % fat)	Requirement (NEL/d)	Percentage of Maintenance (%)	Dry Matter Intake (kg/d; 6.8 NEL/kg)
0	40	100	7.0
10	72	180	10.6
20	104	260	15.3
40	168	420	24.7
60	232	580	34.1

Table 2: Production of SCFA in the rumen according to the calculation of Allen (1997). It is assumed that the organic matter intake (OMI) accounts for some 90 % of dry matter intake.

Milk Production Kg/d (4 % fat)	Dry Matter Intake (kg/d; 6.8 NEL/kg)	Organic Matter Intake (OMI)* (kg/d)	SCFA (Mol/d)
0	7.0	6.3	28
10	10.6	9.5	42
20	15.3	13.8	61
40	24.7	22.2	99
60	34.1	30.7	137

* Assuming a fermentation of 60 % of OMI intake in the forestomachs.

Moreover, the digested organic matter can be used for the calculation of the synthesis of microbial protein (Lebzien and Voigt, 1999). SCFA are absorbed from the forestomachs before reaching the duodenum and there is now growing body of evidence that in contrast to butyrate, acetate and propionate are only marginally metabolised by the epithelia of the forestomachs (Kristensen et al., 2000).

Liver metabolism and blood flow

Ammonia: Ammonia is mainly released by the microbial degradation of protein and the hydrolysis of recycled urea. Despite the fact that ammonia is essential for the synthesis of microbial protein, large amounts of ammonia are absorbed from the gastrointestinal tract of ruminants. As a rule of thumb, some 40 - 50 % of nitrogen intake with the diet is absorbed as ammonia and totally detoxified in the liver to urea (Reynolds, 2003; see table 3).

Turnover of glucose: Net glucose absorption from the intestine is low, despite the significant flow of starch into the intestine (Reynolds et al., 2003). Because of this, the high requirement of glucose for the synthesis of lactose is supplied by gluconeogenesis in the liver. Danfaer (1994) published the following equation for the turnover of glucose in lactating cows: $Y = 1.64 + 0.396x$ ($Y =$ Glucose turnover in Mol/d; $x =$ amount of milk kg/d; see also table 3).

Blood flow and O_2 consumption: Like the detoxification of ammonia to urea or gluconeogenesis, the metabolism of the liver requires a large amount of energy. It is therefore not surprising that the blood flow through the liver and the O_2 consumption is remarkably high and changes with milk production. Reynolds et al. (2003) measured the blood flow, the synthesis of glucose and the removal of ammonia in cows before and after parturition (table 3). The decrease of body weight indicates a NEB up to 83 days after parturition (table 3; see below).

Table 3: Liver metabolism of dairy cows before and after parturition (Reynolds et al., 2003)

Day (- a.p.; + p.p.)	-9	+11	+33	+83
DMI (kg/d)	9.6	14.7	19.5	22.1
Milk (4% FCM kg/d)	0	41.2	44.2	42.4
Liver				
- Blood flow (l/d)	27,528	54,488	53,928	58,488
- O ₂ uptake (l/day)	897	1,659	1,828	2,097
- Glucose (g/day)	1,370	2,708	3,500	3,628
- NH ₃ removal (g/d)	110	186	241	335
Body weight (kg)	766	676	660	647

Summary of important numbers of a lactating cow: The well established physiological facts about a dairy cow with a milk production of 40 l/d can be summarized as follows:

- Energy requirement: 168 NEL MJ/d
- Necessary dry matter intake (6.8 NEL MJ/kg): 24.7 kg/d
- SCFA production: 100 Mol/d
- Ammonia-N absorption: ≈ 300 g/d (≈ 480 l ammonia!)
- Glucose turnover: 17.5 Mol/d (3.15 kg)
- Hepatic blood flow: $\approx 50,000$ l/d
- Blood flow mammary gland: $\approx 20,000$ l/d
- Cardiac output of blood: $\approx 125,000$ l/d
- Cardiac output of blood/l milk: $\approx 3,100$ l/l milk
- Price/l milk (proceeds for farmer): 27.35 cent (Euro; Germany 2006)

Despite the very high nutritional value for man, milk can thus be bought at a shamefully low price owing to the truly remarkable biological performance of the cow. However, both for economical and ethical reasons, the possible health risks for the animal deserve closer attention.

Physiological concerns

Physiological concerns: High culling rates and mortality

The significant increase of milk production in cows during the last two centuries and the remarkable capability of digestion and metabolism of the cows should not obscure the disappointing fact that the modern cow suffers from production diseases around

parturition and in early lactation at rates not seen in previous centuries. These problems have been studied over decades and carefully summarized in recent reviews (Grummer, 1993; Herdt, 2000; Drackley et al. 2005; Ingvarsen, 2006). Furthermore, high mortality is reported (Thomsen and Houe, 2006) and cows with multiple health problems are named “loser cows” (Thomsen et al., 2007). As a consequence of these health risks, dairy cows are culled frequently and the current situation in Germany is given in table 4. 30.9 % of all cows were culled in 1970 and this rate increased to almost 40 % in 2005 (table 4). This means that the average number of lactations in dairy cows in Germany is only 2.5, which is disappointingly low and a definite cause of economic loss. A similar number is given for HF cows in the USA (2.47). Figures for Denmark are 2.5 to 3.0, depending on the breed, while in the Netherlands, the mean number of lactation is 3.4, although the milk production is higher than in Germany. The results of a recent survey of dairy farmers are in good agreement with the statistical data shown in table 4. When to name the major health problems in their dairy cows, farmers named 1. Reduced fertility 56.6 %; 2. Mastitis 43 %; 3. Lameness 41.5 % and 4. Metabolic disorders 25.5 % (Lehnert, 2005).

Table 4: Culling rate of dairy cows per year (%) in Germany since 1970. The major diseases are reduced fertility, mastitis and lameness. (Source: Year book of the Arbeitsgemeinschaft Deutscher Rinderzüchter – ADR).

Jahr	%	Fertility	Mastitis	Lameness	Metabolic Disorders
1970	30.9	31.0*	4.7	2.9	2.0
1975	29.9	33.6	6.6	3.5	1.3
1980	29.8	29.3	8.7	4.4	1.2
1985	33.4	28.5	8.2	4.6	1.3
1990	33.7	26.4	12.3	6.8	-
1995	32.0	21.8	15.3	8.3	-
2000	39.9	19.6	15.2	9.4	-
2005	39.6	20.8	14.1	9.7	3.2

* In 1970 31 % of all culled cows had suffered from disturbances of fertility. The sum of all diseases in line is not 100%, because for simplicity not all reasons are given.

Physiological concerns: The negative energy balance (NEB)

The rapid increase of milk production after parturition causes a negative energy balance, because the intake of dry matter and hence energy is too low and cannot match the metabolic demand. NEB in early lactation is often observed in mammals. Its biological function is to guarantee nutrition of the offspring. However, this priority can lead to metabolic disturbances as for instance milk fever or grass tetany. These diseases of dairy cows are a consequence of maintaining milk production at the expense of the health of the animal.

NEB - Extent and Duration: NEB around parturition and in early lactation is text book knowledge. However, it is rarely quantified and it is believed that NEB is only of short duration and of minor importance. Indeed, it was thought in the nineties that NEB only lasted for a few weeks. However, recent publications clearly show that the extent and duration of NEB have increased dramatically. In cows fed ad libitum before parturition, Van den Top et al. (2005) observed a loss of body weight during the first month p.p. of 114 ± 11 kg or some 4 kg per day. In the experiments of Bulang et al. (2006), cows experienced a NEB of more than 100 days, which is one third of the lactation period of 305 days (see table 3: change of body weight over 83 days p.p).

NEB of this degree is possible because the flexibility of energy metabolism of cows is remarkable. In a recent experiment of Krause and Oetzel (2005), the authors reduced the daily dry matter intake of lactating cows from 25.2 kg to 12 kg per day. No reduction in milk production was observed, instead, productivity was maintained by rapid mobilisation of body reserves. Milk production remained stable even when the re-absorption of glucose was inhibited by application of phlorizin for 7 days, despite the daily loss of 474 g glucose in the urine (Bradford and Allen, 2005).

Despite the maintenance of milk production under a NEB, consequences for the metabolism of the cows and partition of energy for body functions are to be expected. Wade and Jones (2004) proposed a hierarchy of energy partition and distinguished three scales: Oxidizable metabolic fuels are used for 1) essential processes in cell maintenance, circulation and neural activity, for 2) reducible processes as locomotion, thermoregulation and growth and for 3) expendable processes such as reproduction and fat storage.

NEB and Fertility: The negative correlation between the increase of milk production and fertility in dairy cows is well documented (Webb et al., 1999; Lucy, 2001; Beam and Butler, 1999; Westwood et al., 2002; Butler, 2003). Butler (2003) compared the increase of milk production in dairy cows with the conception rate. Milk production increased during the last 50 years (1951 – 2001) from some 5000 l to more than 10,000 l. Concomitantly, the conception rate fell from 65 to 30 %. Corresponding data have been published by Lucy (2001). This negative trend has been observed in various countries. Some data for England comparing the calving interval (CI) and the conception rate of the years 1975 – 1982 with 1995 – 1998 are given in Table 5 (Royal et al. (2000)). A similar increase of the calving interval has been reported in Germany and in the Netherlands.

Table 5: Calving interval and conception rate in England (Royal et al., 2000).

Year	Conception Rate	Calving Interval (CI)
1975-1982	55.6 %	370 Tage
1995-1998	39.7 %	390 Tage

Royal et al. (2000) described an atypical ovarian hormone pattern (progesterone), an increase of delayed luteolysis and ovulation as possible reasons for the extension of CI. Pushpakumara et al. (2003) observed in their study that cows with low progesterone had significantly lower insulin-like growth factor (IGF-1), insulin concentration, reduced dry matter intake, BCS and body weight. Hence, a poor metabolic status (NEB) is associated with decreased fertility. Taylor et al. (2004) confirmed the correlation between a low IGF-1 and reduced fertility and showed that cows with higher milk production in early lactation had lower IGF-1 concentration and prolonged anovulation post partum. This negative correlation between energy demand for milk production and ovulation was also observed by Kawashima et al. (2007b). Furthermore, Kawashima et al. (2006) concluded from their studies that the onset of the ovarian cycle after parturition – within three weeks or delayed – can be used as an index of later reproductive performance. The same group studied in more detail the hormonal background of ovarian functions and observed, that “IGF-1 and insulin represent metabolic signals of the resumption of ovarian functions post partum in high producing cows” (Kawashima et al., 2007a). A possible link between insulin and cystic ovarian disease in high yielding cows was suggested by Opsomer et al. (1999). Taken together, these data clearly show that the NEB in early lactation is characterized by an increase of growth hormone, a decrease of insulin, IGF-1 and glucose and delayed or disturbed ovarian functions.

However, the demonstrated correlations did not offer a causal explanation, since these hormonal changes are probably responses to prior metabolic changes (Wade and Jones, 2004).

There is now a growing body of evidence that NEB primarily influences the regulation of the ovarian cycle via a “fuel detector” in the central nervous system (Wade and Jones, 2004; Schneider, 2004). The suggested fuel detector is located in the caudal hindbrain close to the area postrema (AP), which is located at the floor of the fourth ventricle. The AP is a chemosensory structure with a permeable blood-brain barrier and seems to be important in the control of LH secretion and ovarian cycle. The infusion of the glucoprivic drug 2-deoxy-glucose (2DG) into the fourth ventricle of rat and sheep inhibits pulsatile LH release, which is insulin dependent (Tanaka et al., 2000), and supports the assumption of fuel detectors in the circumventricular organ (see fig. 4 in Wade and Jones, 2004).

The studies about energy intake, the fuel detector, its interaction with hypothalamus and the regulation of ovarian cycle are summarized in table 6.

Table 6: Scheme of the signal cascade for the effect of low oxidizable metabolic fuel on the „fuel detector“ of the area postrema in the hypothalamus, ovarian activity and reproductive behaviour (modified from Wade and Jones [2004] and Schneider [2004]).

Low availability of oxidizable metabolic fuels at the „fuel detector“ of the AP causes:

- Reduced Oxidation (Glucose?) in the fuel detector
- Neural conduction to the hypothalamus ↓
- Transmitter: NP Y and catecholamine
- Transmission in the hypothalamus directly on
 - GnRH neurons (↓) and/or indirectly
 - via CRH neurons on GnRH neurons (↓)
 - Pulsatile LH release (↓)
 - Ovary: Inhibition of follicle development
Delayed/disturbed ovulation
- Reproductive Behaviour: Reduced

Note that these possible links were already suggested by Schillo in his 1992 review, although the details were not known at that time.

It appears that the documented correlation over several decades between energy intake and fertility in cows can now be explained in more detail. It must be emphasized that the reduced activity of the ovarian cycle under energy restriction is a physiological response. Pregnancy under conditions of NEB is probably undesirable for both the mother and the fetus.

NEB and Immune Response: It is well known that NEB predisposes animals and man to infectious diseases (Buttgereit et al., 2000). Vice versa, an immune response against an infection requires energy, reduces growth rates and reproduction (Klasing, 2006). An impaired immune competence in cows in early lactation has been discussed for many years and is currently emphasized by Goff (2006). Results from recent publications support the assumption that the hormone leptin is involved in the interaction between nutrition and the immune system. Leptin is produced in adipocytes and its effects are mediated via leptin receptors in many cells including cells of the immune system (Matarese et al., 2005). NEB causes a decrease in the blood leptin concentration, a reduced immune response and a predisposition to infectious diseases (Faggioni et al., 2001; Cava und Matarese, 2004). Blood leptin concentration in dairy cows decreases after parturition (Leury et al., 2003; Liefers et al., 2005), mirrors underfeeding (Block et al., 2001) and correlates with insulin concentration (Leury et al., 2003). Corresponding relationships between leptin and the immune system of cows can be expected, but have not yet been demonstrated. Leury et al. (2003) discussed this topic as a proposal for future research.

Physiological concerns: NEB, NEFA, Fat Liver and Insulin Resistance

The pathogenesis of fat liver in dairy cows is explained by the rapid lipolysis as a consequence of NEB (Grummer, 1993; Bobe et al., 2004; Ametaj, 2005; Hayirli, 2006; Geelen and Wensing, 2006; Fürll, 2007). NEFA are released from the adipocytes and the uptake of NEFA by liver cells is higher than extrusion leading to an increase of the fat content of the liver. Fat liver is discussed as a possible reason for a variety of diseases such as reduced fertility, ketosis and immune response (Grummer, 1993; Hayirli, 2006; Geelen and Wensing, 2006). There is no doubt that in NEB, NEFA concentrations in the blood and the degree of fat liver are causally correlated. However, there are other important observations which accompany these alterations and hardly fit into this scheme: i) Insulin resistance (IR) has been reported for many years in cows in early lactation (Holtenius, 1993; Holtenius and Holtenius, 1996; see review Hayirli, 2006). ii) NEFA concentrations have been correlated with displacement of the abomasum (Cameron et al., 1998; Geishauser et al., 2000; Pravettoni et al., 2004), reduced fertility (Canfield and Butler, 1990; Grummer, 1995) and proliferation of granulosa cells and fertilisation of cumulus oocyte complexes (Jorritsma et al., 2004). iii) Moreover, increased blood concentrations of pro-inflammatory cytokines like TNF- α have been reported (Ohtsuka et al., 2001; Amentaj, 2005).

It would appear that these changes are not causally related, however, recent studies in cows and in non-ruminants do support the hypothesis of interactions between NEFA, TNF- α and insulin resistance.

Insulin resistance: IR is characterized by an altered response of insulin to glucose (insulin responsiveness), an altered response of the tissue to insulin (insulin sensitivity) or both (Kahn, 1978). Recent publications suggest that IR in cows is related to fat liver and NEFA.

In 2001 Ohtsuka et al. reported that the degree of fat liver was positively associated with increasing TNF- α (and NEFA) concentrations in the plasma of dairy cows in early lactation. In the same year, Kushibiki et al. (2000) demonstrated that the administration of recombinant bovine necrosis factor α (rbTNF- α) induced a condition of reduced insulin sensitivity with inhibition of insulin-stimulated glucose utilization. In a further study, Kushibiki et al. (2001) showed that the injection of rbTNF- α into steers caused IR which was partially reversed by 2,4-thiazolidinedione (2,4-TZD). The mechanisms by which 2,4-TZD prevented the rbTNF- α induced IR is not clear in ruminants (Kushibiki et al., 2001). It should be emphasized that in the studies of Ohtsuka et al. (2000) and Kushibiki et al. (2000, 2001), IR was accompanied by increased levels of NEFA.

In line with this, increased NEFA concentration as a possible cause for IR has been suggested by Oikawa and Oetzel (2006). These authors evaluated insulin response in a 4 day fasting model in nonlactating and nonpregnant Holstein cows. Four day fasting caused a significant increase of NEFA from 0.21 to 1.24 mmol/l. Plasma insulin concentrations were decreased and insulin-stimulated blood glucose reduction was

lower in fasted cows. Insulin response was negatively correlated with NEFA. In line with these results is the study of Pires et al. (2007) who used a more physiological model. A triacylglycerol emulsion (TG) was infused i.v. in nonlactating and nonpregnant Holstein cows for 11 h. TG infusion led to a significant increase of NEFA, TG, glucose and insulin and caused IR by impairing both sensitivity and responsiveness to insulin. These results clearly show a correlation between NEFA and IR. However, the possible signal cascade between these two parameters remains unclear.

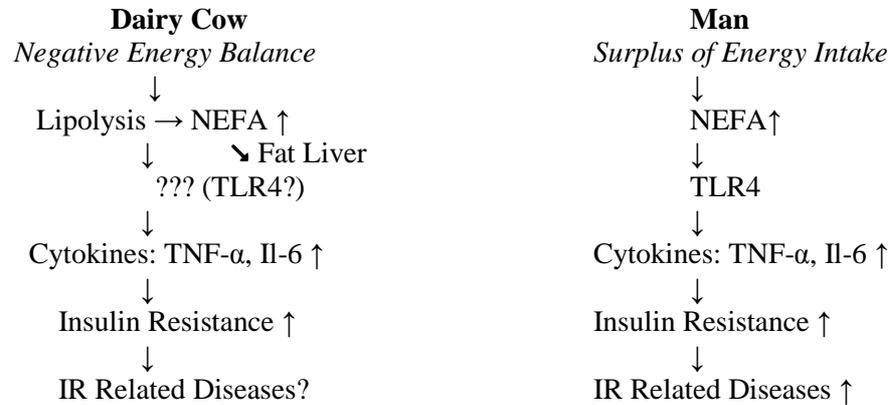
Nevertheless, significant advances in our knowledge concerning NEB, NEFA, fat liver, TNF- α and IR have been made, despite the fact that the possible sequence and causal interactions of the changes are not known. There is no doubt that the key factor is the NEB, which causes the release of NEFA. NEFA are taken up by the liver and an imbalance between NEFA uptake \gg export leads to fat liver. At this point, the underlying causes for the linkage between fat liver, TNF- α , IR, NEFA and IR remain to be elucidated. However, recent studies of the pathogenesis of IR in human diabetic type 2 appear to be helpful in understanding these factors.

NEFA, toll-like receptors, cytokines and insulin resistance: Obesity is one of the most important factors in the pathogenesis of insulin resistance in human patients suffering from diabetes type 2 (Kahn et al., 2006). The possible reasons for linking obesity to IR have been studied for many years and it appears that levels of NEFA, TNF- α and Il-6 play a causative role (Wellen and Hotamisligil, 2003 + 2005). In many cases, obesity is accompanied by chronic activation of inflammatory pathways linked to IR (Wellen and Hotamisligil, 2003 + 2005) in a manner that is still poorly understood. NEFA as possible mediators have been discussed, because NEFA infusion leads to IR (Lam et al., 2003; Boden et al., 2005). Intracellular pathways have been studied as mediators between NEFA and IR and intracellular kinases of inflammatory pathway have been activated upon NEFA infusion (Hirosumi et al., 2002). Shi et al. (2006) suggested that toll-like receptor 4 (TLR4) could be involved and they studied this hypothesis *in vitro* and *in vivo*. The authors clearly demonstrated that in macrophages and adipocytes, NEFA activate TLR4 inflammatory signalling with an increase of TNF- α , Il-6 and activation NF- κ B. These effects were blunted in the absence of TLR4. These *in vitro* findings were confirmed in *in vivo* studies with mice, in which NEFA infusion reduced the ability of insulin to acutely activate signalling via insulin receptor substrate (IRS-1). Mice lacking TLR4 were protected against effects of NEFA. Taken together, these results support the assumption that TLR4 is the receptor and the link between NEFA, inflammatory response and IR.

The parallels between the altered metabolism in diabetes type 2 and in the periparturient cow are intriguing and summarized in table 7. NEFA are elevated, pro-inflammatory cytokines are increased and IR is observed despite the fact that the starting position is very different. The increase of NEFA is caused in man by a surplus of energy intake and in cows by rapid mobilisation of fat. Of course, the role of TLR4 has not been demonstrated in cows, but it appears that the involvement of this receptor of the innate immune system is very likely. IR the kidney and the eyes. The

consequences of IR in cows remain to be elucidated, but it appears that many of the health problems of the periparturient cow could be related to IR. However, it must be emphasized that a better understanding of the effects of NEB, the increase in NEFA and IR should not lead to a simplified scheme of the pathogenesis of periparturient disorders or diseases in cows.

Table 7: Putative scheme of development of IR in dairy cows and in man (diabet. type 2)



Conclusions and Perspectives

The performance of a cow with a daily milk production of 40 kg is remarkable regarding the energy requirements, fermentation in the forestomachs, blood flow and the O₂ consumption of the liver etc. (see table 2 and 3). There is no doubt that this high milk production is a challenge for the physiological and biochemical capabilities and the endocrine system of the dairy cow and at times overwhelms the adaptation capacity at the onset of lactation in some animals. A high incidence of “production diseases” is the consequence of this metabolic overload and this is associated with severe economic losses. Because very different diseases such as displacement of the abomasum, fat liver and reduced fertility occur, a close and clear *cause and effect* could not be determined for these health problems and as a result terms like periparturient diseases or disorders or metabolic syndrome were coined to cover this group of diseases.

However, recent studies have led to a much better understanding of the fundamental problems involved. NEB clearly modulates the ovarian cycle and fertility via a “fuel receptor” in the hindbrain, which can be considered as a physiological response. Furthermore, it is well established that NEB around parturition and especially during the first 2 to 3 months of lactation causes a rapid and long lasting lipolysis and an increase of the NEFA concentration in blood. These are partly taken up by the liver, metabolised or used for the synthesis of TG. Since in many cases the secretion of TG is less than its synthesis, fat liver occurs, which is related to the incidence of numerous diseases in the periparturient disorders (Grummer, 1993; Ingvarsten, 2006; Geelen and Wensing, 2006). NEFA have other effects independent of fat liver and mouse model

studies have shown that NEFA activate toll-like receptor 4 (TLR4), which induces the synthesis of pro-inflammatory cytokines such as TNF- α or IL-6 (Shi et al., 2006). TNF- α causes IR and is a major factor in the pathogenesis of diabetes type 2 in man. Because a correlation between NEFA, TNF- α and IR has been demonstrated in cattle, we suggest that a similar signal cascade via TLR4 maybe operating here. IR in diabetes type 2 is associated with numerous diseases in man; corresponding data are not available in ruminants, but the positive effect of insulin on IGF-1 (Butler et al., 2003) or on the fat content in the liver in cows (Hayirli et al., 2002) show that IR may have similar negative consequences on the health of cows. Cytokines and NEFA are considered the “key players of biological functions” in the periparturient cow (Loor et al., 2005 + 2006).

NEFA as a risk factor was suggested as early as 1997 by Kaneene et al. and a recent publication of Hachenberg et al. (2007) supports this assumption for NEFA concentrations higher than 0.5 mmol/l. In line with this conclusion is the observation of Wehrend (2005) concerning the incidence of mastitis and increased NEFA concentrations. To some extent, these suggestions are still speculative, but should encourage future research about the pathogenesis of the “periparturient diseases”.

Our improved understanding of NEB, NEFA, fat liver, cytokines and IR and their associated health hazards should be taken into account in future breeding strategies in dairy cows. Dairy cows in which an increase in milk production is more related to fat mobilisation than to a corresponding increase of feed intake should not be selected for breeding purposes because it aggravates the problem. Vice versa, not all cows suffer from severe NEB, fat liver and hence, from “periparturient diseases”. These cows could serve as a control for the hypothesis of this publication that the magnitude and extent of NEB with its consequences are the key factors in the pathogenesis of “periparturient diseases” of the dairy cows.

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1.2 HUMANS NEED, NUTRIENT ECONOMY AND ECOLOGY-CHALLENGES FOR ANIMAL PRODUCTION

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The production of edible protein of animal origin is the primary objective of livestock husbandry. The protein intake of people in developed countries is high (more than 50 g per capita daily from animal origin) and rising incomes in developing countries lead to elevated demand and consumption of meat, fish, milk and eggs. Based on this, the following questions as asked by Wennemer et al. (2005) must be answered: “Can the earth feed everyone in the long term?”, “What are the real human needs?”, “Are we making efficient use of the earth’s natural resources?”, “What role do animals play in all of this and how should they be treated?”, “How can we improve the nutrient economy during the production of food (protein) of animal origin?” and finally, “Do we have the potential to reduce the excretions of N, P, CH₄ or trace elements by food-producing animals?” Human needs, nutrient economy and environmental aspects of food production of animal origin are discussed in the paper more in detail. The increase of animal performance and a decrease of animal numbers seems to be the most efficient way for a better nutrient economy and lower excretion per product of animal origin.

Introduction

The increase in world population has dramatic consequences for available resources per inhabitant as shown for arable land in Figure 1 (Steinfeld et al. 2006).

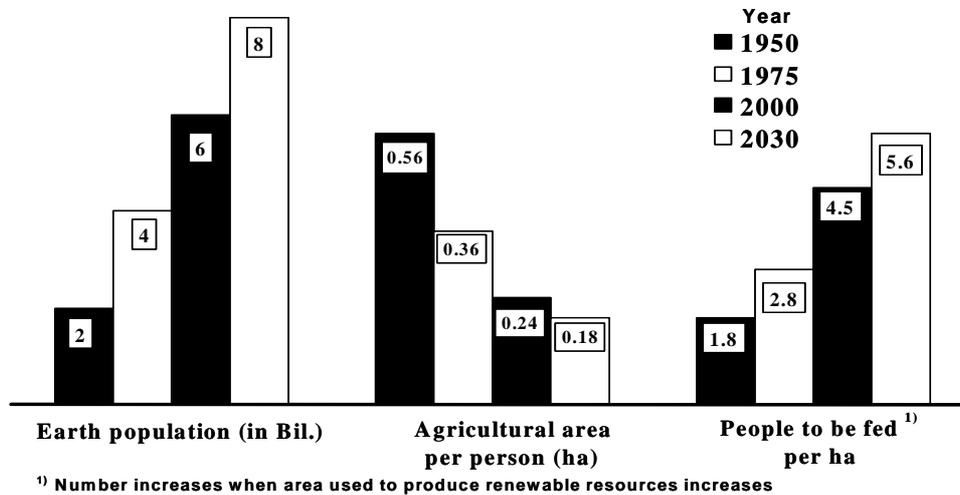


Figure 1: Development of the earth's population, the land area available and the number of people to be fed per hectare (according to FAO yearbooks)

Food of animal origin is a real resource consuming material, if we consider the dry matter intake of food producing animals (Table 1) and compare it with human consumption all over the world.

Table 1: Estimated dry matter (DM) consumption by humans and farm animals

Species	Number (billions) FAO Stat 2005	Consumption (DM)	
		(kg/day)	(billion t/year)
Humans	6.3	0.45	1.0
Cattle, buffaloes, horses, camels	1.6	10	5.8
Sheep, goats	1.8	1	0.6
Pigs	0.95	1	0.35
Poultry	17.4	0.7	0.45
Total (animals)			7.2

The expectations on animal production have changed in recent years. Figure 2 shows some examples in Europe after the Second World War.

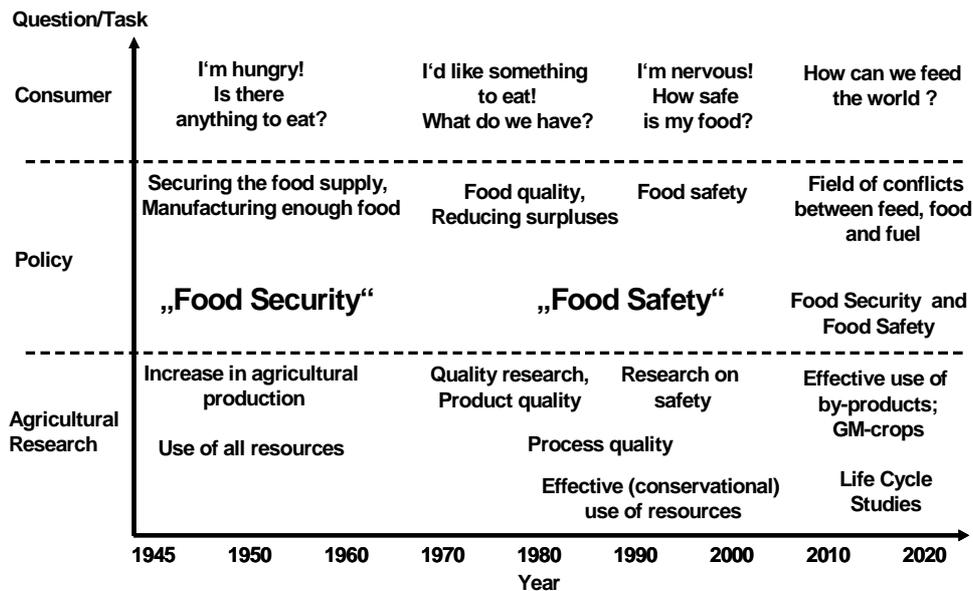


Figure 2: Dominating questions of society as well as tasks of policy and agricultural research after the 2nd World War in Europe, presently and in future

A number of social questions have emerged, and many more are expected in the future, as shown in Table 2.

Table 2: Challenges for animal production or Livestock's long shadow (Steinfeld et al. 2006)

Year	Presently	2050
World population (Mrd.)	6.5	9.0
Meat production (Mio. t)	229	465
Milk production (Mio. t)	580	1043

Therefore animal production is presently being considered in a new framework. It is not only asked to produce high amounts of milk, meat, fish and eggs and other food of animal origin. It is also expected to use resources efficiently, consider the environmental, ethical and socio-economic aspects, food safety and quality as well as some other influences on animal sciences (Figure 3). Some of those aspects will be discussed in the paper.

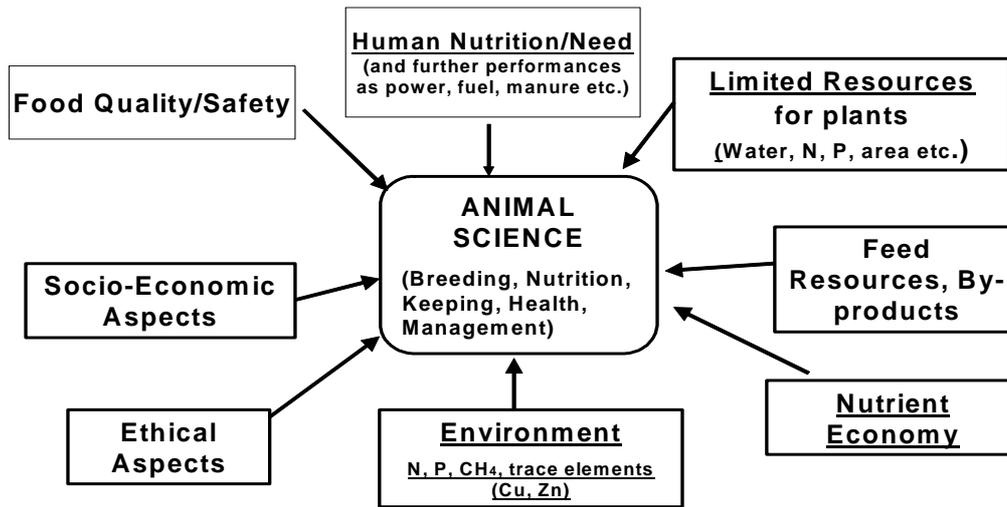


Figure 3: Conditions influencing Animal Science

Human Needs

What do we really need? Humans need adequate and safe food. What does that mean?

- About 500 g dry matter (DM) day⁻¹ or about 2500 kcal or \approx 10 000 kJ or
- 300 g meat, 1 l milk, 1 egg, bread, vegetables, fruits, etc., day⁻¹ or
- 20 g edible protein of animal origin plus all other essential nutrients day⁻¹ or
- 60 g edible protein or what else?

Protein of animal origin is a suitable measurement to compare various forms of animal production. It offers essential amino acids and many important trace nutrients to human beings (Wennemer et al. 2005). Protein of animal origin is not considered to be essential for human beings, but it contributes to an adequate human nutrition, esp. during critical phases of human development (pregnancy, lactation, childhood, youth, etc.).

About 20 g protein of animal origin per day may contribute to an important supply of such nutrients. Presently there is a wide range in the daily protein intake of people from different countries and within different countries. Until 2050, the world's population will be increased from about 6.5 billion people to about 9 billion, but meat and milk requirements will nearly double (Table 2). The reason for this is a higher demand with increased income in many developing countries (Keyzer et al. 2005). We may observe the same development worldwide as immediately after the Second World War in Europe (see Figure 2).

It seems to be difficult to avoid such a critical development. Both possibilities *to reduce or to stop population growth or to stop or to reduce consumption of protein of animal origin* are extremely complicated and are not presently on the agenda of policymakers. This situation is a real challenge for animal science and animal production, but apart from the amount of protein/food of animal origin, safety needs also demand significant attention (Figure 4). The quantitative issue of food security and the qualitative issue of food safety and quality are key elements of human health and well being as shown in Figure 4.

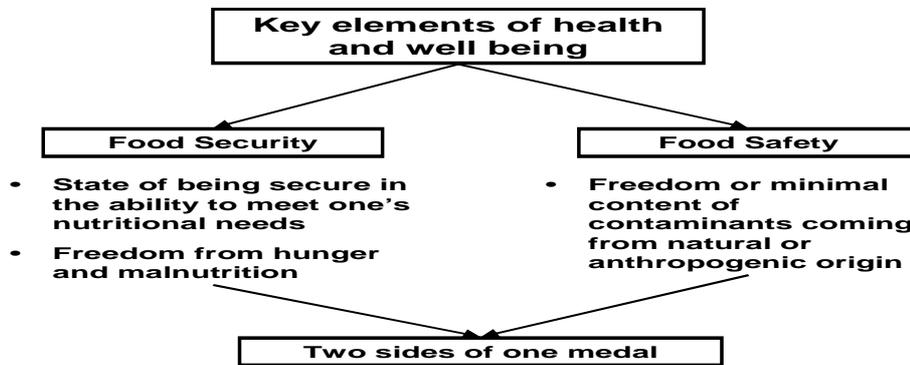


Figure 4: Food security and food safety as elements of health and well being (Flachowsky and Dänicke 2005)

Food security and safety include fair distribution of available food, increased food production in deficit regions (»helping them to help themselves«), application of current scientific knowledge and investment in research to secure food for a growing world population. Increasing demand for high quality animal-based protein should be taken into account as a reality.

Safety means a minimum or the lowest practical relevant content in undesirable/anti-nutritive substances, but it means also a content of essential nutrients, so that tolerable upper levels in human nutrition not to be exceeded if people consume higher amounts of certain food. Food of animal origin may contribute to avoiding deficiencies in some trace nutrients (e.g., iodine, selenium, vitamins A, D) which are characterized by the so-called supply category I, but the tolerable upper level of some nutrients is only 3 to 5 times higher than the human need. For this reason such micronutrients as I, Se, vitamins A and D are grouped to Risk Category I (Table 3). Therefore, for reasons of preventive consumer protection, animal nutritionists reduced the upper levels for some nutrients (e.g., iodine, vitamin A) which are characterized by high transfer rates (from 10 to 30 % for iodine in milk and eggs) from feed into food of animal origin.

Table 3: Supply and risk categories for various trace elements and vitamins under consideration of intake and requirements in humans (by BfR, 2004; EFSA, 2006; Gassmann, 2006)

Nutrient	Supply category	Risk category
Cu	3	high
Fe	1 / 2	high
I	1	high
Se	1/2	medium – high
Zn	2	high
Vit. A	2 / 3	high
Vit. D	1	high
Vit. E	2 / 3	medium
Vit. B₆	4	medium
Folic acid	1 / 2	medium
Niacin	3 / 4	medium

Table 4: Production of edible protein of animal origin and corresponding N excretion from different animal species with various performance at recommended N supply (Flachowsky, 2002)

Protein source (average body weight)	Production per day	Edible fraction %	Protein content in the edible fraction (g per kg fresh substance)	Estimated food competition to humans (% of feed) ³⁾	Edible protein		N-excretion Percentages of intake
					g per day	g per kg body weight	
Milk							
Cow (650 kg bw)	10 kg	95	34	0	323	0.5	75
	20 kg			(20)	646	0.9	70
	40 kg			(40)	1292	2.0	65
Goat (60 kg bw)	2 kg	95	36	0	68	1.1	70
	5 kg			(30)	170	2.8	60
Beef (400 kg bw)	500 g	50	190	0	48	0.12	90
	bwg ¹⁾			(20)	95	0.24	84
	1000 g			(40)	143	0.36	80
	1500 g						
Lamb (40 kg bw)	200 g	50	200	0	20	0.5	85
	bwg			(30)	40	1.0	80
	400 g						
Pork (80 kg bw)	500 g bwg	60	150	(30)	45	0.55	85
	700 g bwg			(50)	63	0.8	80
	900 g bwg			(60)	81	1.0	75
Poultry meat (1.5 kg bw)	40 g bwg	60	200	(40)	4.8	3.2	70
	60 g bwg			(70)	7.2	4.8	60
Eggs (1.8 kg bw)	50 %	95	120	(35)	3.6	2.0	80
	lp ²⁾			(50)	5.1	2.8	65
	70 % lp			(65)	6.6	3.7	55
	90 % lp						

¹⁾ Body weight gain ²⁾Laying performance ³⁾ Depends on amount of roughage and by-products in the diets

Nutrient Economy

Nutrient economy deals with the conversion of feeds/nutrients into animal products. Under consideration of the outstanding significance of protein, all expenditures are related to the production of edible protein. For eco-balances or so-called life cycle studies, all expenditures for feed production, incl. water, fuel, etc., and all emissions (CO₂, CH₄, N, P, Cu, Zn etc.) should be considered.

The conversion losses in food production from animal sources are a main point of criticism from the public. On the one hand, these losses contribute to considerable resource consumption (e.g., 3 - 5 kg grain to produce one kg pork), on the other hand to the excretion of nutrients that pollute the environment (Flachowsky and Lebzien, 2006; Verstegen and Tamminga, 2006). As shown in Table 4, protein production via milk and eggs is more efficient and more environmentally friendly than via pork and beef. As the feed conversion into food improves, the excretion decreases with higher animal performance.

Lower plant yields and lower animal performance require more land to produce a certain amount of protein of animal origin. The land area needed per inhabitant and year is calculated in Table 5 under consideration of plant and animal performances, the ratio of protein from meat and milk, and the level of consumption of protein of animal origin.

Table 5: Influence of the yield level of plants, the performance level of animals, the ratio of protein from meat and milk, and the level of consumption of protein of animal origin on land area needs (m² per capita and year, basal data by Flachowsky 2002)

Consumption (g protein/day) Yield level	10		20		40		60	
	A ¹⁾	B ²⁾	A	B	A	B	A	B
Ratio between protein from meat ³⁾ and milk (% of protein)								
70 : 30	260	105	520	210	1050	420	1560	630
50 : 50	225	95	450	190	900	380	1350	570
30 : 70	190	85	380	170	760	340	1140	510

¹⁾ Yield level A per hectare: 4 t DM of cereals, 10 t DM of forage; performance level A per day: 15 kg milk; live weight gain: beef cattle: 600 g; pigs: 400 g; poultry: 30g

²⁾ Yield level B per hectare: 8 t DM of cereals, 15 t DM of forage; performance level B per day: live weight gain: beef cattle: 1200 g; pigs: 800 g; poultry: 60 g

³⁾ Ratio between protein from beef, pork and poultry (in %): ≈ 15 : 60 : 25

In addition to the traditional competition of land use between production of vegetarian food for human consumption and feed production for animal production, land area is increasingly being used for bio-energy/fuel production in response to the challenge of global warming (Keyzer et al., 2005). Possible strategies to overcome this situation include:

- Continued investments to increase plant yield and animal performance with traditional and innovative biotechnology.
- Improved efficiency of utilizing limited resources (land, water, fertilizer etc.).
- Lower consumption of animal protein by people with current over consumption.

About two thirds of the world's ruminants are kept in tropical and subtropical regions, but these animals account for only about one third of the ruminant protein intended for human consumption. The lion's share of the world's meat supply is produced by a much smaller number of animals in the world's temperate zones. Improvements in efficiency, including optimal feeding of animals (e.g., to meet their energy and protein/amino acids requirements) must be recognized as a top priority for farmers and researchers.

Environmental aspects

Presently, the excretion of nitrogen, phosphorus, methane and some trace elements (e.g., Cu, Zn) is being intensively discussed within our society. Some substances are of more local environmental significance (e.g., for soil, ground or surface water), others have the consequence of their global warming potential (see Table 6), and also their global significance. Therefore animal scientists have to contribute to the decrease of excretion.

Under consideration of the ways of protein production of animal origin and performances of animals, we can expect various amounts of N, P and CH₄ excretion by food producing animals. Protein from milk, eggs and broiler meat is produced with lower N- and P-excretions than beef and pork, but food from ruminants caused a methane excretion because of the microbial processes in the rumen.

Table 6: Local and global environmental aspects

S u b s t a n c e s	S i g n i f i c a n c e	
	l o c a l	g l o b a l (G l o b a l w a r m i n g p o t e n t i a l)
N i t r o g e n (N)	x	N ₂ O Laughing gas, nitrous oxide (310 x as CO ₂) ¹⁾
P h o s p h o r u s (P)	x	-
M e t h a n (C H ₄)	-	x (23 x as CO ₂) ¹⁾
T r a c e e l e m e n t s (e.g. C u , Z n)	x	-

¹⁾ According to ICPP (2006)

Animal scientists have a large potential to decrease the excretions of N, P, CH₄ or trace elements. One of the most important tools to reduce the excretion per animal product (per kg milk, meat or eggs or per kg edible protein) is an increase in animal performance (see Tables 4 and 7 and Figure 5) and a reduction of animal numbers as a consequence of the higher yields. But some more general possibilities exist to reduce excretions per animal and/or per product such as to:

- Develop more accurate requirement values
- Avoid excesses in nutrient supply (N, P, trace elements); meet the requirements of animals
- Use rapid analyses for P- and trace element determination in feed and consider native contents of feeds under consideration of bioavailability of nutrients.
- Reduce animal losses, keep animals healthy and reduce the duration of (inefficient) rearing periods.

Furthermore, much detailed information on reducing the excretion of N, P, CH₄ and trace elements by various animal species or categories exists, such as:

- Using knowledge on rumen metabolisms to optimize N-utilization in ruminants (see Flachowsky and Lebzien 2006),
- Adding crystalline amino acids to the diet if necessary, using phase-feeding depending on age; calculating amino acid requirements on the basis of praecaecal digestible amino acids (see GfE 2006) or on the basis of P-availability of P and trace elements,
- Some activities (e.g., high propionate production by ration, fat supplementation, addition of propionate precursors or other feed additives) may decrease methane emissions, but not more than by 20-30 % (e.g., from 20-25 g to 15-20 g CH₄ per kg DM-intake).

Table 7: Excretions per kg edible protein of animal origin by various animal species/ categories

Protein source	Production per day	Excretion (kg/kg edible protein)		
		N	P	CH ₄
Milk	10 kg	0.65	0.10	1.0
	20 kg	0.44	0.06	0.6
	40 kg	0.24	0.04	0.4
Beef	1000 g	1.3	0.18	1.5
	1500 g	1.1	0.14	1.2
Pork	700 g	0.8	0.12	0.08
	900 g	0.6	0.09	0.05
Broilers	40 g	0.35	0.04	0.01
	60 g	0.25	0.03	0.01
Eggs	70 %	0.4	0.07	0.02
	90 %	0.3	0.05	0.02

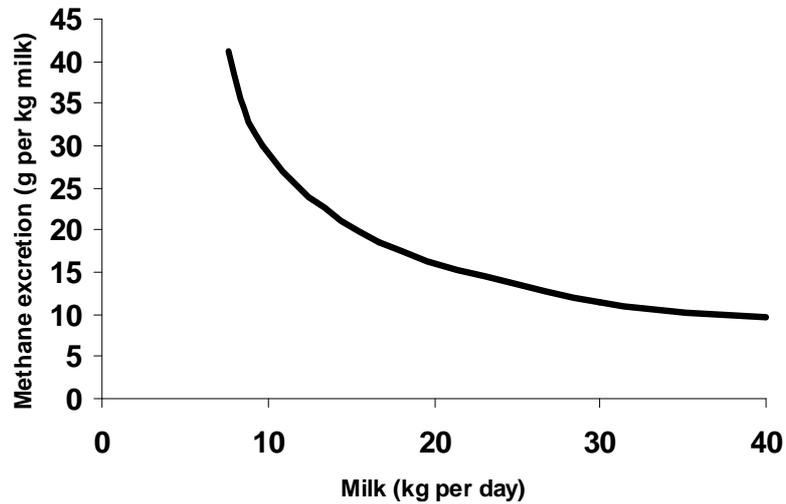


Figure 5: Methane excretion per kg milk in dependence of daily milk yield of cows

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2 EPIDEMIOLOGY OF PRODUCTION DISEASES

2.1 EPIDEMIOLOGY OF PRODUCTION DISEASES – PIGS

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Introduction

As in human populations, disease patterns in animal populations are changing over time and highly dependent on factors such as living conditions (especially the quality of shelter, nutrition and hygiene), population density and the frequency of interactions between individuals (in humans: global tourism, in animals: worldwide trade). With the steep increase of the productivity in animal production and the improvement of the animals' performance, however, the disease patterns in food producing animals have changed drastically: some of so far minor diseases were intensified and new diseases and disorders came into being. Thus, the term "production diseases" was coined, which seems to stem from the observation that with increasing milk yield in cows (i.e. with increasing "production" per animal) so far unknown or very rare diseases and metabolic disorders came to the fore: milk fever, ketosis, and new forms of mastitis. Thus, in the beginning, the term "production diseases" was more a "single animal term", since the health of single animals was impaired by an extreme high production performance of the animals.

Today, however, due to the growing herd and flock size, especially in the animal species pig and poultry, the term "production diseases" has to be seen as an "animal population term". Whereas in individual animals a disease and health impairments are characterised by qualitative terms such as a "mild", "moderate", "serious", "severe", "latent", "chronic" or "acute" disease, in animal populations (herds and flocks), disease and health impairments, but also health itself, are characterised by quantitative terms such as a "high", "medium" or "low" herd health. In the light of this quantitative approach to animal disease and/or health of populations, figure 1 shows a graph that tries to illustrate that animal health is not a "Yes" or "No" issue, but a quantitative issue that can be classified as "low" or "high", which makes it possible to estimate the changes of the health status over time and to measure improvements.

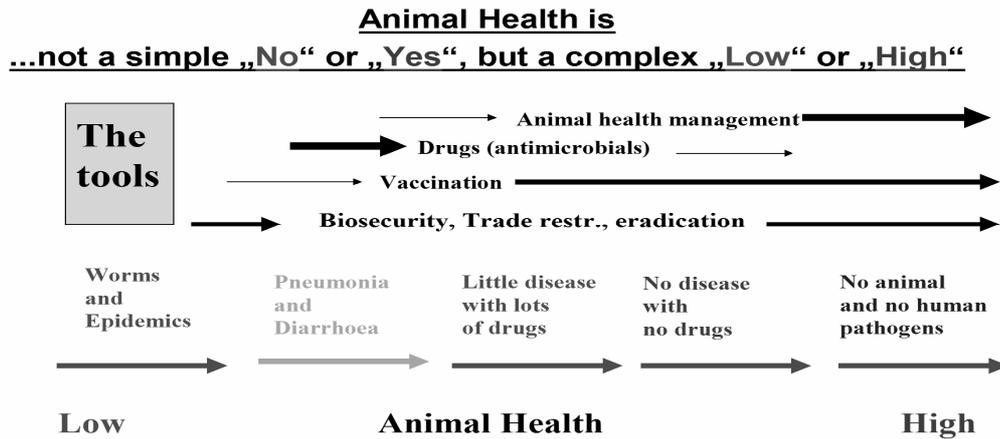


Figure 1: Improvements of animal health over time in relation to the major tools of animal health management

The above illustrated changes over time in the health status of food animal populations have several determinants such as technological progress, application of better and better “animal care tools”, population size, management improvements, structural changes in the agricultural organisation of animal production, and consumer demands.

Therefore, the term “production diseases” in pigs can and should be subdivided into the following disease categories:

- *animal performance associated* (e.g. daily weight gain, lean meat percentage, piglets per sow and year),
- *husbandry and housing system associated* (e.g. injuries, lameness, respiratory diseases, behavioural disorders),
- *management associated* (e.g. cannibalism, suboptimal nutrition, inadequate immunological status),
- *regionally and nationally organisational structure associated* (e.g. mixing of animal groups, lack of vertically coordinated animal health measures, uncoordinated animal transports).

In the following, examples for the four “production disease” categories in pigs are given and a strategy for implementing a concept for the continuous improvement in controlling the various “production disease” complexes in pigs is described.

The complexity of “production diseases” in pigs

Animal performance associated diseases

In pigs, the two major negative effects of the increase of the animals' performance (increase of the muscle mass per animal and the “speed” of the growth) are 1) a significant deterioration of the stability of the locomotive apparatus of the animals, 2) a significant decrease of the average longevity of sows, and 3) a significant decrease of the overall resistance of the animals.

Ad 1) In the early 70's of the last century, when breeders started selecting for lean meat percentage and an additional rib, locomotion disorders such as epiphysiolysis and arthrosis occurred with a frequency that turned out to be a major problem for the pig industry. It took almost two decades to “fix” the problem so that today most modern pig breeds and lines have, despite a very high lean meat percentage, a more or less satisfactory stable locomotive apparatus. However, the “leg weakness” still contributes to the shortening of the life expectancy of sows.

Ad 2) Along with increasing life performance data in sows (up to 30 piglets per sow and year and up to 20 litres of sow milk per day), the life span of the female pig used for reproductive purposes has dramatically decreased like in cows. Whereas single animals easily can give birth to 14 litters, the average number of litters in modern sow herds is often less than 5 litters. The major reason is a “premature burn-out” leading to reproductive failures and/or a drop in the milk yield leading in its turn to weak piglets and a high piglet mortality rate. However, diseases such as lameness, injuries, abscesses etc. also contribute to the high culling rate in sows of today.

Ad 3) The overall impression of a weaker resistance of the pig of today is not very well measured, i.e. there are only weak data on a higher disposition for disease in general. However, it is an internationally agreed upon consensus, on which many an attempt to select for a higher disease resistance is based.

Husbandry and housing system associated diseases

The way pigs are kept in confinement is the major determinant for many “man-made” diseases and disorders as well as behavioural abnormalities. Among the manifold reasons for diseases and health impairments due to the husbandry system and the housing conditions are: too high *a stocking density per pen* (cannibalism, ranking fights, stunted pigs, “support” of infectious diseases etc.), *poor ventilation* (respiratory diseases, cannibalism, dirty pigs etc.), *poor flooring* (claw deformities, bursitis, leg injuries, shoulder ulcers, fractures, abscesses etc.). These health impairments have the most significant impact on the animals' well being, i.e. improving the husbandry and housing systems is a major demand of animal protection.

For quite a long time, the public impression was that the bigger a herd becomes, the less healthy the animals are. This perception must be corrected: it is true that in continuously growing herds by adding more and more animal places to an originally small premise without appropriate biosecurity measures and with mixing animals age groups, bigger herds have more health problems than smaller herds; however, in big herds that are kept in new premises that had been planned for a modern biosecurity system, for an all-in/all-out animal flow and a separation of the age groups (e.g. three site production systems), the animal health status can be very “high”.

Management associated diseases

Although the husbandry and housing conditions have an enormous effect on the herd animal health and welfare, man is the major determinant for the occurrence and severity of health and well being impairments. It is well-known that two pig herds kept under similar housing conditions can have both a quite satisfactory and a very poor health status. The difference in these cases is mainly the quality of the animal care. In the same barn arrangement pigs can be moved in an all-in/all-out or in a continuous flow mode. The continuous flow system, mixing animals of different age groups, leads mostly to a much higher “infection pressure” in the herd than in a comparable herd with an all-in/all-out system. But even the animal observation and, thus, the timeliness of beginning a treatment can be of decisive impact on the disease level in the herd. Whether sick animals are recognised very early and removed to separate “sick pens”, or whether sick animals are left in the pens allowing them to infect their pen mates directly and to be “molested” by their still healthier companions, makes a huge difference. In the same way, the ability of the animal caretaker to apply correctly and timely the appropriate vaccination scheme is of great importance for the overall herd health.

Thus, an experienced and knowledgeable “stockmanship” coupled with a high-quality veterinary care is as important as an appropriate husbandry and housing system (see above).

Regionally and nationally organisational structure associated diseases

The regional and/or national structure of pig production has another quite important impact on the pig herd health. Regions or countries with uncoordinated production systems, e.g. small sow herds supplying big fattening herds by mixing various weaning pig groups, uncoordinated and uncontrolled animal movements, animal trade between regions and countries with an unknown health status, suffer from much more and severe animal diseases than those regions and countries that have built up an intelligent and coordinated animal health prevention and reduction scheme. This is true both for notifiable epidemic diseases and for non-notifiable endemic diseases. It is striking that countries such as the Scandinavian countries Sweden, Finland and Norway, as well as Switzerland have been free from Classical Swine fever and Aujeszky’s Disease for

several decades, and they managed to remain free from the PRRS-Virus and/or the PCV2-Virus. Switzerland has been even successful in eradicating *Mycoplasma hyopneumoniae* and is close to finalising the eradication of *Actinobacillus pleuropneumoniae*, both of them not being notifiable pathogens.

The question, whether a country or a production chain is or will be able to monitor and reduce the salmonella load of its pig herds, is also dependent on the degree of a regional or national coordination in the pig production.

Animal health quantification and benchmarking

The composition of a Herd Health Score (HHS)

In order to quantify the herd health status as the basis for benchmarking herds regarding their “disease load”, the following 4 parameters were gathered, rated, and combined to an HHS:

- 1) *The mortality rate,*
- 2) *The frequency of pathological findings in carcasses of previous meat-inspections,*
- 3) *The animal-treatment-index (ATI), and*
- 4) *The duration of the fattening-period.*

1) The mortality rate

If the mortality rate of a fattening group did not exceed 2% at the end of the fattening period this parameter was rated as mortality score 0. A frequency between 3% and 5% resulted in score 1, between 5% and 10% in score 2, and if the percentage of deceased pigs exceeded 10% the mortality score was 3.

2) Herd prevalence of pathological findings during previous meat-inspections

The frequency of gross pathological lesions found in carcasses and the organs of slaughter pigs of a herd is a quite objective indicator of the occurrence and severity of most diseases in pigs. Based on the Organ-Lesion-Index according to Blaha (1994) the diagnostic findings of the meat-inspections of the slaughter pigs of each pig farm in the last six months were summarized in an index which varies from 0 to 10. Thereby, the frequencies of pleurisy, pneumonia, liver-lesions and pericarditis per slaughter pig batch, and per herd (= cumulative index from consecutive batch) were rated and summarized in values from 0 to 10.

Organ-Lesion-Index according to BLAHA (1994)

pleurisy	frequency	<1%	1-10%	11-30%	>30%
	points	0	1	2	3
pneumonia	frequency	<1%	1-10%	11-30%	>30%
	points	0	1	2	3
liver lesions	frequency	<1%	1-10%	11-30%	>30%
	points	0	1	2	3
pericarditis	frequency	<1%	≥ 1%		
	points	0	1		

For calculating the HHS, a “Blaha-Index” less than 2 results in a lesion frequency score of 0, values from 3 to 5 were rated as score 1, points between 6 and 8 resulted in score 2, and a “Blaha-Index” higher than 8 leads to a lesion frequency score of 3.

3) *Animal-Treatment-Index (ATI):*

The Animal-Treatment-Index (Blaha et al., 2006) stands for the average frequency of medicating every pig in the group with antimicrobial substances, based upon the hypothesis that healthy pigs get less medication than pigs with a poor health and clinical illness. The formula is:

$$ATI = \frac{\text{No. of animals treated} \times \text{No. of treatment days}}{\text{Total - No. of animals in the herd or group}}$$

If the ATI does not exceed 10 days of medicating, this ends up in 0 points. An ATI between 11 and 20 days results in an ATI score of 1, ATI-values from 20 to 40 lead to an ATI score of 2. If a fattening group of pigs has been medicated with antimicrobial substances more than 40 days, the ATI score is 3.

4) *Duration of the fattening period*

On the study farms, all farmers start fattening piglets with a body weight between 27 and 31 kg and intend to send the slaughter pigs to abattoir with a body weight of 115kg to 121kg (= window of best price). If the time for fattening a batch of slaughter-pigs is less than 100 days this is calculated with score 0. A fattening duration period between 100 and 120 days results in score 1, a duration from 121 to 150 days accounts for score 2, and if the fattening took more than 150 days this is taken into account with score 3.

In the end, the sum of the 4 single scores is added to the Herd-Health-Score (HHS) which can vary between 0 and 12 points. The HHS can be calculated for single batches, but also for herds for whatever period of time.

The chosen criteria for indirectly measuring the health of pig groups (slaughter batches, finishing groups and/or herds) seem to provide a rather accurate reflection of the health history of pigs sent to slaughter.

Calculating a Herd Health Score for herds either of the herds supplying a slaughter plant, of a region or of the national pig population supports greatly the estimation of the herd health of slaughter pigs, which can be used for the *risk-based meat inspection*, for *benchmarking the herd health status of herds supplying the same slaughter house (as tool for the improvement of animal health and animal welfare)*, and for other *risk-based decisions such as targeted residue testing*.

Applying the general strategy of benchmarking the herd health status and helping the herds with the relatively lowest health status will lead to an overall continuous improvement. Fig. 3 shows the change of the animal care management systems over time from curing single diseased animals to the *sustainable production of high quality food from healthy animals*.

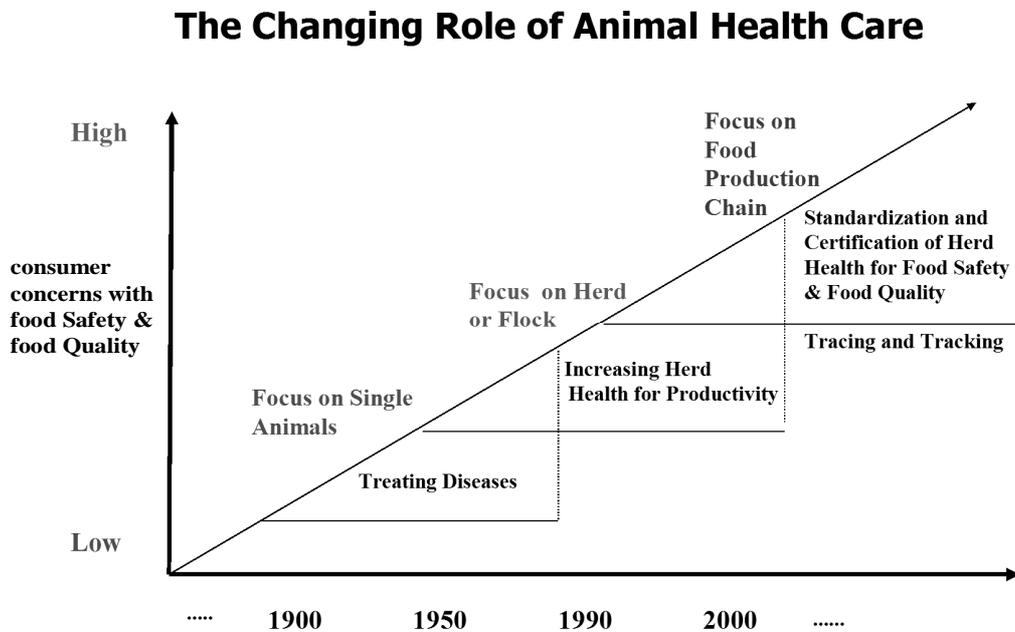


Figure 3: The changing animal care systems over time

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2.2 EPIDEMIOLOGY OF PRODUCTION DISEASES – SHEEP AND GOAT

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Many of the diseases seen in sheep and goats have a worldwide distribution. Disease severity and incidence may be affected by local factors including geography, weather, management systems and breed types.

Disease types can be divided up into those which are important and common, important but less common and unusual. Important and common conditions from a laboratory base include: parasitic gastro-enteritis, clostridial disease, infectious abortion (EAE, *Toxoplasma*), fasciolosis, sheep scab and lice. Sheep and goat flock health services and local practitioners indicated that they would also include lameness (virulent footrot), cervical /vaginal prolapse, gid, hypocalcaemia, tick pyaemia and other tick induced diseases, mastitis, pasteurellosis and listeriosis as conditions which are important, but for which a laboratory diagnosis is not always sought (Davies 2004).

If you look at statistics concerning routine post mortems in sheep worldwide you will find nearly the same distribution of diseases.

About half of the sheep and goats die of diseases of the alimentary tract, mainly due to Endoparasitosis, especially Helminthosis, followed by Coccidiosis, Cryptosporidiosis, and Taeniosis.

It is disappointing that clostridial diseases continue to be diagnosed despite the availability of effective vaccines. Pulpy kidney disease, lamb dysentery and other clostridial diseases are still the most frequent infectious diseases, respectively toxaeemias. Depending on the year, season and region the frequency of clostridial diseases varies between 1 and 12% in the UK (Davies 2004). On US feedlot in 29% of dead lambs the cause was enterotoxemia (APHIS 2004).

In adult small ruminants Ovine Johne's disease (OJD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is of major concern in some countries due to the possible cross-infection between sheep, goats and cattle, as well as the possible linkage of the agent to human Crohn's disease.

The annual mortality rate was calculated by Bush et al. (2006) over 3 years in 12 flocks infected with MAP. Mortality ranged from 1.8 % to 17.5%. The average decrease in gross margin due to OJD infection ranged between 1.5 % and 15.8%. OJD losses

accounted on average for two thirds of the total estimated financial loss associated with sheep deaths.

Own serological investigations in 98 sheep flocks in northern Germany in 2004 revealed an overall prevalence of 3%, with reactants in 16.3% of the flocks. The prevalence was higher in large commercial flocks than in the small hobby flocks. Only in 3% of the flocks were clinical diseases obvious. In North Rhine-Westphalia the seroprevalence in migrating commercial flocks was 4.16% (Vogel et al. 2005).

MAP DNA has been detected in raw goat's milk in the UK (81.1% positive, Grant et al. 2001), Norway (7.1% positive, Djonne et al. 2003) and Switzerland (23.0% positive, Muehlherr et al. 2003) by IS 900 PCR, but neither culture was attempted nor was the presence of viable MAP confirmed in any of these studies. Individual milk samples of goats were negative in northern Germany (Runge 2007). Raw sheep's milk was also tested. In the UK study there was no cultural or PCR evidence of MAP in sheep's milk (Grant et al. 2001), whereas in the Swiss study 23.8% of raw sheep's milk samples tested MAP positive by PCR (Muehlherr et al. 2003). Ikonomopoulos et al. (2005) tested 42 samples of retail feta cheese, made from a mixture of sheep's and goat's milk from Greece and reported the detection of MAP by PCR in 50% of samples and isolation of viable MAP from one sample.

The incidents of caseous lymphadenitis (CL) in sheep (as a percentage of diagnosable submissions varied between 0.3 and 1.7% in the VLA statistic over the years (Anonymous 2007a). However, the flock prevalences are much higher, for example 39% of the clients of the sheep and goat flock health service Hannover are CL positive (Ganter 2007). In Poland the number of serological CL positive goat flocks increased from 13 to 66.2% between 1997 and 2004, respectively (Kaba 2007).

Diseases of the respiratory system are more common than the post mortem statistics show with about 20% in the UK (Anonymous 2007a). Etiological agents for these respiratory diseases are primarily infections by *Mannheimia haemolytica*, *Pasteurella trehalosi* and *Mycoplasma species* (*M. ovipneumoniae*, *M. agalactiae* and others). In US feedlot lamb respiratory disorders including shipping fever pneumonia was the most frequent cause of death (41.8% of lambs that died) (APHIS 2005).

Contagious caprine pleuropneumony (CCPP), induced by *Mycoplasma capricolum* subsp. *capripneumoniae* is notified in Pakistan, Afghanistan, Iran, Saudi Arabia, Oman, Eritrea, Ethiopia, Kenya, Tanzania, Uganda, and Kameroun (Anonymous 2007b). Local outbreaks occur from time to time. Acute CCPP is seen in goats and is of similar economical relevance as CBPP in bovines (Younan 2007).

Important, but less common conditions include ovine pulmonary adenocarcinoma (Jaagsiekte), and Maedi-Visna. Australia and Iceland are regarded as being free of both diseases. Seroprevalence and clinical signs of Maedi-Visna depend on management and housing conditions and also very much on the breed.

Within Europe Small Ruminant Lentivirus (SRLV) infections (Maedi-Visna and Caprine Arthritis and Encephalitis) are enzootic in most European Mediterranean countries, with up to 96.8% of the intensively managed milking sheep flocks being seropositive (Sotelo 1998). Within these intensively managed flocks even the incidence of clinical visna might be high and in some flocks this form of the disease is considered to be the main cause of mortality among adult sheep (Benavides et al. 2006). In Italy, presumably the infection is widespread over the country and in some areas it is very hard to find a seronegative flock. There the seroprevalence inside the flock ranges between 60% and 90%. In central Greece, 17 % of examined adult sheep-lungs were found with gross lesions of Maedi in an abattoir survey (Christodoulopoulos 2005). Despite clinical cases being reported in most Central European countries the Maedi-Visna seroprevalence is low especially in the northern countries, or the disease has even been eradicated as in Iceland. In the UK the SAC Maedi Visna accreditation scheme has increased to 3000 member flocks. In 2006 three flocks failed the first qualifying blood test (Anonymous 2007a) In Germany 352 sheep flocks and 604 goat flocks joined the voluntary accreditation scheme. Concerning sheep, local epidemiological studies in some federal German States revealed Maedi seroprevalences between 3.6% and 29.9%, with flock prevalences between 12.8% and 67%. In the non accredited flocks clinical MaediVisna occurs (Graber and Ganter 2005). The central nervous form of the disease is very sporadic in Germany (Ganter et al. 2007). Outside Europe Maedi- Visna was reported from Argentina, Brazil, Chile, Paraguay, Canada, the USA, and Ethiopia. Some of these countries, e.g. the USA and Brazil run a voluntary accreditation scheme. With the exception of Australia, where CAE but not Maedi-Visna is notified, in most of the countries where Maedi-Visna was reported also CAE was notified (Anonymous 2007b). In Israel, a serological investigation in 2003 revealed 25% - 75% intra flock prevalences in all 10 flocks (Christodoulopoulos 2005).

The prevalence of ovine pulmonary adenomatosis (SPA) is relatively low but intra flock incidences of up to 30% (Voigt et al. 2005) can occur. According to OIE notification (Anonymous 2007b) OPA seems to be present only in South and North America, some European countries, in Uzbekistan and South Africa (Anonymous 2007b). Recently OPA was introduced into the flocks of pastoralist sheep in semi-arid North Kenya with huge losses (Younan et al. 2007).

Pest des petits ruminants is notified in several African countries as well as in Iran, Afghanistan, Pakistan, Nepal, Israel, and Guinea. Within the last decade PPR is spread over the African continent from east to west and has reached now the Indian Ocean. For Eastern Africa was originally free of PPR new outbreaks were accompanied by numerous losses.

Clinical cases of *Rift valley fever* were notified in Yemen in 2005. (Anonymous 2007b).

Bluetongue and *Nairobi-Sheep disease* are endemic in some local African regions. Clinical outbreaks are seen very sporadically, especially when sheep from outside (e.g.

from Europe) come into the region or when pastoralists change their migration tours (Younan 2007)

After several outbreaks of Bluetongue between 2002 and 2004 in Mediterranean countries, Bluetongue occurred in August 2006 for the first time in Central Europe. The Netherlands notified the first outbreak in two farms at the border to Germany. Some days later bluetongue was diagnosed in Belgium, followed by Germany. After the first outbreaks near Aachen the disease spread quickly to several German federal States. Until the end of 2006 306 sheep flocks and 564 cattle herds were infected. Morbidity (3.24%), Mortality (1.5%) and Letality (46.43%) was much higher in sheep than in cattle (2.09%, 0.7%, 3.28%, respectively) (Conraths et al. 2007). Serotyping of the agent revealed Bluetongue Virus serotype 8 (BTV8). This serotype had been isolated before only south of the Sahara, South and Middle America, India and Pakistan. The virus is transmitted by *Culicoides* spec. endemic in Central Europe. How BTV 8 was imported could not be clarified.

Anthrax is still a seasonal problem in some African countries.

Skin diseases

Sheep and goat pox were reported from several African countries and from the Arabian peninsula, Turkey, Iran, Pakistan, Nepal, Tadjikistan, Kyrgyzstan, and Vietnam (Anonymous 2007b).

There are no epidemiological data about *Orf* virus infections available but the disease is very widely distributed in Central Europe. Orf as well as Sheep and goat pox induce sporadically severe outbreaks with numerous losses in young lambs especially when the infections are newly introduced into the flocks.

Louse infestations are very common in sheep and goats in the whole of Europe. There are no statistics reflecting the real situation as practitioners may confirm infection locally or farmers may administer treatment without seeking laboratory diagnosis. The same applies for sheep scab. In the VLA Report for 2006 5% of the diagnosable submissions were positive for *Psoroptes ovis* (Anonymous 2007a). In Germany chorioptic mange is more prevalent especially in goats than psoroptic and sarcoptic mange.

Due to the hot weather in 2005/2006 the incidents of Blowfly Strikes increased (Anonymous 2007a).

CNS disorders

The most common causes of neurological symptoms in small ruminants in Germany are inflammatory- infectious (34%) and metabolic-toxic (53%) diseases. Traumata

caused 13% of the disorders. Within the infectious CNS-diseases Listeriosis was the most frequent followed by Encephalitis due to other bacteria and Myelitis. Otitis interna, Tetanus and Scrapie were sporadic. In Listeriosis an association with poor quality silage is common. Encephalitis due to Listeriosis is diagnosed more frequently in small ruminants than in cattle. (Anonymous 2005, Schenk 2005). Within the group of metabolic-toxic CNS disorders hypocalcaemia and nutritional muscle disease were most frequent followed by cerebrocortical necrosis, pregnancy toxicosis, sway back, and hepatoencephalopathy (Schenk 2005).

Scrapie: Beginning in 2002 active monitoring of Transmissible Spongiforme Encephalopathies (TSEs) was performed in the European Union, according to the Regulation EC 999/2001 followed by various modifications over time. In 2005 an increased testing of goats began due to a confirmed BSE case in a French goat. Sheep monitoring requires testing of all clinical suspect cases plus a minimum of 10,000 sheep over 18 months slaughtered for human consumption in all Member States with more than 750,000 breeding ewes, plus a “minimal sample size” of sheep over 18 months that leave the population but are not slaughtered for human consumption, plus “minimal sample size” of animals older than 12 months from known infected flocks. The goat monitoring requires testing of all goats over 18 months slaughtered for human consumption in the 19 Member States with small goat populations, and a “minimum sample” in the other Member States, plus all breeding goats over 18 months up to 200 or “a minimum sample size” of risk animals / fallen stock, plus tests from animals from infected flocks as for sheep. Within the EU15 more than 2.1 million sheep and 0.65 million goats were tested from 2002 to 2006. 7,446 sheep and 254 goats were tested positive. By this sampling method intensity of testing is much higher in states with small SR populations like Luxembourg or Denmark, where 4-5 % of the population was tested; in contrary less than 0,5% of the population was tested in the member states with huge populations like Spain, the UK or Greece. Differences in the scrapie incidence within the different national flocks depend also on the amount of fallen stock and other animals at risk tested. The TSE prevalence in SR varied from 0 (in Luxembourg, Denmark and Austria) to less than 0.001 in Sweden, Germany, Portugal, and Finland and up to 0.008 in the UK. An extraordinary scrapie incidence was found in Cyprus with more than 100 cases per 100,000 healthy-slaughtered adult sheep and more than 1,000 cases per 100,000 sheep at risk (Doherr 2007).

In the USA the surveillance within the scrapie eradication program in 2005 revealed in total 598 scrapie cases among 35,447 tested animals. 165 newly identified infected flocks were reported. Testing was performed not only by immunohistochemistry on the obex or a peripheral lymph node taken by third eyelid biopsies (APHIS 2005).

In all confirmed German TSE cases the Prion-protein (PpP) genes were genotyped at the codons 136, 154 and 171. The classification into Scrapie risk categories based on the UK National Scrapie Plan (NSP) (Anonymous 2007c) scheme where the animals with the homozygote alleles ARR/ARR are regarded as most resistant (= R1) and the homozygote alleles (VRQ/VRQ) are regarded as most susceptible for scrapie (=R5). Based on this genotyping the NSP for the UK was established also in other Member

States. The NSP is a strategic long-term plan which consists of a breeding programme to increase the number of sheep that are genetically naturally resistant to scrapie.

In 1998 Benestad and Coworkers (Benestad et al. 2003) found the first case of a sheep with atypical scrapie, showing neurological signs dominated by ataxia, and a PrP genotype, which was rarely associated with scrapie. Brain histopathology revealed neuropil vacuolisation essentially in the cerebellar and cerebral cortices; vacuolation was less prominent in the brainstem, and no lesions were observed at the level of the obex.

The discriminatory testing between BSE and scrapie, where one BSE positive goat was found in France (Eloit et al. 2005) was supplemented by discriminatory tests between classical and atypical scrapie following the finding of atypical scrapie, according to the Commission Regulation (EC) No 214/2005 (Gretschel 2006, Buschmann 2007). According to the discriminatory tests atypical and classical scrapie was found in most Member States with the exception of Austria and Greece, where only classical scrapie was found, and Portugal and Denmark, on the other hand, where only atypical scrapie cases were detected. Outside the EU atypical scrapie has been detected in Iceland and on the Falkland Islands up to now. In Germany 123 out of 249 scrapie positive sheep, detected in the time span from 2002 until May 2007 were diagnosed as atypical scrapie. On flock bases these 123 atypical scrapie cases originated from outbreaks in 118 different flocks, respectively, despite 126 sheep with classical scrapie originating from 23 flocks. That shows that the prevalence of atypical scrapie in affected flocks is much lower compared to classical scrapie. The finding of atypical scrapie in cohort animals of affected flocks does not allow transmissibility of atypical scrapie to be excluded (Buschmann 2007).

15 of the German sheep with atypical scrapie carried the ARR/ARR allele (Buschmann 2007). Therefore, it can be concluded that the breeding program based on the NSP is not suitable for elimination of atypical scrapie. Future scrapie eradication measures of the EU will focus on the discrimination of BSE, classical scrapie and atypical scrapie. In cases of BSE the whole flock will be culled without any exception. In cases of classical scrapie all genetically highly susceptible animals, that means all VRQ carriers and all animals lacking at least one ARR allele will be culled or slaughtered. ARR carriers can be used continuously and leave the flock only for slaughter, breeding only with ARR/ARR rams. In cases of atypical scrapie culling is not compulsory, but all animals in the flock have to be identified individually. An intensified surveillance including rapid testing of all slaughter animals has to be performed on all animals over 18 months. Additionally, transport of live animals or embryos or ova to other Member States or non-EU countries is prohibited.

Reproductive disorders

Enzootic abortion of ewes (ovine chlamydiosis) is the most common aetiological agent found in aborted placentas in Northern and Central Europe. Outside Europe ovine chlamydiosis is only registered in Mediterranean parts of Africa and Asia (Anonymous 2007b). In 2006 Murer reported abortions occurring in Switzerland caused by *Salmonella abortusovis* with abortion rates of 14% to 60% per flock. *S. abortusovis* also induced abortions in France, Italy, Croatia, Portugal, Palestina, Mongolia, Uzbekistan, Angola, Indonesia, and Uruguay (Anonymous 2007b)

Brucellosis (*B. melitensis*) is prevalent in numerous Mediterranean countries in Europe, Africa and Asia, the Arabian Peninsula, South America and some Central Asian countries. Sporadic infections have occurred in the UK and in Germany within the last years. In most of the countries where Brucellosis is notified **Ovine epididymitis** (*Brucella ovis*) is also present. Additionally, Ovine epididymitis occurs in Australia, New Zealand and Canada (Anonymous 2007b).

Large outbreaks of human **Q fever** in Germany in 2003 and 2005 were connected to sheep flocks without any clinical signs or significant increase in abortions. In Lower Saxony (northern part of Germany) the seroprevalences of ovine chlamydiosis (19.3% of animals and 55% of the flocks were positive), toxoplasmosis (53,6% animals and all flocks positive), and Q fever (4% of the samples and 10 % of the flocks were positive) was evaluated in 2004. In a positive flock *Coxiella burnetii* could not be found by PCR in the fetus and placentas of abortus but in the placentas of normal births (Ganter et al. 2005). A meta-analysis revealed percentages of seropositivity against *Coxiella burnetii* varying between 0.2 and 48.6% in various countries in different species (Manteca et al. 2005).

In New Zealand the bacterium ***Campylobacter fetus*** subsp. *fetus* is the leading cause of diagnosed sheep abortion (Mannering et al. 2005).

In Norway **Mastitis** is the most frequently reported disease in a recording system with 4,600 members, which makes up 26% of the registered flocks. The cumulative annual incidence of mastitis was 2.4%. The occurrence of severe mastitis was highest during the first week after parity. The cumulative incidence increased with the number of lambs born (Vatn 2005).

Metabolic disorders are seldom in comparison with infectious diseases in post mortem statistics and make up less than 10% of the cases (Winkelmann 2007). Within these "Metabolic disorders" cachexia, due to malnutrition is the most frequent problem followed by copper disorders (deficiency as well as poisoning), Selenium deficiency, Rumen Acidosis and Ketosis. Infrequent diseases are Urolithiasis, Infections of the urinary bladder, renal failure and renal cysts, Goiter, Sarcocystosis and Malformation in Germany. Despite clinical diseases due to deficiencies in copper, selenium and vitamin E being very seldom, routine liver sampling reveals subclinical Vitamin E

deficiency in 39% of the samples, selenium deficiency in 45% and copper deficiency in 45% of the samples in Northern Germany (Humann-Ziehank, and Ganter 2006). In the VLA-report metabolic disorders were counted under the “Systemic and Miscellaneous” Syndrome, which attributed to 43% of all submissions in 2006. Within this syndrome complex 5% were diagnosed as Pine/Cobalt deficiency, 4% Hypocalcaemia, 3% Copper poisoning, 3% pregnancy toxemia, and 3% malnutrition (Anonymous 2007a). In the 2005 US Animal Health Report, metabolic disorders are counted as the cause of death in 3.7% of adult sheep and in 2.8% of lambs (Anonymous 2005b).

General management trends and risks

Production in sheep and goat is diverging. In Europe production with sheep and goat is becoming more and more extensive with the main aim of landscape protection. On the other hand, an intensification can be observed especially in milk production in small ruminants not only in the Mediterranean but also in northern countries.

Both intensive and extensive productions are facing a severe crisis in the industry due to the enormous increase in resistance to endoparasites against anthelmintics. Resistance to all the main anthelmintic classes has been exhibited by nematode populations in most sheep-rearing countries over the last twenty years. Multi-resistance to all anthelmintic classes makes lamb production impossible in some regions of the world.

Resistance is the heritable ability of the parasite to tolerate a normally effective dose of the anthelmintic. The parasite is considered resistant if it survives exposure to the standard recommended dose of the anthelmintic and its ability to survive is passed on to its offspring. Factors that influence the rate at which anthelmintic resistance (AR) appears in a worm population are as follows:

1. One of the important factors influencing the rate at which resistance develops in a worm population is the relative size of the exposed population in relation to the unexposed or in refugia population. In general, the larger the in refugia population in comparison to the exposed population, the more slowly resistance will develop.
2. The more frequently treatment is given, the faster AR develops.
3. Dose and movement gives the resistant survivors after dosing a longer period of reproductive advantage.
4. In the past, under-dosing of sheep with anthelmintics was probably common-place, because, either the weight of the sheep was under-estimated, instructions for dose calculation were misleading or dosing equipment was faulty. This is now recognised as a very significant factor in the development of resistance to Benzimidazoles and Levamisole anthelmintics in particular (Abbot et al. 2004).

Extensivation

The idea behind extensive production for landscape preservation is to keep a landscape formed by more or less intensive grazing by sheep and goat in former times in this form. Effective farming is only possible if there are sufficient subsidies to equalise the production losses and the increase in work load. Extensive production with small ruminants faces several problems:

- Undernutrition especially during the dry seasons.
- Endo- and Ectoparasites are a main problem in extensive production.

Imbalances in trace element metabolism are common in farm animals in northern Europe and other countries like e.g. Canada. Deficiencies were frequently found in grazing sheep and goats, whereas overload appears once in a while resulting from disarrangement of food composition or for example industrial pollution. In general, trace element deficiency remains without severe clinical signs for a long time. The main clinical signs may be bad body condition, reduced infection resistance and/or poor breeding results. The ability and effort of the owner to offer a commercial mineral supply is variable because of costs and presumed effort. Mineral supply prevails, but discussion regarding its necessity is common, particularly in some organic farming systems, due to the comparison to non-supplied and supposed healthy wild ruminants in the same region. Nevertheless, due to the opportunity of selective browsing, only vitamin E supply is advantageous in roe deer compared to sheep, whereas selenium, copper and zinc are unaffected or even lower (Humann-Ziehanek et al. 2007).

Intensivation

Especially milk production with small ruminants is a growing market and has turned from familiar production to industrial units especially in goats but also to a lesser amount in sheep. Intensive milk production calls for continuous intake of well-balanced feedstuff. Due to the behaviour of goats this seems to be more warranted by transponder feeding with numerous small doses of concentrates combined with continuous offer of roughage than total mixed ration. In the case of transponder feeding, losses due to Clostridiosis and sub-clinical rumen acidosis can be reduced in spite of increased intake of concentrates and increased milk yield. By the abdication on silage, on which total mixed ration bases, the risk of Listeriosis is reduced.

Intensive milk production is only effective if the flocks are free of chronic infectious diseases. Therefore, great efforts are made to free the flocks from Small Ruminant Lentivirus infections, Caseous lymphadenitis and Johne's disease. To avoid losses due to endoparasites there is the need for total indoor production. In biological farming a total indoor production is not permitted according to the EU-legislation.

To improve milk yield and to reduce losses due to obstetric problems an increasing number of goats are milked over a period of up to three years without mating and lambing.

In lambing goats, kids are reared directly after birth and fed by milk replacer to improve the milk yield from the dam, and to interrupt vertical transmission of infections.

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2.3 EPIDEMIOLOGY OF PRODUCTION DISEASES IN HIGH-PRODUCING DAIRY COWS – HUNGARIAN EXPERENCES

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Introduction

Metabolic disorders or production diseases have an increasing significance in high producing dairy herds and remain a critical problem for the livestock industry (Drackley, 2006). During the second half of the last century the Western and Eastern parts of Europe were characterized by dairy herds of different sizes: in the West, smaller family farms were common while in the East dairy herds with 800-1000 cows were more frequent. In the nineties this changed as bigger farms appeared in the West and smaller ones developed in the East. Both sized farms are found in Hungary and production diseases are common here. Our experiences might therefore be interesting for the participants of this congress. Results shown here reflect partially conditions of the surrounding countries. For comparison, some data of other European countries are also described.

Hungarian experiences

From the late nineteen seventies, some 30.000 Holstein-Friesian heifers/cows were imported to Hungary. More than 350,000 Hungarian spotted, Simmental-based, dual-purpose cattle were crossed during 15 years. Due to this breeding policy milk production nearly doubled from 4138 kg/lactation in 1980 to 8122 kg in 2006 (National Institute for Agricultural Quality Control, NIAQC). Unfortunately, farmers only slowly understood that these cows had requirements different from the traditional, dual purpose breeds especially during the transition period. This was probably the main reason why the frequency of metabolic/production diseases substantially increased in the country and why we have been working in the field of production diseases since the early 1980s using experiences of European, mainly British researchers (Gaál et al. 1983a, 1983b). Comparable situations were seen in some surrounding countries where similar cross-breedings happened.

Prevalence of production diseases

Annual reports of the NIAQC Hungary clearly show that, during the past few decades, the importance of production disorders has become more substantial than that of any

other diseases. Culling rate in high producing dairy herds has tended to increase. As the Hungarian Central Statistical Office and Livestock Performance Testing Ltd. reported, in 1996 culling rate in 379,000 Holstein Friesian cows was 22.8% while in 2006 it was 33.2% in 268,000 cows. Cullings are often consequences of primary metabolic disorders. The most common reasons of removal/culling in large dairy herds are low milk production and infertility (Ózsvári, 2004). In spite of the fact that milk production and culling rate increased in parallel, we do not think that high milk yield alone caused increasing number of production diseases and cullings. Rather, we agree with Drackley (2006) that the conceptual view that high-producing cows are more susceptible to environmental insults disturbing their homeostasis and causing production diseases is more attractive than the notion that production disease is caused by high production *per se*.

Clinical production diseases

Among production diseases with clinical signs and often death or emergency slaughter, fatty liver is the most common while milk fever, ketosis and abomasal displacement are less frequent. However, overt clinical disease seems to have declined while culling for subclinical forms and consequences has increased.

At a large dairy herd of some 1000 Holstein Friesian cows between 1996 and 2006 milk production increased from 8971 kg to 9435 kg. In 1996 16% of 924 cows showed clinical signs and died or had to be slaughtered. One-third of these clinical diseases were fatty liver, milk fever, and ketosis with dominance of the first one. As further 16% of the cows had to be removed due to low milk production, chronic lameness, and infertility (“subclinical” diseases), total culling rate was 32%. Ten years later in 2006 among 1103 cows at the same herd only 60 cows (5.4%) died or had to be slaughtered. Half of these cows were suffering from metabolic disorders. A further 26% of cows had to be removed from the herd for “subclinical” problems, so the total culling rate was similar to that in 1996.

At another dairy farm of 800 Holstein Friesian cows between 2000 and 2006 milk production increased from 6300 to 8600 kg/lactation. During these 7 years at this farm total culling rate increased from 24 to 31%. There was a similar tendency as in the first farm: rate of clinical production diseases decreased while subclinical cases increased. Among clinical production diseases in 2000 fatty liver was represented by 35% while in 2006 it dropped to 15%. Milk fever and ketosis was represented by 10 and 12% in both years.

Subclinical production diseases

Subclinical metabolic disease frequently goes unnoticed and may be associated with clinical disease risks, impaired production, reduced reproductive performance, and increased risk of culling (Duffield, 2006). For this reason subclinical forms of

production diseases were studied by several Hungarian companies in the nineteen eighties and nineties using the classic or modified Compton Metabolic Profile Test (Payne et al., 1970). Currently, most data are provided by the workgroup of Brydl at our Faculty who regularly visits most large-scale dairy farms in Hungary where 500-1000 dairy cows are kept and grouped according to the stage of lactation and reproduction. With the modified metabolic profile test they analyze individual blood and urine samples in 4 groups of cows: dry cows in the close-up period i.e. 2-3 weeks prior to calving; fresh cows (4-7 days in milk, DIM); cows in early lactation (10-30 DIM); cows in peak lactation (<100 DIM). Numbers of the randomly sampled, healthy cows depends on the size of the group but is 5-10% of the total. Body condition score (BCS) is always evaluated. Blood plasma metabolites used in evaluation are glucose, aceto-acetate (AcAc), beta-hydroxy-butyrate (BHB), NEFA, urea, total carotene, total Ca, inorganic P, Mg, and activities of AST as well as glutathione-peroxidase enzymes of red blood cells. In the urine pH, net acid-base excretion, urea, Na and K are measured. They use the following reference points for normality in blood: glucose >2.3 mmol/L; AcAc <0.1 mmol/L; BHB <0.8 mmol/L; NEFA <0.2 mmol/L; AST <80 mmol/L; increased lipid mobilization with elevated AST: subclinical lipid mobilization disease; urea: 3.3-5.0 mmol/L; total carotene: > 5.6 micromol/L; total Ca 2.1-3.0 mmol/L (in fresh cows 1.75-2.1 mmol/L); inorganic P 1.6-2.3 (in fresh cows 1.0-1.6 mmol/L); Mg 0.8-1.25 (in fresh cows 0.6-1.25) mmol/L; GSH-Px >10 U/g haemoglobin. Reference points in urine: pH 7.8-8.4; net acid-base excretion >100 mmol/L; urea 130-300 mmol/L; Na 20-80 mmol/L; K 140-320 mmol/L.

Based on his reports (Brydl et al. 2003, 2006) the following subclinical metabolic diseases were the most common:

Dry cows: BCS >3.5 (25%); increased lipid mobilization (10%); protein overfeeding (25%); protein deficiency (25%); ketonaemia (1-3%); aciduria (60%); carotene deficiency (70%); Na-deficiency (40-50%)

Fresh cows: BCS <3.0 (15-20%); increased lipid mobilization with elevated AST activity (25-30%); protein overfeeding (40-50%); ketonaemia (20%); aciduria (60%); carotene deficiency (80%); hypocalcaemia (2-4%); hypophosphataemia (10-20%); Na-deficiency (40-50%)

Early lactation cows: BCS <3.0 (40-45%); increased lipid mobilization with elevated AST activity (5-10%); protein overfeeding (45-50%); ketonaemia (5-10%); aciduria (40-50%); carotene deficiency (75%); hypophosphataemia (15%); Na-deficiency (20%)

Peak lactation cows: BCS <3.0 (60-70%); protein overfeeding (45-50%); ketonaemia (5%); aciduria (30-40%); carotene deficiency (60%); hypophosphataemia (10-15%); Na-deficiency (10%)

In summary, the most common subclinical metabolic disorders are carotene deficiency, protein overfeeding and aciduria. As these conditions are not necessarily connected

with marked, direct clinical consequences, their significance is often underestimated. In contrast, laboratory results linked to NEB are often associated with development of clinical symptoms and even with death of cows. Probably this is the reason why emphasis is generally given to the NEB-related disorders.

Experiences with negative energy balance (NEB) and fatty liver

NEB is one of the most important factors in the development of several metabolic disorders. Increasing metabolic activity during the transition period might be associated with several consequences that are routinely rarely studied such as changes/adaptation of the ruminal epithelium to subclinical acidosis or changes in the redox system of the body as well as changes of leptin metabolism.

Parakeratosis, hyperkeratosis and subclinical rumen acidosis

Subclinical rumen acidosis is thought to be a major problem on many dairy farms but is difficult to measure and very little controlled research exists for this syndrome (Duffield, 2006). It was observed also that the susceptibility of animals to rumen acidosis varies highly (Bevans, 2005). One of the possible physiological backgrounds of the subclinical rumen acidosis and of the various susceptibility of the ruminal mucosa may be the rate of keratinization affecting the intensity of VFA absorption in the rumen as the thick keratin layer serves as a physical barrier for VFA absorption. Gálfi et al. (1981a, 1981b, 1988) established that the function and structure of the rumen epithelium is deeply affected by the speed of epithelial cell division (mitotic index), the migration time of basal cells into the stratum corneum (transit time) and, in close connection with this, by the number of cells in the stratum spinosum. They also found that the concentration of certain end products of ruminal fermentation such as butyrate influenced cell division. In vitro and in vivo experiments of Gálfi (2005) defined three basic types (A, B, C) of the stages and adaptation of the ruminal mucosa that were in close connection with the diet and, indirectly, with production.

Stage A: physiological stage, a resting/silent period. Mitotic index, number of cells in the stratum spinosum and butyrate concentration in the rumen fluid is low. Rumen pH is around 7. This stage is observed when the diet is low in energy/concentrate and rich in roughage.

Stage B: Parakeratotic adaptation. Mitotic index is high, number of cells in the stratum spinosum is higher than in Stage A and progressively increases, butyrate concentration is high and fluctuating, but always less than 15 mmol/L. Rumen pH drops temporarily for 1-2 hours below 6 (short-term, controlled acidosis). This progressive adaptation can be observed as a consequence of the energy-rich diet.

Stage C: Pathological hyperkeratosis. Mitotic index is low, number of cells in the stratum spinosum decreases, butyrate concentration is continuously higher than 15

mmol/L. A drop in rumen pH (<5.8) is commonly present for 12 hours/day or longer (long-term, uncontrolled acidosis). This pathological stage is the consequence of an energy-rich diet without the ability of the animal to adapt. The rumen epithelium enters Stage C after a period in Stage B. Signs of moderate parakeratosis might be seen even in Stage A.

Potential role of free radicals

Increasing milk production is associated with intensive oxidative metabolism. The oxidative stress caused by calving and introduction of milk production in 58 dairy cows was studied before calving, at parturition and after calving (Gaál et al., 2006). Higher superoxide dismutase activity in red blood cells and lower antioxidant capacity was found at calving compared to the average of all pre- and post-calving results. We concluded that stress of calving and starting milk production had an influence on the antioxidant system of cows.

In another experiment (Gaál et al., 2004) 3 weeks prepartum and 7, 21, 35 and 56 days post partum liver biopsy samples were collected and their free radical concentration was measured by electron spin resonance spectrometry (ESR). During the dry period and 7 days after calving when the lipid mobilization and fatty infiltration of the liver was the highest surprisingly the concentration of free radicals in the liver was relatively low. However, as milk production and metabolic activity of the liver increased the free radical concentration in the liver – most probably due to the accelerated oxygen utilization and leakage of ROS – also elevated and reflected in a 2.5-fold increase by the end of the experiment.

NEB and leptons

Leptin is one of the cytokine-like protein hormones of the white adipose tissue. The triglyceride content of lipid depots associated with the current feeding level (energy balance) is the primary determinant of leptin gene expression in adipocytes, and the circulating leptin level (Chilliard et al., 2005; Zieba et al., 2005). Leptin plays an important role in signaling nutritional status to the central regulation of reproduction (hypothalamic GnRH-producing neurons). It appears to be a permissive factor in modulation of ovarian function shifting from anovulatory-acyclic to ovulatory-cyclic (Meikle et al., 2004; Zieba et al., 2005; Kulcsár et al., 2006). In dairy cows the endocrine signals that most likely could inform the reproductive axis regarding the postpartum negative energy balance and the level of body reserves include IGF-I and leptin (Meikle et al., 2004). The subclinical form of bovine (Huszenicza et al., 2006) and ovine ketosis (Kulcsár et al., 2006) is characterized by complex endocrine alterations reflecting the lactation- or pregnancy-associated energy imbalance, which include a decrease in plasma leptin (Kulcsár, 2007). During the periparturient period clear differences were demonstrated between the circulating leptin levels of normo-

and hyperketonaemic dairy cows, with lower leptin content in the plasma of those which have had >1 mmol/L BHB since calving (Kulcsár, 2007).

Diagnosis of NEB

Early diagnosis of NEB during subclinical stage and prevention of its further consequences is essential as treatment of clinical symptoms is often questionable. Minimizing the occurrence, severity, and consequences of NEB has become an important issue for the dairy industry (Geishauser et al., 2000). Most commonly studied parameters of NEB are mobilized non esterified (=free) fatty acids (NEFA, FFA) and ketone bodies (LeBlanc, 2006)

1. Cow-side tests

As energy imbalance is associated with increased lipid mobilization and ketogenesis, it is obvious that indicators of these processes are often evaluated. Ketone body concentration in urine, blood plasma and milk have been measured for many years by clinicians with available ketone tests (Geishauser et al. 2000; Carrier et al. 2004; Oetzel et al., 2004) while measurement of NEFA with an accurate portable monitor (DVM-NEFA®, Veterinary Diagnostix Inc., USA) was introduced recently and found to be a useful tool for prediction of cows with increased risk of postpartum subclinical ketosis (Goojer et al., 2004).

Ketonuria-index: Increased ketogenesis due to NEB is reflected firstly in ketonuria. Detectable amounts of aceto-acetate (AcAc) appear first in the urine (ketonuria) then in the blood plasma (ketonaemia) and at last in the milk (ketolactia). Total ketone body concentration is reported to be 4 times higher in urine than blood (Schultze, 1971). In our experiences even in the best dairy herds a temporary, minor ketonuria may develop for 1-2 days during the first week after calving (Tóth et al., 1989). We agree with others (Duffield, 2000; Carrier et al. 2004; Oetzel et al. 2004) that urine tests are not specific enough for detection of subclinical ketosis as they report too many false positives. However, we think that early recognition of energy deficiency by assessment of ketonuria gives us a good tool to prevent more severe consequences. Excellent sensitivity of nitroprusside-containing reagents makes them a useful test for evaluating individual sick cows for whom a false positive result is preferred to a false negative one (Oetzel et al. 2004).

A crucial point in assessment of ketonuria is that its severity and occurrence relative to calving are equally important. A moderate ketonuria before calving might be more significant than a severe one after calving. For exact evaluation of both intensity and occurrence of ketonuria, we worked out an equation that expresses in one number these two components: the ketonuria index (Kégl and Gaál, 1992).

Intensity of ketonuria was measured twice weekly in 643 Holstein-Friesian cows during 3.5 years with Ketostix® (Bayer) dipstick 1 week before and 3 weeks after

calving. Intensity results were marked on a scale of 0-5. Clinical signs and treatments of all animals with ketonuria were recorded.

Calculation of ketonuria index in 2 steps:

1. Examination of the basal value: $10 \times \text{intensity (0-5)}$ of ketonuria. This gives a basal value between 0-50.
2. Days related to calving.
 - a. before calving: basal value + $2 \times \text{days to expected calving}$
 - b. after calving: basal value - $2 \times \text{days after calving}$

Example: In a dairy cow intensity of ketonuria is 3, i.e. the basal value = $10 \times 3 = 30$

- a. If she is 5 days *before* expected calving, the ketonuria index = $30 + (2 \times 5) = 40$
- b. If she is 5 days *after* calving, the ketonuria index = $30 - (2 \times 5) = 20$

The calculated ketonuria index is interpreted as follows:

Group	Ketonuria index	Interpretation
1	< 20	physiological
2	20 - 29	slightly risky
3	30 - 39	dangerous
4	> 39	life threatening

Treatment of Group 1 is not necessary but care is needed, ketonuria should be tested repeatedly. Cows in Group 2 need extra feeding and care while in Group 3 medical treatment is also essential. Treatment of cows in Group 4 is often unsuccessful.

NEFA measurement in field conditions

Since measurement of NEFA in blood serum with DVM-NEFA® is available, several papers have reported its usefulness in dairy herds (Goojer et al., 2004). We have only preliminary results using this test. Correlation between cow-side DVM-NEFA results and two laboratory methods (colorimetric, non-enzymatic: Duncombe, 1964; colorimetric, enzymatic: Matsubara et al., 1983) on a RANDOX Daytona® chemistry analyzer in 24 blood samples was acceptable but between the results significant differences was found. DVM-NEFA produced significantly higher NEFA than the other two methods (Gaál, unpublished). Detailed evaluation validation of DVM-NEFA and comparison of methods is needed in the near future to enable laboratories to compare their results.

2. Evaluation of NEB in laboratory

Most laboratories are able to measure parameters of energy metabolism in the blood such as glucose, NEFA, ketone bodies.. and will not be discussed here. Evaluation of milk parameters is less common.

Milk urea and acetone

At the central milk testing facility of Livestock Performance Testing (LPT) Ltd. (Gödöllő, Hungary) milk production of several dairy herds is measured monthly and

individual milk samples are analysed on a Fossomatic 4000 MilkoScan® milk analyzer (Foss Electric, Denmark) using infrared technology for lipid, protein, and somatic cell count. On request urea concentration is also measured (Grappin et al., 1980; Tömösközy et al., 1998; Fekete et al., 1998). Recently, acetone measurement with an injection flow spectrophotometric system (Marstorp et al., 1983) has also been introduced on a EnviroFlow 5012 analyzer (Foss-Tecator, Sweden).

Results achieved with milk analysis in January 1998 on 94,000 cows showed that 38% had normal protein and energy balance. Some 6% of investigated animals expressed moderate protein and energy deficiency and 8% were slightly energy-deficient. Nine years later in January 2007 40% of 128,000 cows belonged to the normal group while 5% were protein and energy deficient. Energy deficiency alone was present in 17% of cows in the study (Kerényi, personal communication; LPT Ltd. Newsletters).

Milk citrate determination

As the Fossomatic 4000 analyzer has the capability to measure milk citrate it was obvious that raw milk samples analyzed on a regular basis at the LPT Ltd might be evaluated for citrate too. In theory, during energy deficiency/subclinical ketosis, the lack of oxaloacetic-acetate may explain the simultaneous decrease of citrate and increase of ketone body production. We presume that the concentration of citric acid in milk decreases after calving faster in energy deficient animals than in those with normal energy status due to the competition between the ketogenic biochemical pathway and TCA-cycle. Immediately after calving relatively high citric acid concentration is in the milk 10 mmol/l (Souci et al, 1994) and lower levels might be found later on (Illek et al., 1997). Unfortunately only few data are available on using milk citrate for characterizing the energy status of dairy cows. Duffield (2000) did not find milk citrate as a useful parameter for monitoring subclinical ketosis.

We have evaluated the connection between milk citrate, acetone and BHB concentrations in 41 high producing cows with elevated milk acetone concentration (>0.4 mmol/L) 10-90 days after calving (Baticz et al., 2004). These cows were in an energy-deficient, hyperketonaemic status (Gustaffson, 1993). Seventy-eight cows with lower (<0.4 mmol/L) milk acetone concentration served as controls. In the milk of cows with higher acetone content a significant negative correlation existed between milk citrate and BHB ($r=-0.579$). Similar connection was not found in the control cows.

Critical evaluation of milk citrate as a parameter of energy status is necessary. We hope that the rapid, automated determination of acetone and citric acid in raw milk samples might be introduced in milk testing laboratories.

Prevention of fatty liver

Effect of rumen-protected choline

Choline and methionone metabolism are closely associated and as much as 28% of absorbed methionine is used for choline synthesis (Emmanuel and Kennelly, 1984). Methionine plays a direct role in very low-density lipoprotein (VLDL) synthesis in cattle (Durand et al., 1992). Thus, supplying choline directly may enhance synthesis of phosphatidylcholine and increase VLDL synthesis or serve to increase methionine availability for lipoprotein synthesis to indirectly stimulate liver TG clearance as VLDL (Hartwell et al., 2000).

The effect of rumen protected choline on the liver lipid content of periparturient lactating cows was studied by Elek et al. (2004, 2007). Thirty high-producing multiparous Holstein cows were randomly assigned to two equal groups. Fifteen cows were fed 0 (control) or 100 g/d rumen protected choline (RPC) from 21 d prepartum until calving and 0 or 200 g/d RPC from calving to 60 d postpartum. All cows were offered a total mixed ration (TMR) of identical ingredient composition. Liver biopsies were taken at -21, +7, +35 and +60 d relative to calving.

Total lipid and TG content of the liver were significantly lower in RPC group at d 7 and d 35 after parturition. Glycogen concentration was higher in the RPC group on d 35.

Feeding RPC around calving had a positive effect on liver metabolism as evidenced by lower lipid and higher glycogen content. Supplementation of cow's ration with RPC is a potential tool in preventing fatty infiltration of the liver.

Effect of protected lipids

It has become practice to balance energy deficiency by adding protected fats to the diet of high yielding dairy cows. Only a few data are available about these fats on the overall lipid metabolism.

The effect of two kinds of protected fats were studied by Karcagi et al. (2007). In groups of 10 Holstein Friesian dairy cows the diet was supplemented with calcium soaps of palm oil fatty acids (CAS), hydrogenated triglyceride (HTG) or no protected fats. All diets were fed from some 3 weeks prior to the expected calving to 3 months postpartum. Total lipid and glycogen concentration and fatty acid composition of liver lipids in liver biopsy samples were determined. The glycogen concentration slightly decreased in the liver of each treatment from d 14 prepartum to d 5 postpartum, however, this decrease was more intensive in both control and CAS groups than in the HTG group. At d 5 postpartum both control and CAS cows had higher liver lipid and lower glycogen concentrations than cows in the HTG group. The variations in the liver lipid concentrations were accompanied by significant changes in the proportion of several fatty acids in the liver lipids analyzed by gas chromatography. Results showed that HTG-supplementation provided more advantageous effects on liver lipid and glycogen metabolism than CAS.

3. *Statistical data from European countries*

Differences in climatic conditions, landscape and husbandry systems cause variable disease problems in Europe. Annual milk yield per cow increased during the last decades in all countries. While the number of dairy farms and dairy cows are expected to reduce, an increase in dairy cows per farm might be predicted (Wentink and de Kruif, 2004). As national statistics on metabolic disorders are not always available in European countries the following reports often represent only some dairy herds in a country.

In The Netherlands the number of dairy cows in 1995 was 1.708.000 while in 2006 it was 1.420.000. During the last decade milk production increased from 7500 to 9100 kg (CBS Holland). Culling rate varied between 25-35% with the latter ratio at high productive farms Fatty liver and milk fever were reported at some 54% and 28% of dairy farms, respectively. (Counotte, personal communication). In 8272 cows at 9 dairy farms during a 10-year period fertility, udder, locomotory, and metabolic problems were represented by 35, 25, 37 and 20%, respectively. The metabolic problems included 5.5% acetonaemia and 14.4% milk fever. (Wentink and de Kruif, 2004).

In Germany Fülll (2005) evaluated morbidity rate at 4 dairy farms with 6800-8000 kg lactational milk production. Milk fever varied between 2-7%. Everts (2006) in a dairy farm with 12000 kg yearly milk production (55 cows and 33 heifers) found 9% milk fever among cows and 0% among heifers. Abomasal displacement was also more frequent in cows (7%) than in heifers (3%) while puerperal disorders dominated in heifers (27% vs. 15% in cows). At another farm with 9600 kg milk production/lactation in 969 cows and heifers Hadrich (2007) found 2,8% milk fever, 45-75% subclinical ketosis (BHB > 0.62 mmol/l) and 3-25% cows with liver damage. Lober (2007) compared data of 182 cows with 5900 kg milk production in 1996 to 177 cows with 8600 kg milk production in 2006. Culling rate increased from 29 to 38%. While milk fever remained at 5% and subclinical ketosis at 20-35%, liver damages increased substantially (36% vs. 45%) based on elevated serum AST, GLDH and bilirubin. Acidosis similarly increased from 19% to 28%. Beta-carotene deficiency dropped from 51% to 28%.

In Slovakia in 198,600 dairy cows (not only Holstein Friesian) with 5927 kg milk yield/lactation the culling rate was 26,6% and deaths were 5%. In 2006 at the biggest Slovakian slaughterhouse in 2988 cows with liver tissue damage the dominating abnormality (67%) was fatty degeneration. Leading metabolic disorders in Slovakia are energy imbalances resulting in lipid mobilisation, fatty liver, abomasal displacement, milk fever, mastitis, lameness and reproduction disorders (Kovac, personal communication).

In Serbia data of 1996 and 2006 are available from 7 dairy farms (Samanc et al. 2006; Samanc, personal communication). In 1996 milk production was 5600 kg/lactation in 10300 cows while ten years later it was 7200 kg in 8500 cows. Culling rate increased from 20 to 32%. Rate of subclinical ketosis, milk fever and acidosis relative to other

diseases increased from 6, 2 and 13% to 26, 3, and 30%, respectively. In 2006 at these 7 farms more than 40% of clinical diseases were metabolic including 17% ketosis, 8% milk fever, 10% fatty liver.

In Czech Republic there were 651,000 dairy cows (50% Holstein Friesian) in 1996 with 4620 kg milk production/lactation. In 2006 the cows' number was 424,000 with the same percentage of Holstein Friesian and milk production increased to 6254 kg. Based on data of 20 large dairy herds the rate of metabolic diseases relative to other diseases increased from 66% to 74%. Prevalence of fatty liver, ketosis, milk fever and acidosis relative to metabolic diseases increased from 30, 16, 6, and 22% to 34, 18, 8, and 26%, respectively (Illek, personal communication).

Fatty liver in fallow-deer

A surprising and unique finding was reported by Zomborszky and Husvéth (2000) in fallow-deer males. During the rutting season these animals do not eat for weeks, their body condition dramatically decreases and for compensation they mobilize body reserves as dairy cows do after calving. In hunting season fresh liver tissues were removed and analyzed for lipid content from 21 fallow-deer immediately after shooting them. Results showed that the liver of these wild deer contained 410 ± 112 g total lipid/kg wet liver weight. This huge lipid accumulation is rarely found even in dairy cows that died due to acute fatty liver and hepatic coma.

Conclusions

Metabolic/production diseases both in clinical and subclinical forms dominate among diseases in high producing dairy herds all over the world. Clinical investigations, herd-based and laboratory testing are equally important in diagnosing these conditions. Further fundamental and applied research needs to be done to decrease the losses caused. The author hopes that results provided here can contribute to this progress.

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2.4 CURRENT UNDERSTANDING OF THE EPIDEMIOLOGY OF INTESTINAL COCCIDIOSIS IN MAMMALIAN LIVESTOCK

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In young mammalian livestock, intestinal coccidiosis plays a considerable role as it does in poultry farming. For various reasons, awareness of the significance of coccidial disease and knowledge of and methods for assessing coccidiosis are limited (Table 1). This article will look at experience with the occurrence of coccidiosis in piglets, calves and lambs, its course and its identification, and will aim to describe a logical path through this complex subject matter. This makes it easier to assess the problem in the species mentioned here, and can also be applied to other species by analogy, at least with respect to the fundamental considerations.

Table 1: Reasons why coccidial infections are so difficult to assess

Incomplete awareness of problem due to inadequate sampling (sampling not representative, too few samples, wrong sampling time etc.)
Inadequate diagnosis without pathogen differentiation (pathogenic – non-pathogenic species)
Correlation between clinical picture and parasitological findings often not good
Secondary infections can mask the clinical picture (multifactorial disease, doubts about primary pathogenicity of coccidia)
Often no correlation between hygiene status and the occurrence of coccidiosis
Other causes of diarrhoea (enteropathogenic or nutritional causes) often difficult to differentiate in the history

Taxonomy and development

Coccidiosis in livestock is caused by pathogenic species of the genus *Eimeria* in cattle and sheep and infection by *Isospora suis* in swine (Tenter et al., 2002).. Typical features of both genera are their pronounced host specificity and a reproductive cycle that takes place in a single host. The parasites reproduce asexually (schizogony) and sexually (gamogony) in the intestinal mucosa of the host species, where

they form oocysts that are subsequently passed with the faeces into the environment. They sporulate in the open and thus become infectious. Coccidia have several general features which are important in determining the nature and extent of the coccidial disease and, indeed, whether or not it develops. Coccidia have a considerable reproductive potential (a single oocyst of *E. bovis*, for example, can produce up to 24 million oocysts). Oocysts are exceptionally resilient to various external factors, i.e. they persist for months, if humidity and temperature are favourable, as a potential source of infection in the stable or on pasture and can even survive periods of frost. The different species differ considerably with respect to certain biological features, and this determines the coccidial infection and its development. The main aspects here are the length of the prepatent period, the primary site of reproduction and, in conjunction with the tissue lesions, the nature and time of occurrence of clinical symptoms. These features are summarized in Table 2.

Table 2: Characteristics of pathogenic intestinal coccidia in mammalian livestock

Host	Cattle		Sheep	Swine
Coccidia	<i>E. alabamensis</i>	<i>E. bovis</i> <i>E. zuernii</i>	<i>E. crandallis</i> <i>E. ovinoidalis</i>	<i>I. suis</i>
General characteristics				
Enormous reproductive potential				
Very high resistance of oocysts to environmental factors and disinfectants				
Sporulation favoured by temperature, aerobic conditions and high humidity				
Special characteristics				
Length of prepatent period (days)	short (6-11)	long (16 – 23)	long (10-20)	short (4–5)
Main location of development	Small intestine	Large intestine	Large intestine	Small intestine
Onset of diarrhoea (days p.i.)	Schizogony (3-4)	Gamogony (16-23)	Gamogony (10-14)	Schizogony (3)
Length of sporulation (days)	(5-8)	(2-3)	(1-3)	(1-2*)

* considerably shorter in some cases

Pathomorphology of coccidiosis

The proliferation of the various developmental stages of coccidia causes typical lesions in the host tissue (intestinal mucosa) with the attendant clinical symptoms. Clinical symptoms may appear during schizogony as a result of tissue damage (piglet, *I. suis*; calf, *E. alabamensis*) or not until the end of the prepatent period. Table 3 summarizes some of the typical changes. Since intestinal coccidiosis is a self-limiting infection,

spontaneous healing with restoration of morphological and functional integrity occurs at the end of the patent period in all forms of the disease (Dauguschies et al., 2002, Daugschies and Najdrowski, 2005). The course of coccidiosis, with the changes caused during the various phases, is shown in Figures 1 to 5 using the example of *I. suis*. Species such as *E. bovis* and *E. zuernii* cause particularly severe damage as they reproduce deep in the wall of the intestine or in the intestinal crypts (Figures 6 to 10).

Table 3: Pathomorphological changes in intestinal coccidiosis in mammalian livestock*

Host	Coccidia	Pathomorphological changes	
		Small intestine	Large intestine
Swine	<i>I. suis</i>	Necrotizing enteritis, villous atrophy and villous fusion	-
Cattle	<i>E. alabamensis</i>	Catarrhal enteritis	-
	<i>E. bovis</i>	-	Haemorrhagic typhlitis and colitis
	<i>E. zuernii</i>	-	Catarrhal-haemorrhagic typhlitis and colitis
Sheep	<i>E. crandallis</i>	-	Catarrhal enteritis and focal epithelial desquamation
	<i>E. ovinoidalis</i>	-	Haemorrhagic typhlitis and colitis

* Sources: Bach et al., 2003; Daugschies et al., 1998; Hooshmand-Rad et al., 1994; Mundt et al., 2004; Mundt et al., 2005 a; Taylor and Catchpole, 1994



Figure 1: Normal villous structure of jejunum of piglets (SEM)

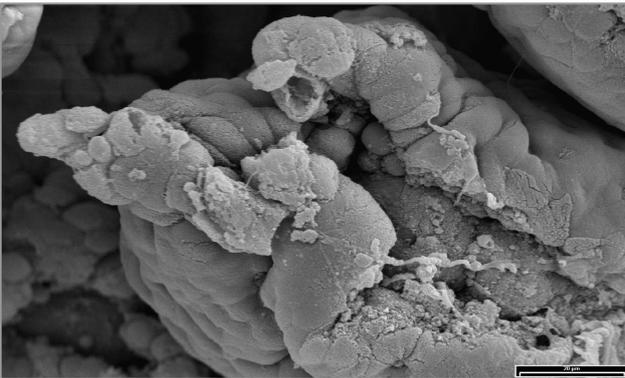


Figure 2: epithelial desquamation following infection with *Isospora suis* (SEM): detached epithelium with empty epithelial cell (parasitic stage released and fibrin)

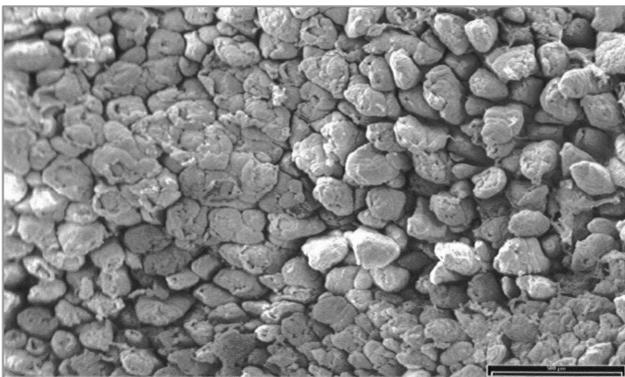


Figure 3: villous atrophy 5 days after infection with *Isospora suis*

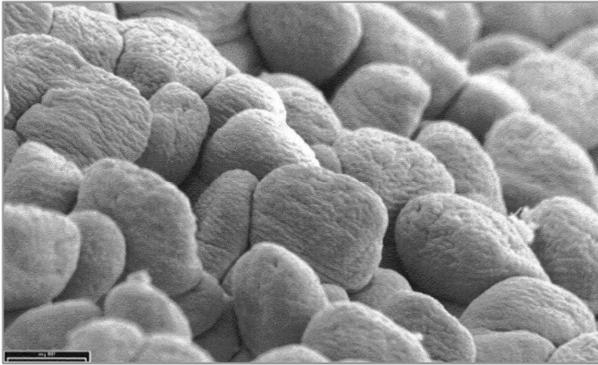


Figure 4: Re-epithelialisation of villi 7 days after infection with *Isospora suis* (SEM)

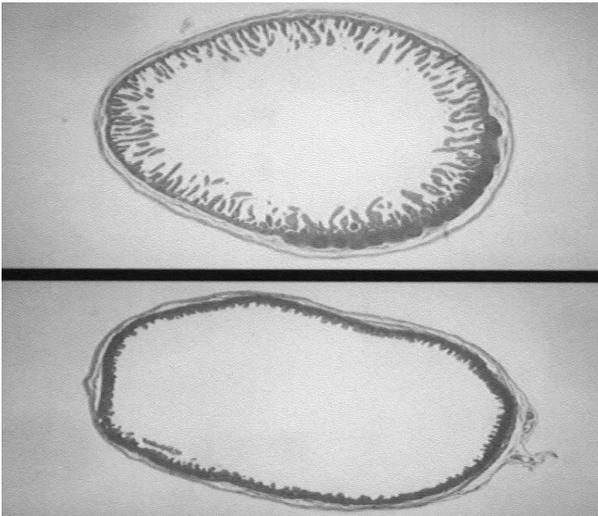


Figure 5: Small intestine: normal villous length (above) and severe villous atrophy day 5 after infection with *Isospora suis* (H&E stain, 12x)



Figure 6: Normal villous of calf jejunum with schizont of *E. bovis* in the pre-patent period of infection (H&E stain)

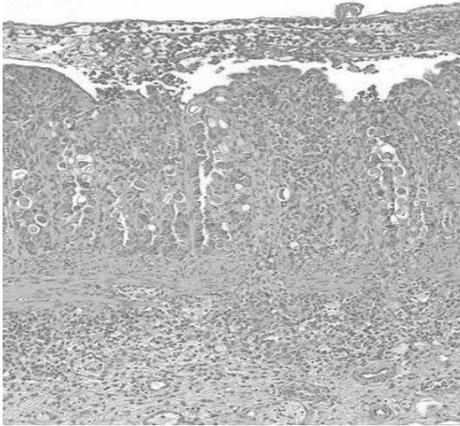


Figure 7: Severe fibrinoid enteritis of calf caecum during patency of infection with *E. bovis* (H&E stain)

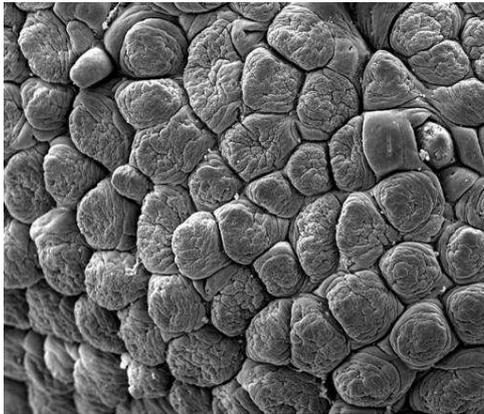


Figure 8: Normal appearance of villous structure of ileum of calf during the pre-patency of infection with *E. zuernii* (SEM)

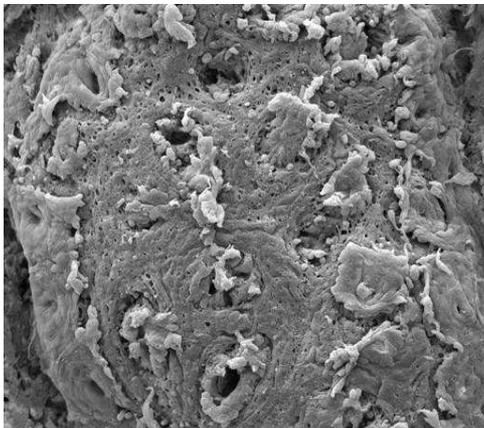


Figure 9: Superficial epithelial loss of calf caecum during patency of infection with *E. zuernii* (SEM)

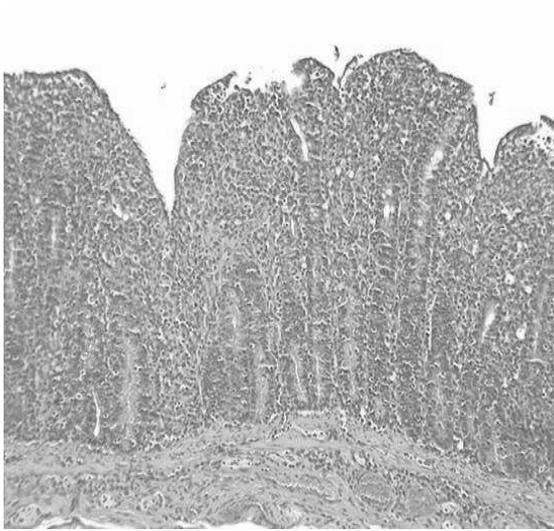


Figure 10: Superficial epithelial loss of calf caecum during patency of infection with *E. zuernii* (H&E stain)

The pathomorphological changes lead primarily to diarrhoea which, depending on the species of coccidia involved and the severity of the infection, may vary in appearance (Table 4). In any case, the intestinal lesions lead to impairment of digestive function, which manifests as reduced digestion of nutrients and reduced nutrient absorption (Dauguschies et al., 1998, Alzieu et al., 1999). The protective function of the intestinal mucosa is also severely impaired. This frequently leads to secondary bacterial infections on the damaged mucosa which can affect the pathomorphology of the disease and the clinical picture (Mundt et al., 2003; Figure 11).

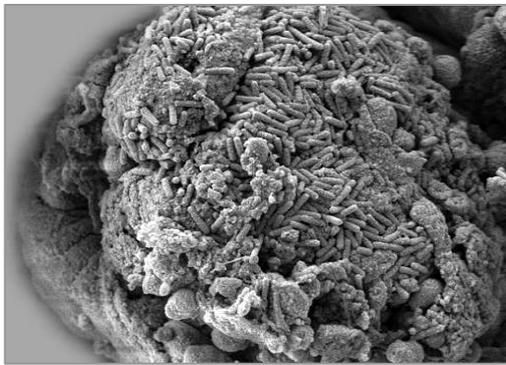


Figure 11: Secondary bacterial infections of piglets' jejunal stroma following infection with *I. suis*

Table 4: Clinical symptoms of intestinal coccidiosis

Host	Coccidia	Diarrhoea	Inappetence	Dehydration/ Mortality	Live weight
Swine	<i>I. suis</i>	pasty - watery yellowish	±	±	+++
Cattle	<i>E. alabamensis</i>	catarrhal - watery greenish-brown	+	+	+++
	<i>E. bovis</i>	catarrhal - haemorrhagic (with fibrin)	+++	+++	++
	<i>E. zuernii</i>	catarrhal - haemorrhagic	+++	++	++
Sheep	<i>E. crandallis</i>	catarrhal - watery grey-green	++	++	+++
	<i>E. ovinoidalis</i>	catarrhal - (haemorrhagic)	+++	+++	+++

Infection with coccidia

Susceptible animals always become infected by ingesting sporulated oocysts from a contaminated environment, i.e. coccidiosis is a contamination-borne infection. It is generally only young animals which are susceptible. Once they have survived an infection, they usually develop immunity. This is not sterile immunity, i.e. immunized animals are protected against the disease but can still excrete oocysts. If the pressure of infection is great enough, or if the animals' immune competence is suppressed, for example as a result of transport stress, the immunity may still be breached (Taylor and Catchpole, 1994; Cornelissen et al., 1995; Dauguschies et al., 1986; Svensson et al., 1996; Faber et al., 2002; Bohrmann, 1991; Matjila and Penzhorn, 2002). Cross-immunity does not develop. Piglets rapidly develop resistance with age; initial infections have no clinical manifestations from as early as 4 weeks of age although oocyst excretion can be considerable.

Under normal breeding and management conditions, animals become infected either if they are born into a contaminated environment or if they are transferred to one. Coccidiosis is always the sum of numerous factors determined by the parasites, the host and the environment (Figure 12). However, the species of coccidia mentioned here are always primary pathogens, i.e. it is not vital for other factors to co-exist if susceptible animals are infected with a certain dose of infective oocysts.

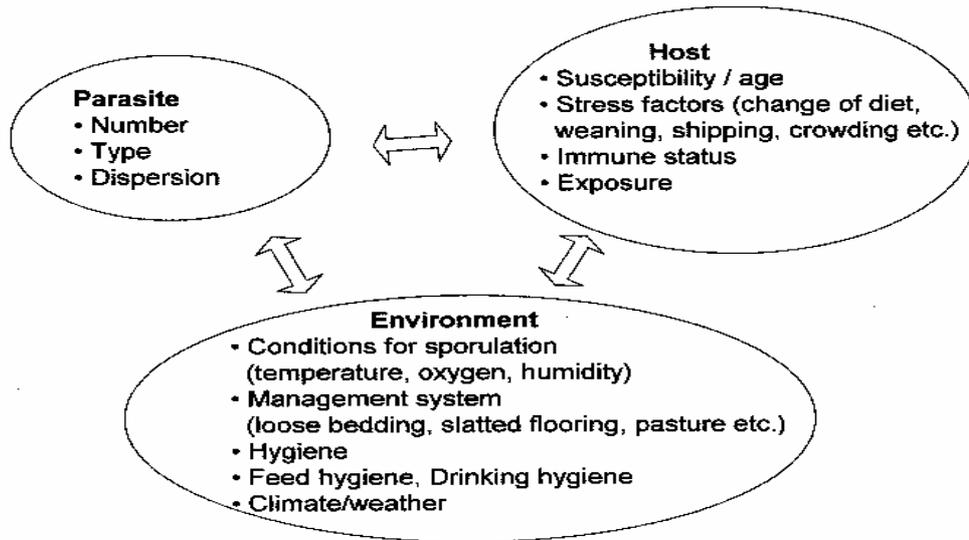


Figure 12: Contributory factors in the development of coccidiosis

Conclusions about the number of oocysts that an animal needs to ingest in order to develop the corresponding clinical picture can be derived from experimental infections (Table 5). In piglets, a single inoculation of 10^4 sporulated oocysts at the age of 3 days produces typical clinical symptoms. Considerably lower numbers of oocysts inoculated at an earlier age produce comparable symptoms, i.e. the age at infection is evidently more important in piglets with respect to clinical coccidiosis than the infectious dose.

Single experimental infections with 5×10^4 sporulated oocysts of *E. bovis* and 1.5×10^5 sporulated oocysts of *E. zuernii* produce the typical picture of severe clinical coccidiosis. There is no reproducible experience concerning the infectious dose of *E. alabamensis* (the pathogen involved in pasture coccidiosis in cattle) required to produce a clinically relevant infection. Infections with extremely large doses of up to 400 million sporulated oocysts were needed to produce clinical symptoms.

Table 5: Intensity of infection in experimental infections with pathogenic intestinal coccidia

Host	Species of coccidia	Infectious dose*	Age of animals when infected	Source
Swine	<i>I. suis</i>	10 ⁴	3 days	Mundt et al., 2006
Cattle	<i>E. alabamensis</i>	10 ⁷ – 4 x 10 ⁸	2 months	Hooshmand-Rad et al., 1994
	<i>E. bovis</i>	5 x 10 ⁴	2 weeks	Mundt et al., 2004
	<i>E. zuernii</i>	1.5 x 10 ⁵	2 weeks	Mundt et al., 2005a
Sheep	<i>E. crandallii</i>	10 ⁴	4 weeks	Catchpole and Gregory, 1985
	<i>E. ovinoidalis</i>	10 ³	5-13 weeks	Gregory and Catchpole, 1987

* number of sporulated oocysts; oral inoculation

It is known that the strain virulence of some of the coccidia species that occur in poultry (*E. brunetti*, *E. tenella*, *E. maxima*, *E. necatrix*) can vary considerably and it is probable that this also applies to mammalian coccidia, however, respective studies have not been published so far.

Course of the infection

The course an infection takes within a group of animals depends on various factors (see also Figure 12). Specifically in terms of the parasite, the determining factor is whether a certain infectious dose can be ingested at a certain time. The variability of the course of coccidiosis, measured primarily by oocyst excretion, is striking even under experimental conditions with identical infection parameters. This variability has been demonstrated clearly in infections with *I. suis* and *E. zuernii* which have been studied more closely. During an observation period of 7 days (days 5 to 11 p.i.), piglets excreted on a varying number of days; one animal only excreted once (Figure 13). An inconsistent course was also observed in a group of 11 animals following experimental infection with *E. zuernii* (Figure 14); consequently diarrhoea was evident on different days in different animals and in varying severity (Figure 15).

The conditions under which animals are infected in the field differ from those that prevail in experimental infections. The global prevalence of the coccidia in mammalian livestock is very high and it can be assumed that each individual animal will acquire infection sooner or later (Barutzki et al., 1990; Mundt et al., 2005b; Pfister and Flury, 1985; Gräfner et al., 1982; Gräfner et al., 1985; Fox, 1985; Cornelissen et al., 1995). If exposure is low, most infections will remain subclinical and will thus remain undetected. In regions and farms/herds with a high density of susceptible animals higher infection pressure increases the risk of infection and subsequent disease. There are, however, considerable differences between animal species.

Coccidiosis basically takes the same course in all populations in piglet-rearing facilities. It is currently assumed that small numbers of *I. suis* oocysts persist in the farrowing pens even if they are cleaned thoroughly and disinfected, and that these infect individual piglets immediately after birth, thus initiating the first reproductive cycle. This only causes a small amount of damage, and it is unusual to see sick piglets in the first week after birth. However, there is a sharp rise in oocyst contamination at the end of the first week; the pressure of infection increases considerably and clinical coccidiosis is seen in the second and third weeks after birth.

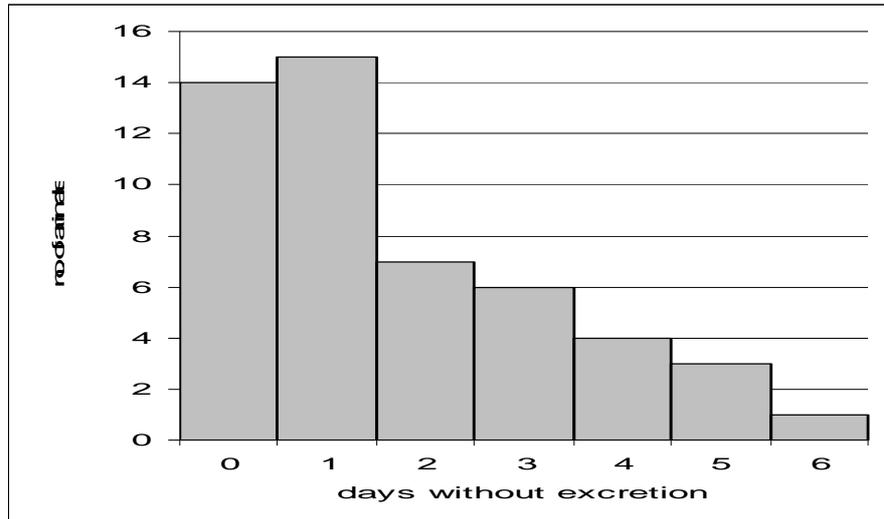


Figure 13: Distribution of „no-excretion days“ in a group of piglets (n=50) infected with 10,000 *I. suis* oocysts on the 3rd day of life, irrespective of the onset of diarrhoea

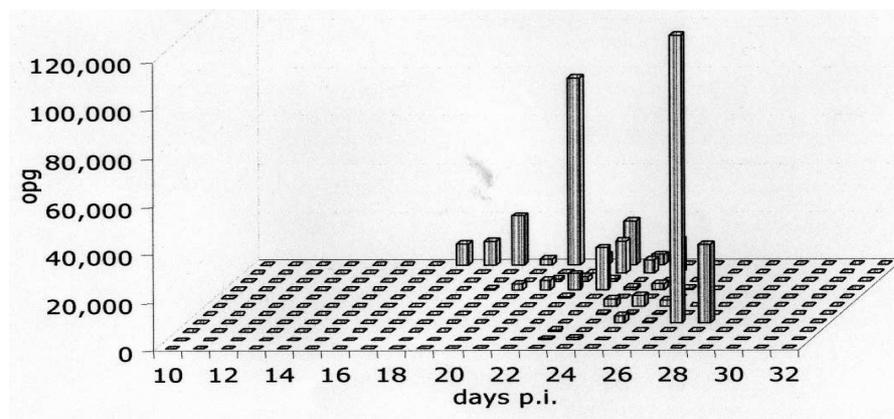


Figure 14: Excretion of oocysts following experimental infection with *E. zuernii*

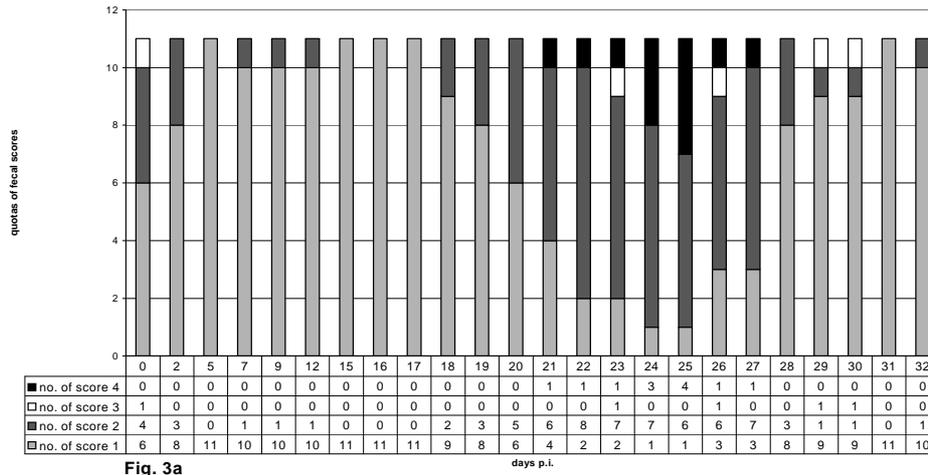


Fig. 3a

Figure 15: Distribution of faecal score following experimental infection with *E. zuernii*

The conditions prevailing for ruminants are different. The frequently high density of animals and the limited possibilities for ensuring hygiene in sheep-rearing make it extremely likely that fully susceptible lambs will be born into a highly contaminated environment and may fall ill at an early stage. They may also become ill later on when older animals are transferred to a highly contaminated environment (e.g. when they are turned out to pasture). Young calves are fairly unlikely to be exposed in the first few weeks after birth because they are kept individually in stalls or hutches (iglus), or in spacious group pens with ample bedding. Calves can be infected later on in life if they are regrouped into a contaminated environment. The course of bovine coccidiosis can be extremely variable depending on management conditions and the type of production. The initial pressure of infection plays a role in determining how rapidly a coccidiosis problem develops.

On problem farms, coccidiosis manifests clinically or can lead to financial losses even without causing clinical symptoms, e.g. failure to gain weight (Fitzgerald, 1980; Gräfner et al., 1985, Bürger, 1983, Fox, 1985, Cornelissen et al., 1995; Matjila and Penzhorn, 2002; Balicka-Ramisz A., 1999; Gjerde B., O. Helle, 1991; Alzieu et al., 1999; Maes et al., 2007, Bach et al., 2003). It can be assumed that the animals in a group will be infected at the same time, or at around the same time, under field conditions. In contrast to experimental infections, however, it can be assumed that the infectious doses, i.e. the number of oocysts ingested, under field conditions vary more widely. Moreover, the animals in a group are exposed for a longer time.

It has been shown in trials with piglets under field conditions that infections with *I. suis* behave in a comparable way to infections under experimental conditions. At the start of the episode of coccidiosis in the second week after birth (days 7 to 10), 3 out of

10 piglets were excreting oocysts; later on (days 10 to 12) the figure was 8 out of 10 piglets in a litter. The number of oocysts excreted increased in parallel (Martineau and Castillo, 2000).

Trials with cattle under various management conditions have shown that the animals in a group are evidently infected with stable coccidiosis within a very short space of time. The animals were typically excreting oocysts three to four weeks after being transferred to the contaminated stable or part of a stable. The infection may, however, manifest at an earlier stage, for example if animals which are already infected are transferred to the stable. If the initial pressure of infection is very low, the animals may not start excreting until later (Figures 16 to 18). This makes it difficult to predict the probability and course of stable coccidiosis (see Diagnosis).

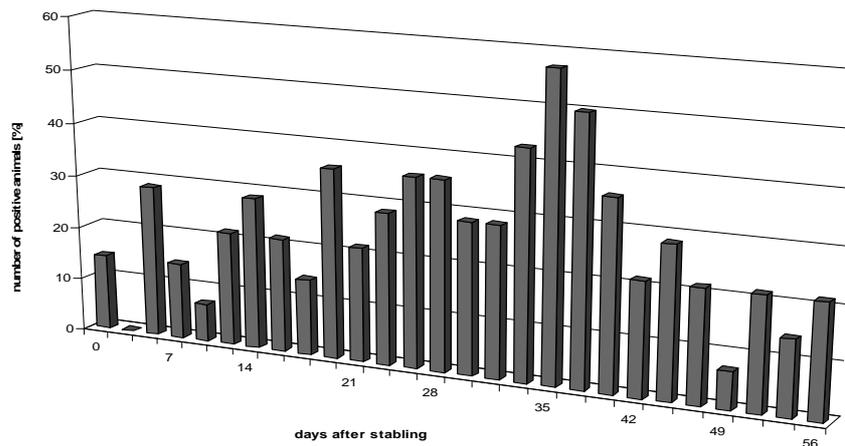


Figure 16: Extensity (%) of *E. bovis* and *E. zuernii* oocyst excretion (example 1)

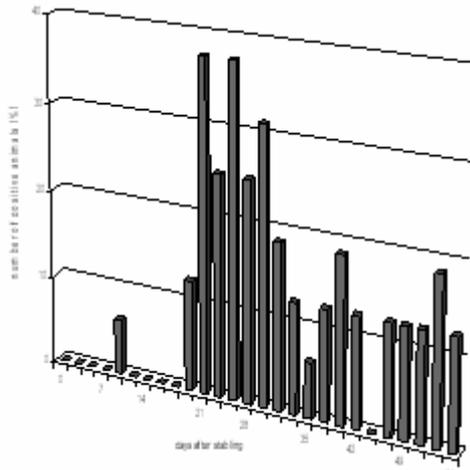


Figure 17: Extensity (%) of *E. bovis* and *E. zuernii* oocyst excretion (example 2)

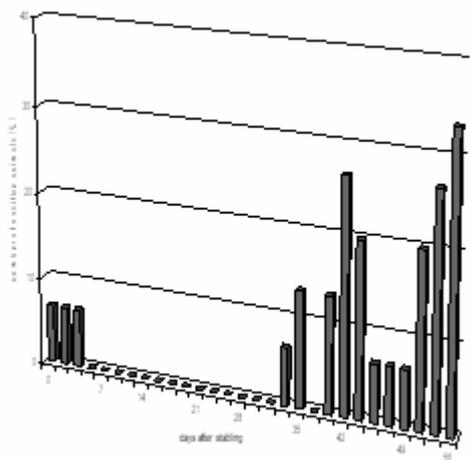


Figure 18: Extensity (%) of *E. bovis* and *E. zuernii* oocyst excretion (example 3)

The prevalence of oocyst excretion may remain more or less consistently high at 20 to 50 % of animals over an extended period of time. Both the pathogens implicated in stable coccidiosis behave in a basically comparable manner. The prevalence of oocyst excretion, and to a limited degree the intensity as well, correlate positively with the clinical picture. A prevalence of around 60 to 70% in a herd and a high number of *E. bovis* or *E. zuernii* in the faeces are indicative of a high pressure of infection. If this pressure is low, the prevalence tends to be around 20 to 30%, with fewer than 1000 or 500 pathogenic oocysts being excreted by individual animals.

Calves become infected with the pathogen that causes pasture coccidiosis (*E. alabamensis*) the first time they are turned out. The animals in a group are infected at about the same time, and the infection is extensive (practically 100%). Since the prepatent period is very short, the patent phase of the infection and the subsequent normalization of the clinical picture (spontaneous healing) are fairly indistinguishable.

Infections in lambs caused by the pathogenic species *E. crandallis* and *E. ovinoidalis* take a similar course. Animals become infected in a heavily contaminated environment (stable or pasture) immediately after birth or shortly after they have been introduced to the contaminated environment. The infection is very extensive, as is the degree of oocyst excretion. Trials with lambs carried out on several farms found that practically all animals were excreting at all the times at which they were examined (3 to 4 times per week) over a period of five weeks.

Diagnosis

Diagnosis of coccidial infections or clinical coccidiosis must address a number of questions. The usual approach is to examine oocyst excretion in the faeces (semiquantitatively or quantitatively) and to differentiate the coccidial species (pathogenic and non-pathogenic species).

The identification of coccidial oocysts in the faeces or in the bedding shows the general prevalence on a farm or in a section of a stable; if the pathogens are differentiated, this approach shows the prevalence of a certain species. More extensive diagnosis (sampling) is generally necessary in order to correlate oocyst excretion with existing clinical symptoms, or to characterize an infection on the basis of oocyst excretion in a group of animals over time. This is the only way to provide a basis for recommendations on optimal control, which in turn includes a recommendation on the optimum time for treatment.

In general, this requires repeated samples to be taken, e.g. at weekly intervals. The lower the extent of the infection and the lower its intensity, the more samples will need to be taken in order to gain a conclusive picture. This applies particularly to stable coccidiosis in cattle since the course of the infection can be very variable.

Correlation between parasitological and clinical picture

Oocyst excretion basically correlates well with the clinical picture. This can be demonstrated clearly under defined experimental conditions in an infection model. Under natural infection conditions the correlation between the clinical and the parasitological picture is better the more animals in a herd or group are affected (extent) and the more intense the infection is. However, oocyst excretion is very variable and the extent of excretion does not always reflect the severity of the clinical disease. The correlation is often much weaker, and in many instances almost impossible to discern, if the infection is weak, particularly if other factors (infectious and non-infectious) contribute to the development of diarrhoea or induce enteritis irrespective of coccidia.

Hygiene

Since coccidiosis always develops as a result of infection by a contaminated environment, it appears reasonable to strive for control by improved hygiene measures. In practice, however, this is often not feasible. Hygiene measures are not sufficient on their own, although they should always be considered in coccidiosis control. Even when attention to hygiene is rigorous, as in piglet rearing (all in-all out, cleaning and appropriate disinfection of farrowing pens etc.), clinical coccidiosis may still develop. This is mainly due to the nature of the parasites (see Table 2) and the fact that just a few oocysts are all it takes to initiate the development of an infection. Given the common housing conditions in ruminant management, the hygiene measures that can realistically be applied are very limited and areas once contaminated with oocysts (e.g. pastures, stables with deep litter) will remain so for weeks or months.

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2.5 FIELD STUDIES ON EFFECTS OF FEEDING COARSELY GROUND DIETS (CONTAINING ORGANIC ACIDS) ON SALMONELLA PREVALENCE IN WEANED PIGLETS, FATTENING PIGS AND PIGS AT SLAUGHTER

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Problem: Salmonella is the most important bacterial cause of acute, food-borne disease in humans (1). There is also a strong relationship between the Salmonella (S.) status of pig finishing units and the prevalence of S. in pigs at slaughter (2; 3).

Aim: The purpose of current studies was to measure the efficacy of a dietetic strategy to reduce the prevalence of S. on the level of piglet production, in the fattening period and in pigs at slaughter.

Material and Methods: Two farms producing piglets (f1-f2) and three fattening units (f3-f5) which had a high S. prevalence were chosen. S. status of a total of 20 control (cg) and experimental groups of piglets (eg, 100 pigs per group) as well as in 12 groups of fattening pigs (200 animals each) was tested several times by cultural technique from the suckling period until piglets entered the fattening unit (f1-f2) and from the start of the fattening period up to slaughter (f3-f5), respectively. At slaughterhouses, samples of caecal contents were taken from about 30 pigs of each group for microbiological analysis; S. antibody status was determined in meat juice taken from the same pigs. The diets of the cg's had grinding intensities generally used on the farms. The eg's were fed a coarsely ground diet made up of the same ingredients and with the same chemical composition. Also organic acids were used in the trials (4; 5).

Results: During the suckling period S. has not been culturally detected in suckling piglets (n=1700). The use of a low grinding intensity and of organic acids reduced the S. prevalence in weaning piglets on f1 about 68 % and on farm 2 about 86 % (positive/total, f1/cg: 69/1021, f1/eg: 21/973; f2/cg: 28/432, f2/eg: 4/432). On f3-f5 piglets entering the fattening unit were the main source of infection. Here a combination of coarsely ground diets in addition with organic acids and potassium diformate, respectively, resulted in a continuous reduction of S. shedding up to the end of the fattening period. An increased particle size within diets (f3-f5) resulted in significantly lower S. seroprevalence in meat juice. The use of coarsely ground diets reduced S. prevalence in caecal content of pigs from all three farms, in two of three cases significantly.

Conclusions: In general, the dietetic concept is suitable for weaner and fattening pigs to reduce the S. prevalence. Finally specific feeding concepts are an adequate tool to improve food safety. (1) Todd 1997, World Health Stat Q. 50 (1-2), 30-50; (2) Belceil et al. 2004, Prev. Vet. Med. 63, 103-120; (3) Srensen et al. 2004, Vet. Microbiol. 101, 131-141; (4) Visscher 2006, Diss., Tierrztl. Hochsch., Hannover; (5) Offenberg 2007, Diss., Tierrztl. Hochsch., Hannover.

2.6 AN EPIDEMIOLOGICAL STUDY OF THE PREVALENCE OF CHLAMYDOPHILA SPP. IN RANDOMLY SELECTED DAIRY FARMS IN THE WESTERN PART OF GERMANY

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Infections with intracellular bacteria of the genus ‘Chlamydophila’ are associated with various symptoms such as infertility in cattle. Serological studies suggested a high level of exposure to ‘Chlamydophila spp’, but systematic epidemiological investigations for the DNA-based detection of ‘Chlamydophila spp’ are only scarcely available.

The objective of our study was to characterize the prevalence of ‘Chlamydophila spp’ in dairy cows in the western part of Germany (North-Rhine-Westphalia) since the epidemiological status of dairy cattle infection with ‘Chlamydophila spp’ in North-Rhine-Westphalia (NRW) was unknown.

Material & Methods: In total, 100 dairy farms were randomly selected. For this purpose, the dairy cow stocking rate in the different administrative districts of NRW was taken into account. Ten dairy cows per farm or at least 10% of the stand density per farm were sampled. For the detection of ‘Chlamydophila spp’, vaginal swabs from non-pregnant, early lactating dairy cows were analyzed using an established highly sensitive genus-specific real-time PCR. In consideration of the discontinuous shedding of the pathogen, a farm was classified as positive if at least one animal per farm was tested positive for ‘Chlamydophila spp’. **Results:** All in all, samples from 1074 individual dairy cows were analyzed. Positive testings were observed in 13.5% of the cows. In 61 of the 100 dairy farms, positive testings were found resulting in a per farm prevalence of 61%.

Conclusions: The lower prevalence observed on a per cow basis is attributed to the discontinuous shedding of the pathogen. Nevertheless, our results suggest that ‘Chlamydophila spp’ are widely spread in NRW. To evaluate the impact of ‘Chlamydophila’ infections on herd health and fertility and to develop strategies to counteract ‘Chlamydophila’-associated health disturbances, further investigations are needed.

2.7 PERIPARTURIENT CONCENTRATIONS OF IMMUNOGLOBULINS IN DAIRY COWS

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Periparturient concentrations of immunoglobulins in dairy cows M Herr, H Bostedt Clinic for Obstetrics, Gynaecology and Andrology of Large and Small Animals with an Ambulatory Service of the Justus-Liebig-University, Giessen, Germany The incidence of periparturient diseases of dairy cows (retained fetal membranes, puerperal toxicaemia with septicaemia and simple chronic endometritis) can be as high as 37%.

The aim of this study was to evaluate the influence of birth on the peripheral concentrations of immunoglobulin G and M in dairy cows. In the current clinical trial, 45 dairy cows, 11 with eutocia and 34 with dystocia, were examined and analysed both antepartum, intrapartum and in the postpartum period up to the tenth day, dairy cows after eutocia were examined up to the 28. day p.p.. The type of assistance, mild and severe conservative birth-assistance or a caesarean section, and occurrence of postpartal complications were recorded. Plasma IgG and IgM were measured by ELISA. At partus, a decline in the concentration of IgG from an average of 33,47 mg/ml eight weeks a.p. to 12,98 mg/ml ($p < 0,001$) is evident, while the IgM concentrations first decrease from 5,71 mg/ml to 3,91 mg/ml two weeks a.p. and than reascent until the birth. Post partum the IgM increase further to concentrations about 7 mg/ml. While the IgM concentrations rise the IgG decline after parturition. They stay at this low (IgG) respectively high (IgM) level until the fourth week p.p.. At this time the concentrations are equal to the ones eight weeks a.p. Further it could be recognised that the IgG- and IgM concentrations after conservative birth- assistance and after a caesarean section are in a range of physiological concentrations, if there are no complications in the postpartal period. Furthermore it's of interest that cows with postpartal complications (retained fetal membranes, puerperal toxicaemia with septicaemia and simple chronic endometritis) have a stronger and longer lasting immunosuppression.

As a conclusion it is to be regarded that an eutocia has a suppressive effect on IgG concentrations, which is compensated by IgM. In addition a dystocia with postpartal complications has a larger suppressive effect on IgG and IgM concentrations. This leads to an immunosuppression of the maternal organism, which explains the increased susceptibility to infections in the postpartal period.

2.8 REASONS FOR CULLING OF HOLSTEIN DAIRY COWS IN NORTH EAST OF IRAN

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This study was designed to determine the culling rates of 23 Holstein dairy herds (with an average size of 180 cows per herd) in Neishaboor area in the Northeast Iran over a period of three years from 2001 to 2003. The average total annual culling rate was 13.3 % (98.5 % involuntary and 1.5 % voluntary). Of the total disposals, 53.48 % occurred by the end of five years of age. Poor fertility was the most important reason for culling (34.9 % of disposals), followed by digestive disorder (12.6 %), alimentary problems (10.85 %), mastitis (9.6 %) and lameness (8.3 %). It is concluded that more detailed epidemiological studies addressing the incidence of diseases are a prerequisite if profitable farming and preventive measures are planned.

2.9 THE GENETIC RESISTANCE TO GASTRO-INTESTINAL STRONGYLIDS IN APPENNINICA SHEEP: RELATIONSHIP AMONG PARASITIC LOAD AND HAEMATOLOGICAL PARAMETERS IN LAMBS

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Faecal Egg Count (FEC) is generally considered a simple but effective parameter for estimating resistance to strongylids, due to its phenotypic relationship to the total number of parasites present in the animal. Nevertheless, also haematological indicators have been shown to be useful in measuring gastro-intestinal nematode resistance in sheep, such as Packed Cell Volume (PCV), number of leukocytes, proportion of eosinophils and plasma albumin.

The study was conducted in a single herd of Appenninica sheep situated in Tuscany (Italy) with the aim of investigating the relationship among parasitic load of gastro-intestinal Strongylids and haematological parameters in 20-30 days-old lambs grouped accordingly to the parasitic burden of their mothers. Faecal samples from 108 subjects belonging to the Appenninica sheep breed (54 daughter-mother pairs) were collected in four different withdrawals (April, May, September and November 2004) and analysed in order to determine parasite EPG (Eggs Per Gram). At the same time as the faecal samples, blood samples were also collected in order to determine PCV, total proteins, complement proteins, plasma albumin, erythrocytes and eosinophils. Considering the low infection level in spring, it was believed that adult females with a positive EPG in this season could already be considered 'not resistant', although a limited infection (EPG<200) might serve as an index of host's capacity to keep the parasite population under control. So, we defined as 'resistant subjects' (RS) adult females with an average value in the spring sample equal to zero EPG, 'intermediate resistant subjects' (IRS) ewes with an average value under 200 EPG and 'not resistant subjects' (NRS) ewes with an average value over 200 EPG. Then we defined three corresponding groups of lambs: 'daughters of the resistant subjects' (DRS, n=14), 'daughters of the intermediate resistant subjects' (DIRS, n=29) and 'daughters of the not resistant subjects' (DNRS; n=11). Regarding parasitic load, DRS animals resulted significantly more resistant to strongylids than the other two groups in September, while all the lambs, including the DRS group, were equally infected in the November withdrawal. As to haematological parameters, DNRS animals showed an higher value of total protein (April and May) and eosinophils (May) and a lower value of complement (May and September) compared to the other two groups. In the higher infestation period (November), the DNRS group showed lower values of PCV and plasma albumin (indicators of anaemia) compared to the other groups.

Results highlight that FEC and haematological parameters do not vary consistently among groups.

2.10 A SURVEY ON PREVALENCE OF SUBCLINICAL KETOSIS IN SOME OF DAIRY CATTLE FARMS AROUND ARAK (IRAN)

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Ketosis is a metabolic disease characterized by abnormally elevated concentrations of the ketone bodies in the body tissues and fluids. In cattle, ketosis is a disease of dairy herds and is prevalent in most countries where intensive farming is practiced. Ketosis occurs in two forms: clinical or sub-clinical. Subclinical ketosis; occur more commonly than clinical ketosis and have more significant economic importance. This study was carried out to find the occurrence of subclinical ketosis in four dairy herds around Arak in Iran. Milk samples were taken from 394 dairy cows in 2-8 week after calving. The samples were examined with Rothera's test.

Results showed subclinical ketosis in 6 cases (% 1.5). Although no positive case was found during the spring, in each of other seasons, 2 cases of subclinical ketosis were found. In each groups of first, second and third calving, 2 cows were positive in Rothera's test. Daily milk production in 5 positive cows was 20-30 Kg/day and in 1 cow was 30-40 Kg/day. It seems the ketosis is not a serious problem in Arak.

2.11 SEASONAL CHANGES OF VITAMIN A AND BETA-CAROTENE LEVELS OF SERUM IN ARAB HORSES IN AHVAZ (IRAN)

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Vitamin A is one of the fat-soluble vitamins. Because of its particular role in different tissues and organs, in deficiency conditions various clinical signs are seen. In addition, sometimes the marginal deficiency is present that clinical signs are not visible but performance defects, such as infertility is seen. In present study seasonal changes of serum vitamin A and b-carotene in 22 Arab horses was investigated in Ahvaz between July 2002- June 2003. A simple and cheap method (spectrophotometry) was used for measuring vitamin A and b-carotene. The results were analyzed statistically by multifactorial repeated measure (ANOVA) and pearson's correlation.

Results showed that the values of mean of serum vitamin A (20.37 ± 1.21 mg/dl) was within normal range. This value in different seasons and two sexes were normal, too. Serum vitamin A and beta- carotene in spring was significantly higher than other seasons. The mean of serum beta- carotene was 8.02 ± 0.48 mg/dl.

2.12 RESEARCH REGARDING THE EPIDEMIOLOGICAL ROLE OF THE 'OVINE FECES - VEGETABLE REFUSAL' COMPLEX IN TRANSMITTING THE ENDOPARASITOSIS TO SHEEPS, DURING PASTURING SEASON

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The research were developed during one year, on a plain pasture situated in Moldavia, with the purpose of determining the presence, the spreading, the development and resistance of the parasitic elements (sporozoans, oocysts, eggs and helminths larvae), on a pasture where there have also been other herbivorous besides sheeps; of surprising the complexity of the relationships established in the 'sheep faecals-vegetable refusal' complex and the role of this complex in the perennialization of the parasitosis and their transmission to sheeps. In this direction of study, from the pasture, there have been gathered monthly 10 samples of faecals and 10 samples of vegetation on a radius of 0-30 cm around the feces and with a height of the grass of 0-22 cm, that have been analyzed through the Willis, Mc.Master oviscopic methods and larvosopic Baermann and Euzebiy. The climatogram of the time interval comprised into the study (T, U.R., precipitations), intensivity (OPG, LPG) and extensivity (E%) of the parasite elements, both in feces and in the vegetation of the vegetable refusal. We have studied the monthly dynamics, seasonal and on a yearly basis of the parasite elements in the 'sheep feces-vegetable refusal' complex. In the climate conditions of the time interval comprised into the study, the monthly, seasonal and yearly dynamics was oscillating. In the feces there were identified eggs of nematodes of the Trichostrongylidae fam., Nematodirus, Trichostrongylus, Ostertagia, Haemonchus genus; Strongyloides genus, cestodes, Moniezia genus, sporozoans, oocysts of the Eimeria genus, with variable medium OPG from 0-540 for Trichostrongylidae, exception, the Nematodirus genus with OPG:1750. L1-L3 larvae belonging to gastrointestinal nematods, Trichostrongylidae, Strongyloides, and to pulmonary nematods, Protostrongylus, Dictyocaulus, as well as terrestrial adults nematods, were identified, both in feces and vegetation. Quantitative and qualitative variations of invasionally elements in ovine feces-vegetable refusal complex, are adjusted especially by the oscillations of endemical and climate factors with intervention of biotics factors: acarians, coleopters, gasteropods, anellids. The knowledge of the monthly, seasonal and yearly dynamics of the parasitary elements on pasture in 'ovine feces-vegetable refusal complex', contribute to epidemiological estimation of minimal and maximal moments of parasitic pollution and infestation of receptive hosts with effectiveness establishment of control measures, prophylaxie and disproof of endoparasitosis on sheep.

Key words: pasture, 'ovine feces-vegetable refusal complex', sheep, endoparasitosis, perpetuity.

2.13 DEVELOPMENT OF A PHAGE TYPING SYSTEM FOR SALMONELLA ENTERICA SEROVAR INFANTIS FOR EPIDEMIOLOGICAL PURPOSES

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The EU demands a decrease in diseases that are transferable from animals to human (zoonosis) for consumers protection. *Salmonella infantis* is one zoonotic agent, causative of human enteritis that during the last four years has increased. The incidences in humans show that *S. Infantis* is a new emerging pathogen [1]. An EU-wide *Salmonella* study of the prevalence of *Salmonella* (*S.*) in hen flocks, initiated by the European Food Safety Authority (EFSA) was carried out in all member states. The three most frequently serovars in the EU-study were in descending order: *S. Enteritidis*, *S. Infantis* and *S. Typhimurium* [2]. There are phage typing schemes for *S. Enteritidis* and *S. Typhimurium* which are used worldwide for outbreak investigations, but not for *S. Infantis*. Therefore, we develop a phage typing scheme for *S. Infantis*, necessary for epidemiological surveillance, and control of this pathogen. The phages were isolated from lysogenic strains isolated from 1973 until 2004. 119 typing phages were used to type 100 *S. Infantis* strains and the most discriminating phages were used for the final typing scheme (22 phages) and further validation of the scheme. Additionally we investigated the presence of the phage encoded virulence gene *sopE* in *S. Infantis* strains by PCR as an additional epidemiological marker and sequenced some PCR-products. [1] SurvStart@RKI [2] <http://www.efsa.europa.eu>

2.14 A CASE-CONTROL STUDY ON FACTORS ASSOCIATED WITH MASTITIS IN DAIRY COWS IN THE SUBURB OF KARAJ CITY-IRAN

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Problem: Mastitis is one the most important diseases in dairy cows industry. In Iran with more than 6 million cattle, mastitis can generally be considered the most serious disease of dairy cows.

Aim: In order to identification of effective factors in mastitis and determine the range of somatic cell count (S.C.C.) for diagnosis of mastitis.

Material and methods: A case-control study was conducted. CMT was carried out as screening test for isolating cases and controls. In this study 157 cases (Milking cows were infected at least one quarter with sub clinical or clinical mastitis) and 300 controls (Milking cows were not infected) were allocated in two groups. Independent variables were age, record, yielding, parity (stage of lactation), history of mastitis, teat dipping, and distance from parturition and S.C.C. for two groups in this research.

Results: Age, parity of cows and distance from parturition in cases were significantly greater than controls ($p < .01$). In contrast mean of milk production in cases {27.28(SD=9.26)} was less than controls {31.2 (SD=7.91)}, ($p < .001$). Also we found a positive association between preceding mastitis and didn't use T.D. with current mastitis. Mean of S.C.C. for healthy quarter was 51700/ml, while it was 725000/ml, 2400000/ml, and 6215000/ml for quarters positive 1, 2 and 3 CMT, respectively.

Conclusions: Because of high prevalence and importance of mastitis in dairy herds, we recommend that analytical studies perform in different areas of country for detection of vastly infection and its risk factors.

3 DEVELOPMENT IN THE YOUTH AND PRODUCTION DISEASES

3.1 „METABOLIC PROGRAMMING“ AND POST-WEANING DEVELOPMENT: BACKGROUND AND CONSEQUENCES FOR FUTURE PRODUCTIVE PERFORMANCE OF RUMINANTS

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Key words: insulin resistance, maternal nutrition, postnatal nutrition, feed intake capacity, metabolic adaptation

The impact of metabolic disturbances in dairy cows

Metabolic disturbances such as ketosis and fatty liver are a major problem in the dairy industry. On the one hand, milk yield is negatively associated with hyperketonemia, but even more concern raises the fact that clinical as well as subclinical ketosis play a key role in the development of production diseases such as abomasal displacement, metritis and mastitis. In addition, there is growing evidence for a negative impact of ketosis on reproduction performance as well as culling rate. The prevalence of hyperketonemia in the first two months of lactation is unacceptably high and ranges between 8.4 and 34 % (Duffield et al. 2000).

The pathogenesis of ketosis and fatty liver is closely related to an insufficient feed intake during the first weeks of lactation concomitant to rapidly increasing milk yield. The associated negative energy balance (NEB) tends to be more pronounced in high yielding dairy cows as compared to substandard animals. In general, cows are able to cope with NEB. A variety of adaptation mechanisms are needed to deal with NEB because early lactation constitutes a tremendous challenge for the metabolic regulation. Whilst basal requirements of glucose are rather low in cows ($0.5 \text{ mmol/h/kg}^{0.75}$; (Danfaer et al., 1995), approximately 72 g glucose are needed for the synthesis of each litre milk. Thus, in cows producing 50 kg milk per day, approximately 3.6 kg glucose are needed for milk production whereas only 300 g are required for extramammary tissue function. Homeorhesis of lactation favours supply of sufficient energy. Lower insulin and increased growth hormone and glucagon concentrations compared to levels during dry period facilitate lipomobilization and hepatic gluconeogenesis.

There is no inevitable relation between milk yield and incidence of metabolic disturbances per se. Instead, it is general consensus that metabolic disturbances develop as a result of insufficient adaptation capacities of affected cows pointing out an individual disposition (Herdt 2000). A distinct increase in insulin resistance has been demonstrated in cows suffering from fatty liver compared to those being

clinically healthy which was even more profound in ketotic fatty liver cows (Hayirli et al. 2002, Kaske et al. 2004). Thereby, appropriate glucose utilization by peripheral tissues is put at risk and the reduced suppressive effect of insulin on lipolysis may result in an overflow of non-esterified fatty acids (NEFA) to the liver.

Overconditioning of heifers and cows prior to calving increases the risk of metabolic disturbances during early lactation considerably. Compared to moderately conditioned cows, postpartal feed intake of obese cows is lower, NEB more severe, fat mobilization higher, accumulation of triglycerides in the liver amplified, and hepatic function somewhat impeded. A higher risk for further non-infectious as well as infectious diseases complements the susceptible metabolic condition. In addition, obesity is known to induce a chronic inflammatory status which may predispose for further infectious diseases.

At present, main strategies aimed at reducing metabolic disturbances in dairy herds focus on optimizing transition cow management. The definite goal is maximizing feed intake during first weeks of lactation which is the most effective preventive tool to avoid production diseases in general. Therefore, several approaches have been implemented, e. g., specific feeding regimes during the far-off and close-up-period ensuring an appropriate body condition at parturition, prevention of hypocalcemia and dystocia and fresh cow surveillance programs.

In conclusion, important key words discussing etiopathogenesis of metabolic disturbances in dairy cows are feed intake, glucose homeostasis, insulin resistance and obesity. These key words are also in the focus of researchers investigating the background of prevalence of obesity and diabetes, tremendously increasing particularly in Northern America and Europe during the last decades. In this context, the concept of developmental programming attracted more and more attention. The results derived from epidemiological as well as experimental studies may be meaningful also for the future of food animal production.

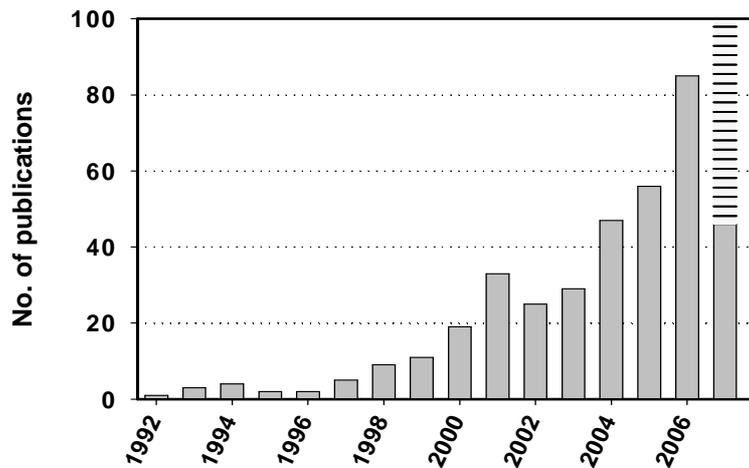


Fig. 1: Number of publications published per year as cited by Pub Med (search words: "metabolic programming", "developmental programming", "fetal programming", "fetal imprinting")

The concept of programming: a historical view

The idea of a possible epigenetic perinatal programming of regulatory systems in the organism has been suggested already 32 years ago (Dörner, 1975) and was substantiated by the concept of a "fuel-mediated teratogenesis" by Freinkel & Metzger (1978). They demonstrated that female pups of diabetic rats developed hyperinsulinemia. Thereafter, it took 15 years until a generally accepted definition of metabolic programming was accepted as a stimulus or insult at a critical period of development with lasting or lifelong significance (Lucas, 1991). Within the past fifteen years, an enormously increasing number of experimental studies were conducted applying animal models (predominantly rodents) which gave a more precise insight into qualitative and quantitative aspects of the impact of metabolic programming. The "thrifty phenotype hypothesis" (Hales and Barker, 2001) suggested that an unfavourable intrauterine supply influences the metabolic and endocrine constellation of the fetus leading to reduced birth weight which favours survival under detrimental nutritional conditions after birth. More recently, the "predictive adaptive response hypothesis" proposed that the degree of mismatch between the pre- and postnatal environments determines the forthcoming disposition for subsequent diseases (Gluckman and Hanson, 2004). It has to be emphasized that fetal programming affects not only the metabolism of an organism but is also related to a variety of further chronic diseases in humans, such as coronary heart disease, and hypertension. However, environmental factors are also able to program regulatory systems of an organism during periods of developmental plasticity in postnatal life; thus, the terms

“developmental programming” and the “Developmental origins of adult health and disease” may be more appropriate (Armitage et al., 2005).

Metabolic programming of humans and rodents

Epidemiological results

An epidemiological study carried out in England with men (50 - 70 years old) revealed evidence that the risk to develop obesity, diabetes type 2 and coronary heart disease is markedly increased if the birth weight of the subjects was below 2.95 kg (Hales & Barker 1993). An impressive retrospective study examined the proportion of the Dutch population which was exposed to famine during World War II (Ravelli et al. 1998). Exposure of pregnant women to famine for 3 - 4 months during the first trimester of pregnancy increased the risk of their offspring to suffer from coronary heart disease in later life compared to non-exposed individuals. In contrast, famine exposure during late gestation tended to impact on glucose-insulin homeostasis.

However, the risk to develop a reduced glucose tolerance is also increased for children of diabetic mothers whose birth weight is mostly above average (Plagemann et al., 1997, Holemans et al. 2003). Also in Pima Indians with the world's highest recorded prevalence of type 2 diabetes, the prevalence of type 2 diabetes in 20 - 29 year old subjects was highest for those born with a birth weight of either < 2.5 or > 4.5 kg (Dabelea et al. 1999).

Experimental results

Maternal malnutrition

Feeding pregnant rats a protein restricted diet became a frequently used model to study intrauterine environmental effects. The birth weight of offsprings which experienced maternal protein restriction is 7 - 35 % lower compared to pups of adequately fed control rats (Desai et al., 1997; Plagemann et al., 2000; Fernandez-Twinn et al., 2005). Offsprings of rats fed a protein restricted diet exhibit morphological and functional peculiarities: the size of pancreatic Langerhans islets and the number of β -cells are reduced, the insulin content of the β -cells is 50 % lower and the insulin secretory response to glucose is impaired compared to control animals (Holness et al. 2000; Bertram and Hanson, 2001; Ozanne, 2001).

After weaning at an age of 12 weeks glucokinase activity in the liver is reduced by 43 % compared to control rats, whereas activity of phosphoenolpyruvate-carboxykinase, a key enzyme in gluconeogenesis, is increased by 50 %. In adult offsprings of rats fed a protein restricted diet activity of insulin signal transducing molecules (protein kinase C, p85 α , p110 β) in fat and muscle tissue are reduced resulting in a diminished glucose uptake of these cells via GLUT-4 (Fernandez-Twinn et al., 2005; Ozanne et al. 2005).

Serum NEFA concentrations are increased, and ketone concentration reduced (“ketosis resistance”) (Ozanne et al. 1998).

All these changes reflect that undernutrition and/or stress during the intrauterine development trigger adaptive responses which support postnatal survival in an environment with scarce nutrient supply. However, if high-quality, palatable nutrients are available in abundance after weaning, this adaptation leads to an increased risk of cardiovascular and metabolic diseases in adult life. Despite a reduced birth weight of pups resulting from maternal malnutrition, these offspring grow faster after birth if a high-quality diet is fed compared with offspring of control-fed rats (“catch-up growth”). The insulin sensitivity is improved in early adulthood, but an insulin resistance develops in later life; reproductive performance and the lifespan of these animals is reduced compared with control animals (Breier et al., 2001; Gafi et al., 2001; Simmons et al., 2001; Vehaskari et al., 2001; Guzmán et al. 2006).

Maternal diabetes

Impaired glucose tolerance of women during gestation as well as gestational diabetes increase considerably the risk for type 1-diabetes development in offsprings. Bilateral intrahypothalamic insulin agar implants on day 3 or 8 of life resulted in an impaired glucose tolerance and increased diabetes susceptibility in adult rats compared with rats who were implanted an insulin-free agar (Plagemann et al., 1992). Thus, fetal and/or neonatal hyperinsulinism during a critical period of brain development triggers a permanent malorganization of hypothalamic regulation centres for metabolism and hence leads to malprogramming of the hypothalamo-pancreatic system (Plagemann et al. 1994). An epigenetic materno-fetal transmission of such acquired persistent modifications can be passed on several generations, mediated by gestational hyperglycemia and fetal or neonatal hyperinsulinism.

Postnatal nutrition

At least in rats a life-long programming of the metabolism can also be induced by postnatal nutrition regime. If pups are nourished via an intragastric cannula between day 4 and 24 of life administering milk formula containing increased lactose concentration (“pup in a cup model”), they develop within 24 h a hyperinsulinemia compared with pups fed an isoenergetic, fat-based milk formula. Despite a uniform diet starting at day 25, a reduced glucose tolerance was still found on day 270; in addition, initially lactose-based milk formula fed pups became obese and insulin resistant compared with their controls (Patel and Srinivasan, 2002). Hyperinsulinemia, hyperleptinemia, early-onset obesity and glucose intolerance were also found in adult rats which were overfed from postnatal day 3 to 21 by adjusting the litter size of the mothers to three compared to rats raised in litters of ten (Boullu-Ciocca et al., 2005).

Programming of feed intake capacity

Already 30 years ago, a study carried out with rats indicated that the amount of feed consumed during the suckling period determines appetite and feed intake capacity in later life (Oscari and McGarr, 1978). Overfeeding of rat pups by adjusting the litter size to four resulted in persisting increased intake capacity after these animals grew up. Further in-depth studies using immunocytochemistry revealed evidence that the percentage of NPY-immunopositive neurones per total number of neurones in the arcuate nucleus of the mediobasal hypothalamus was higher in postnatally overfed rats compared to controls (Heidel et al., 1999; Plagemann et al., 1999). These results may be one explanation for a life-lasting hyperphagia in those rats as NPY is known as a potent orexigenic neurohormone. Postnatal undernutrition, however has been found to result in hypophagia.

Gender differences

Several studies suggest sexually dimorphic responses to metabolic programming. The reported effects of gender are inconsistent. In guinea pigs, fetal growth restriction appears to impair insulin sensitivity in females but not in males (Thavanesaran et al. 2002). In children, girls are known to be intrinsically more insulin-resistant than boys. Whether this phenomenon is due to differences in metabolic programming is discussed controversially (Murphy et al. 2004; Reynolds et al. 2005). In female rats an insulin resistance as a result of intrauterine protein restriction develops later in life compared with male rats (Sudgen and Holness, 2002; Fernandez-Twinn et al., 2005).

Re-programming

Recent studies indicate that intrauterine metabolic programming is potentially reversible. Using the model of maternal undernutrition, Vickers and colleagues (2005) demonstrated in rats that a leptin treatment from postpartal day 3 - 13 resulted in reversing prenatal adaptations.

Mechanisms involved in metabolic programming

The biologic mechanisms of metabolic programming are still poorly understood. According to the present conception, metabolic programming is caused by epigenetic modifications of non-imprinted genes induced by the developmental environment (Wu et al., 2006; Godfrey et al., 2007). As a result, gene expression is life-long modified without altering the DNA sequence. Such a mitotically heritable alteration of gene expression induces a non-genomic tuning of phenotype through developmental plasticity (“Genetic proposes, epigenetic disposes”; Crews and McLachlan, 2006). These effects can even be passed on to more than one succeeding generation.

“Metabolic programming” of ruminants

There is clear evidence that the principle of fetal programming represents a common feature among mammals including ruminants (Taylor and Poston, 2007). It has to be emphasized, however, that most researchers studying metabolic programming focus on reducing incidence of chronic diseases affecting humans in late adult life. These diseases do not represent a key issue for food producing animals. Animal scientists strive primarily for an increase of weight gain in growing animals and an improvement of fertility performance and metabolic resilience in adult food animals. The options to influence these parameters by metabolic programming have not been elucidated extensively until now.

Role of maternal nutrition

Also in ruminants, prenatal nutrition has carry-over effects on growth and body composition. Obviously, the time period during which the fetus experiences maternal malnutrition, seems to be decisive for the type and extent of effects.

Dairy cows are often inseminated the first time 60 days post partum to achieve a calving interval of approximately 1 year. However, at this point of time many cows still have to cope with a more or less pronounced NEB. This metabolic constellation may affect the embryo as demonstrated in sheep. Restricted feeding of ewes (70 % of control feed allowance) from 60 days before until 7 days post mating followed by adequate feeding of the ewes during the remaining gestation resulted in increased fetal ACTH-concentrations between day 110 and 145 of gestation in twin, but not in singleton fetuses compared to lambs from ewes which were fed 100 % during the periconceptional period (Edwards and McMillen, 2002).

Two studies on sheep revealed that an energy restriction during early gestation causes only minor effects on the metabolic constellation of the offspring. A maternal dietary restriction (50 % of recommended allowance) from day 30 - 70 did not affect birth weight compared to lambs delivered from control-fed ewes. Final slaughter weight at an age of 24 weeks was slightly, but significantly lower in lambs of restricted fed ewes

compared to lambs of control-fed sheep. Only small differences were obvious in respect to muscle fiber composition between lambs from either restricted fed ewes or controls (Daniel et al., 2007). These results are in agreement with those of another study, where no significant effects of a comparable feed restriction in ewes between day 0 and 30 of gestation on the offspring were found (Gardner et al., 2005). In a further study, however, maternal undernutrition (50 % of requirements between day 28 and 78 of gestation) induced an increased body weight of the lambs during later life and dysregulated glucose uptake in the absence of any change in birth weight (Ford et al., 2007).

Maternal energy restriction in late gestation exhibits an even stronger influence on offsprings. On day 120 of gestation, fetuses of ewes fed a severely restricted diet from day 100 - 120 of gestation had higher GH- and lower IGF-1, glucose and insulin concentrations than fetuses of control-fed ewes (Bauer et al., 1995) revealing that the fetal somatotrophic axis is nutritionally regulated in late gestation. In addition, the level of maternal nutrition during late pregnancy affects the insulin resistance of the lambs. A maternal feed restriction (50 % of requirements) between day 110 until term did not influence birth weight and growth rates until 1 year of age; intravenous glucose tolerance test at day 63 and 250 revealed, however, a profound dysregulation of insulin secretion in lambs experiencing undernutrition during late intrauterine development (Gardner et al., 2005). These results indicate that intrauterine programming of the metabolism endures readjustment of the metabolic constellation during post-weaning adaptation of ruminants to roughage diets; these are characterized by a significant decrease of blood glucose concentration, reduced intestinal absorption capacity of glucose via SGLT-1 (Shirazi-Beechey et al., 1994), increased hepatic gluconeogenesis and a markedly increased insulin resistance compared to monogastrics (Kaske et al., 2001) and neonatal ruminants being functional monogastrics.

In beef cows, a protein supplementation during late gestation did not affect birth weight of calves compared to those from non-supplemented dams. However, weight at prebreeding was greater for heifers from protein-supplemented dams. Thus, fetal programming affected heifers postweaning body weight and fertility, as pregnancy rates were greater for heifers from cows supplemented with protein during late gestation compared with heifers from non-supplemented dams (Martin et al., 2007).

Finally, birth weight was found to affect postnatal weight gains in lambs fed ad libitum; lambs with a high birth weight gained more weight than substandard lambs ($p = 0.08$; Greenwood et al., 1998). Mid-pregnancy shearing of ewes has been found to consistently increase birth weight and post-weaning survival of twin lambs (Revell et al. 2000). This effect has been explained by several factors including cold exposure, increased feed intake of the ewe and increased nutrient availability for the fetus (Breier 2006). Interestingly, despite a lower birth weight the intermediary metabolism of twin lambs is not generally compromised indicating that developmental programming is not related to birth weight per se but is apparent only when the perinatal environment is specifically inappropriate (Gardner et al., 2005; Bloomfield et al. 2007).

Postnatal feeding

By definition, metabolic programming takes place in a narrow timeframe utilizing the plasticity of regulatory systems in that critical period. Ruminants as nidifugous species exhibit a different pattern of organ development compared with nidicolous species such as rodents and humans. Therefore, the appropriate timeframes for metabolic programming may differ. For example, in rodents hypothalamic nuclei continue to differentiate until postnatal day 20 (Grove et al., 2005); within this period the key regulatory hypothalamic neuropeptides and receptors can still be permanently programmed by dietary-related factors (Taylor and Poston, 2007). Whether the respective systems of ruminants - being more developed at birth than rodents - can still be programmed in the respective postnatal period, is unclear but there are at least several hints that the early postnatal feeding level has long-lasting consequences.

In modern rearing systems restricted feeding of MR and early weaning of calves is practiced to lower rearing costs. There is some evidence that such restricted feeding has a negative impact on the future performance of the forthcoming dairy cow as enhanced early nutrition may improve first lactation milk yield. Allowing Holstein calves to suckle nurse cows three times daily during the first 42 d of life resulted in 30 days earlier calving and 453 kg more milk production in the first lactation compared to calves fed milk replacer in restricted amounts (Bar-Peled et al., 1997). Also intensified MR feeding was found to have a positive effect on milk yield in the first lactation without affecting the weight at calving (Ballard et al., 2005; Drackley et al., 2007).

Consequences of postnatal feeding on the insulin status were investigated in further experiments. Female Holstein Friesian calves were fed from day 2 - 50 either standard milk replacer (MR) or isonitrogenic lactose-reduced MR, both on a low and a high feeding level (lactose supply 8.3, 16.6, 4.8, 2.4 g/kg BW/d). Thereafter, standard MR was fed on a low feeding level to all calves until day 75 followed by a silage-based diet for growing heifers ad libitum (Kaske, unpublished). Growth rates of calves fed low-lactose MR during the first seven weeks of life were still at an age of 1 year significantly lower compared to calves fed standard MR. Peripheral insulin response (as quantified by hyperinsulinemic euglycemic clamps) at an age of 8 months was not affected by the early postnatal feeding regime whereas a statistical trend towards lower pancreatic insulin responses (quantified by hyperglycaemic clamps) in later life was evident for calves fed low-lactose MR compared to calves fed standard MR.

The consequences of the postnatal feeding regime for the metabolic and health status during first lactation are a matter of present investigations. Metabolic problems of dairy cows are caused by their inability to handle the massive changes of the metabolic constellation between dry period and fresh lactation. Metabolic programming has been proven to influence the general basic setting of the metabolism – whether it affects also the short-term ability of the organism to respond to metabolic challenges remains open.

Conclusions

Growth, milk yield and fertility as decisive parameters for productivity of ruminants are influenced by genetic, epigenetic and environmental factors in all mammals. Knowledge about the role of the intrauterine and early postnatal environment on metabolism and health status of humans and rodents in their later life increased considerably within the past decade. Despite striking differences between the metabolic constellation of ruminants and monogastrics, metabolic programming seems to have marked impact also on ruminant species. Further research has to elucidate the potential of specific tools to manipulate metabolic programming in order to improve productivity and fertility in sheep and cattle.

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3.2 INFLUENCE OF THE AGE OF ENTRY IN THE FATTENING UNIT ON THE GROWTH PERFORMANCE AND HEALTH STATUS OF VEAL CALVES

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The mixing of animals from different farms and the confinement procedures of the transport from the farm to the fattening unit often cause respiratory diseases during the first weeks after arrival in the fattening unit. As it coincides with a period of change from a passive to an active immunity (between approximately weeks 4 and 6 of life) the health of the animals could be more severely affected.

The main objective of this study was to determine the effect of different ages of veal calves at the arrival in the fattening unit on their growth performance and health status (group 2-4, 5-7, and 8-10: arrival at an age between 2 and 4, 5 and 7, 8 and 10 weeks; n= 27, 29, and 30). The three groups were submitted to similar experimental conditions. Blood samples were taken on day of arrival, on week 1, 3, 5, and 7 after arrival for the determination of immunoglobulin G (IgG). During a common period for growth data (weeks 10 to 18 of life) mean average daily gain (ADG) was significantly ($P < 0.001$) higher in group 2-4. It could be explained by a shorter period with different feeding conditions between birth and the begin of the trial for the calves of the group 2-4. At least 50% of the total medical treatments for respiratory disorders for group 5-7 and 8-10 were done during the first two weeks after arrival (only 29% for group 2-4) but the sum of antibiotic treatments for pneumonia per group was the same in the three groups. Mean concentrations of IgG at arrival was significantly lower ($P < 0.01$) in group 2-4 than in group 5-7 and mean overall concentration in group 2-4 was significantly lower ($P < 0.05$) than those of group 5-7 and 8-10. No correlations ($P > 0.05$) were found in the three groups between IgG concentrations at arrival and ADG or number of antibiotic treatments for respiratory disorders.

It can be concluded that the lower concentrations of IgG in group 2-4 were not reflected by any differences in health status and that the age of arrival in the fattening unit in this trial had no influence on the growth performance or health status of the veal calves.

3.3 CONCENTRATE SUPPLEMENTED DIET PROMOTING RUMINAL, BUT RETARDING OMASAL EPITHELIAL CELL PROLIFERATION IN YOUNG GOATS

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In this study two experiments were conducted. In each experiment 8 goats (BW 9.05 ± 0.13 kg in EXP1 and 15.79 ± 0.22 kg in EXP2) were assigned to 2 groups ($n = 4$), fed by peanut straw only (PS) or PS supplemented with 100 g (PSC100) or 400 g (PSC400) concentrate per day. The intake of ME, in EXP1, was 0.84 MJ/kg $0.75/d$ (PS) and 1.01 MJ/kg $0.75/d$ (PSC100) and of nitrogen was 1.16 /kg $0.75/d$ and 1.64 /kg $0.75/d$. In EXP2 the ME intake was 0.89 MJ/kg $0.75/d$ (PS) and 1.37 MJ/kg $0.75/d$ (PSC400) and the nitrogen intake was 1.23 g/kg $0.75/d$ and 2.60 g/kg $0.75/d$, respectively. The feeding period was 42 d for each experiment. Body weight was determined weekly. At slaughter the weight of visceral organs was recorded. The cell cycle of fresh ruminal and omasal epithelium was assayed by flow cytometry. The number of cells in S phase and G2-M phases indicated DNA synthesis and cell division, respectively. The average daily weight gain was significantly greater in PSC100 and PSC400 than those in PS groups ($p < 0.01$). Though the weights of complex stomachs, rumen and liver were heavier in PSC100 and PSC400 ($p \leq 0.05$), the omasal organ weight did not differ between PSCs and PS groups in both experiments ($p > 0.5$). These data show that the CSD stimulates the growth of rumen, but failed to stimulate the omasum. The discrepant responses of ruminal and omasal growth to CSD could be explained in part by the pattern of their epithelial cell proliferation. In rumen the epithelial cell number of S and G2-M phases was higher, but G0-G1 phase was lower in PSC100 and PSC400 than those in PS groups ($p < 0.05$), indicating that CSD induced cell cycle acceleration. It is consistent with our previous illustration that the positive response of rumen epithelial cell proliferation to energy-rich diet is associated with increased IGF-1 concentration in plasma and more IGF type1 receptors in rumen epithelium of young goats. Contrary to rumen, in the present study the omasal epithelium of goats in PSC100 and PSC400 groups exhibited less cell number of S and G2-M phases, but more of G0-G1 phase ($p < 0.05$), suggesting that the cell cycle was blocked. This is an unusual observation, because the cell cycle block occurred during an energy- and protein-rich feeding regime where the IGF system was activated.

In summary, this study reveals that concentrate supplemented diet failed to stimulate omasum growth. It is associated with the retarded cell proliferation of omasal epithelial cells.

3.4 METABOLIC AND ENDOCRINE PROBLEMS IN VEAL CALVES

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Introduction

Neonates have to adapt to extra-uterine life and to develop the ability to digest colostrum and milk. Neonatal calves are characterized by inherent and by often marked nutrition-dependent metabolic and endocrine changes (for review: Guilloteau et al., 1997; Blum and Hammon, 1999; Blum and Baumrucker 2002; Blum, 2005, 2006). The metabolic and endocrine status of neonatal calves is in part specific. For example plasma lactate, creatinine, and nitrate and nitrosylated protein concentrations are much higher in neonatal compared to older calves (Blum et al., 2001; Blum, 2006; Christen et al., 2007). After a few weeks of life young calves are either maintained at the preruminant stage (veal) or are weaned. Growth performance of veal calves is very high when compared with weaned calves raised for potential breeding due to greater feeding intensity and use of highly digestible liquid diets. Furthermore, veal the iron (Fe) intake of veal calves is much lower. This review selectively summarizes some of our production-related and nutrition-associated studies in veal calves that evaluated metabolic and endocrine changes 1. during Fe deficiency, 2. dependent on feeding level and feeding frequency, 3. in response to asynchronous protein and lactose intake, and 4. in ruminal drinkers.

Metabolic and endocrine changes in veal calves during iron deficiency

Veal calves are fed mostly insufficient amounts of Fe in order to produce white meat. If the Fe deficiency is severe, this causes not only anemia and cardiovascular adjustments (such as increased heart rate), reduced appetite and diarrhea, but also reduced cell-mediated immune reactions changes (leading to enhanced infection rates), reduced oxygen consumption, transport and utilization, enhanced L-lactate production especially during physical stress (Gygax et al., 1993; Lindt and Blum, 1993, 1994), and other metabolic and endocrine adjustments. Thus, severely Fe deficient calves are characterized by relatively low plasma glucose (G) concentrations, enhanced insulin (I)-dependent G utilization, enhanced plasma clearance rate of growth hormone (GH), reduced plasma insulin-like growth factor (IGF-1) responses to exogenous (recombinant) bovine GH, and in part reduced plasma thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) concentrations (Hostettler-Allen et al., 1993; Ceppi and Blum, 1994; Ceppi et al., 1994). These metabolic and endocrine changes are expected to contribute to reduced growth performance during severe Fe deficiency.

Metabolic and endocrine changes in veal calves dependent on feeding level and feeding frequency

Veal calves are primarily fed liquid diets (milk or milk substitutes/milk replacers) and have usually access to only very limited amounts of roughage and concentrates. They are preruminants or pseudomonogastrics because forestomach development is inhibited. Ingested diets are normally directly transported into the abomasum and not into the rumen. The transfer of ingested nutrients to the digestive and absorptive sites in the small intestine is more rapid if diets contain non-milk proteins (such as soluble whey or soy protein) that do not clot in the abomasums and often dextrose is added to milk replacers. As a consequence, the intestine may become overloaded with nutrients, possibly followed by gastrointestinal problems, such as diarrhea, that may lead to metabolic and endocrine adjustments (Gutzwiller and Blum, 1996). If non-clotting proteins are added to milk replacers this leads to marked increases in absorption rates of amino acids, fatty acids and G. As a consequence, absorbed components appear in the circulation in excessive amounts that may lead to marked metabolic and endocrine changes. In fact, heavy veal calves frequently exhibit postprandial hyperglycemia, glucosuria, galactosuria, hyperinsulinemia and I resistance (Hostettler-Allen et al., 1994; Hugi et al. 1997a,b, 1998; Kaufhold et al., 2000; Vicari et al., 2007a), a phenomenon that is absent in postweaning calves (Hugi and Blum, 1997).

Feeding frequency (FF) and feeding level (FL) influence calf performance and health. It is still common practice to feed veal calves only twice daily at a high FL. This appears un-physiological if one considers calves suckling on their dam ingesting milk several times per day in an *ad libitum* manner. In fact, the metabolic and endocrine status of suckling calves differs in many respect from that in veal calves fed twice daily by bucket (Egli and Blum, 1998). Kaufhold et al. (2000) showed that increasing the FF reduced postprandial G and I responses in heavy milk-fed calves, i.e. improved blood G homeostasis, moderated postprandial changes of other metabolites (reduced urea and lactate) and created an endocrine status (reduced I/G ratio; reduced plasma glucagon concentration; increased plasma GH, IGF-1 and prolactin concentrations) that is favorable for growth. Although various aspects of FF and FL, especially on growth performance, have been studied in veal calves, several things remain unclear. Van den Borne et al. (2006) studied the effects of increasing FF and FL on protein and fat retention using whey proteins as the only protein source in heavy veal calves. Increasing FF increased both nitrogen (N) and fat retention. In the calves used in the study of Van den Borne et al. (2006) we have investigated the effects of FF, FL, and their interactions on plasma concentrations of metabolites and hormones (Vicari et al., 2007a). We hypothesized that an increased FF decreases problems with G homeostasis more at a high than at a low FL in heavy veal calves. We further hypothesized that the greater protein (N) and fat deposition with increasing FF in heavy veal calves are associated and mediated by endocrine changes. Effects of FF and FL on pre- and postprandial hormone and metabolite concentrations were studied in 15 heavy veal calves (body weight >100 kg) fed 1× (FF1), 2× (FF2) or 4× daily (FF4). The adaptation period lasted for 28 d, followed by the experimental (balance) period of 14 d. The study was performed under conditions in which enhanced FF did not affect dry

matter intake, gross energy intake and N intake, but positively affected energy retention and N retention (Van den Borne et al., 2006). Blood samples were collected during the last 2 d of the experimental period. Each calf was studied in 2 periods (P1, P2). In P1, all calves were fed at a low FL (FL_{low} ; $1.5 \times$ metabolizable energy requirements for maintenance, ME_m). In P2, FF2 and FF4 calves were fed at a high FL (FL_{high} ; $2.5 \times ME_m$), although FF1 calves remained at FL_{low} . Postprandial integrated plasma hormone and metabolite concentrations (AUC_{12-18h}) were calculated and for GH the pulsatile secretory pattern was evaluated.

Plasma concentrations of G, non-esterified fatty acids (NEFA), urea, I, glucagon, and leptin changed significantly after feed intake, whereas IGF-1 concentrations remained stable and those of GH did not change consistently. AUC_{12-18h} of G increased with increasing FL, but decreased with increasing FF; AUC_{12-18h} of urea increased with increasing FL, but was unaffected by FF; AUC_{12-18h} of NEFA was unaffected by both FL and FF. AUC_{12-18h} of I decreased with increasing FF and increased with increasing FL. AUC_{12-18h} of glucagon increased with increasing FL and decreasing FF. AUC_{12-18h} of GH decreased, whereas AUC_{12-18h} of IGF-1 and leptin increased with increasing FL, but neither GH, IGF-1 and leptin AUC_{12-18h} were affected by FF. There were no significant effects of FF or FL on basal concentrations, peak amplitudes and peak frequencies of GH. Mean plasma T3 and T4 concentrations were enhanced by increasing FF and FL and were higher in FF4 at FL_{high} than at FL_{low} . Except for plasma glucose (AUC_{12-18h} at FL_{high} at FF4 < at FF2) there were no FF \times FL interactions. Plasma I/G ratios were greater at FL_{high} than at FL_{low} and decreased with increasing FF.

In conclusion, metabolic and endocrine traits were differently affected by FF and FL. With the exception of plasma G, there were no FF \times FL interactions, suggesting separate effects of FF and FL on these traits. An increased FF decreased postprandial plasma G and I concentrations and resulted in lower G/I ratios, indicating enhanced I sensitivity and (or) responsiveness, in part explaining the increased growth performance. Overloading of G metabolism (hyperglycemia), marked postprandial I responses and I resistance were prevented by decreasing FL and (or) increasing FF in heavy veal calves.

Metabolic and endocrine changes in veal calves during asynchronous protein and lactose intake

Heavy veal calves often exhibit postprandial hyperglycemia, glucosuria, and I resistance if the total milk or milk replacer is fed in a small number of daily meals, as discussed above. Insulin (I) resistance can be associated with decreased protein (P) and fat deposition. Nutrient asynchrony by concentrating lactose (L) in one and P in the other daily meal did not alter performance, but reduced heat production, explaining increased fat retention without affecting P retention in heavy veal calves (Van den Borne et al., 2006b). Based on these findings we investigated the underlying endocrine mechanisms that mediate effects of asynchronous L and P supply on nutrient partitioning

and assessed effects of nutrient asynchrony on glucose (G) homeostasis (Vicari et al., 2007b). Holstein-Friesian calves (n = 36) were fed a milk replacer at 06.00 and 18.00 by bucket. Daily nutrient intakes and amounts of fat for each meal were identical, but P and L intakes were gradually separated over two daily meals. Calves were assigned to one of six degrees of nutrient synchrony (SYN 1-6; 6 calves/treatment). They were fed a P-rich (P-)meal and an L-rich (L-)meal at 06.00 and 18.00, respectively (sequence A), or *vice versa* (sequence B). Protein/lactose ratios at SYN 1, 2, 3, 4, 5 and 6 in sequence A were 50/50, 57/44, 64/38, 71/32, 78/26 and 85/20, resp., and in sequence B were 50/50, 43/56, 36/62, 29/68, 22/74 and 15/80, resp. P and L were iso-energetically exchanged between the two daily meals from SYN 1 to 6. Calves were adapted to the treatment for 28 d, followed by a balance period of 8 d. Blood samples were collected every hour during 24 h through a jugular vein catheter on d 5 of the balance period. Urine was collected during the complete balance period. The 24-h means and the integrated 5-h postprandial changes of metabolites and hormones were evaluated by calculation of areas under concentration curves (AUC).

The results showed that nutrient intake was similar across treatments. The digestibility of dry matter, P, L and energy of calves at SYN 5 and SYN 6 were, however, lower than those of calves at SYN 1-4 (Van den Borne et al., 2006b). Because the goal was to compare degrees of nutrient asynchrony at identical intakes of digestible nutrients, regression analyses were performed separately for SYN 1-6 and SYN 1-4. Mean body weights at the end of the experiments (152 kg) and average daily gains (1.20 kg) were not affected by nutrient asynchrony. Plasma G concentrations transiently increased after feeding, AUC were similar after P- and L-meals for SYN 1, but mean 24-h G concentrations and AUC increased along with nutrient asynchrony after the L-meal, whereas AUC decreased with increasing nutrient asynchrony after the P-meal, as expected. The 24-h urinary G excretion increased for SYN 1-6, but was unaffected for SYN 1-4. Mean 24-h I concentrations decreased with increasing nutrient asynchrony. Postprandial I responses (AUC) decreased with increasing nutrient asynchrony after both P- and L-meals. Plasma I responses decreased with increasing nutrient asynchrony, demonstrating that a synchronous provision of P and L in a meal was needed to induce high I responses. Possible explanations for the low I response with increasing nutrient asynchrony include a reduced pancreatic I secretion and an increased I clearance rate. The 24-h I/G ratios decreased with increasing nutrient asynchrony, which is usually considered to be associated with enhanced I sensitivity and (or) responsiveness. This possibly contributed to enhanced fat retention with increasing nutrient asynchrony in SYN 1-4. Mean 24-h NEFA concentrations and AUC_{0-5h} were similar for P- and L-meals. Urea concentrations decreased after the L-meal, whereas they increased after the P-meal. Although within-day variation in plasma urea concentrations increased along with separation of L and P intake, 24-h urea concentrations were not affected. This corresponded with the unaffected P retention (Van den Borne et al., 2006b). Interestingly, nutrient asynchrony did not affect plasma concentrations of IGF-1, glucagon, GH, leptin, T3 and T4. In conclusion, separation of P and L intake over meals affected postprandial responses of urea, G and I, and inhibited I responses despite high plasma G concentrations after high L and low P meals. Separation of P and L intake in different meals inhibited I responses to a

lactose-rich meal in heavy veal calves despite high plasma G concentrations, i.e. dichotomized postprandial I and G responses.

Metabolic and endocrine changes in ruminal drinkers

In un-weaned ruminants, ingested milk normally bypasses the forestomachs and is directly transported into the abomasum whereas in ruminal drinkers (RD), ingested milk is transported totally or in considerable amounts into the rumen instead into the abomasum. This occurs because of lacking or insufficient closure of the oesophageal (reticular) groove. Systemic metabolic effects, such as metabolic acidosis due to accumulation mainly of D-lactate, are often observed, but additional metabolic effects and associated endocrine changes have not yet been studied in RD. Although reduced provision of milk reduces clinical symptoms and weaning onto hay and concentrates allows clinical recovery within days, such procedures provoke considerable problems in the management of veal calf rearing systems. Based on these premises we tested the hypothesis that RD are characterized by distinct pre- and postprandial metabolic and endocrine changes and that these metabolic and endocrine changes are normalized when calves are suckling with a nipple instead of drinking from the bucket (Herrli-Gygi et al., 2006). Unweaned, bucket-fed calves (1 RD, 2 controls) were studied on 7 farms. On d 1, after metabolic and endocrine 12-h profiles were studied, RD and one control calf were fed for 10 d by nipple, whereas the other control calf was fed by bucket. On d 11, metabolic and endocrine 12-h profiles were again studied. On d 1, mean plasma concentrations of G, triglycerides, urea, IGF-1, T3, and T4 were significantly different and leptin tended to be different between RD and controls. Mean concentrations of NEFA, total protein, albumin, and glucagon did not significantly differ among groups. In RD animals, concentrations of G, NEFA, I, GH, IGF-1, and T4 were higher, and of urea lower on d 11 than on d 1. Glucose and I concentrations increased postprandially in healthy calves on d 1, but barely increased in RD and remained lower than in controls. There was no rise of NEFA and triglyceride concentrations on d 1 after the initial postprandial decrease in RD in contrast to controls. However, on d 11 postprandial responses of these measures were similar in RD and controls and urea and T4 concentrations on d 11 became normalized. However, G and T3 concentrations in RD on d 11 were still lower than in one or both control groups.

In conclusion, RD were characterized by significant changes in the concentration of some metabolites and hormones when compared with healthy controls. Glucose, NEFA, triglycerides, urea, I, IGF-1, T3 and T4 concentrations in RD differed with respect to absolute concentrations and (or) postprandial patterns from the healthy control calves. The metabolic and endocrine status of RD calves differed not only from the one in healthy veal calves fed by bucket, but also from the one found for veal calves fed by computer-controlled automates or from the one of calves suckling on their dam. Changes in nutritional technique (drinking by floating nipple instead of drinking from bucket for 10 d) improved the status of several metabolic and endocrine traits (especially postprandial G and I responses).

Conclusions and outlook

Veal calves exhibit several nutrition-related metabolic and endocrine changes and problems that are quite unique. Problems of G handling with I are of special interest because of the importance of G for delivering energy. They express genetic differences in G metabolism between ruminants and non-ruminants (Greger et al., 2006b), that exist already at the neonatal stage (Scheuer et al., 2006).

Intensified studies on receptor and postreceptor effects of the major hormones that are important for rapid and (or) long-term metabolic control in veal calves are needed at the molecular and protein level to fill gaps in our present knowledge. The liver is of central importance for metabolic control (biotransformation, conjugation and transport of xenobiotics and endobiotics). Its function (also as an endocrine organ) is influenced by various hormones through specific receptors. They exhibit ontogenetic changes, likely according to altered needs, as for example shown for α - and β -adrenoceptors (Carron et al., 2005; Ontsouka et al., 2006). Several hepatic nuclear receptors, which in calves have only recently received some interest (Krüger et al., 2005; Greger et al., 2006a,b; Greger and Blum, 2007), are involved in the coordinated transcriptional control of many of genes that encode proteins which are involved in hepatic handling of metabolic products. Studies in these directions can serve as a basis for improvements in health maintenance and performance of veal calves and may even offer basic insights into metabolic control.

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3.5 EFFECTS OF FEEDING HOLSTEIN CALVES ON A COLOSTRUM AND MILK REPLACER SUPPLEMENTED WITH CLINOPTILOLITE ON BLOOD PROFILE

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The objective of this field study was to assess the effect of feeding newborn calves with clinoptilolite in colostrum and milk replacer at the first two months of their life. A natural zeolite, clinoptilolite, was used at two different levels based on the amount of concentrates fed (1 and 2 gr of clinoptilolite/kg of bodyweight). A total of seventy eight (78) healthy Holstein newborn calves were employed in the study. They were divided into three groups (26 calves each), so that sex and parturition season were equally represented in each group. The calves of the first group (A) were fed colostrum for the first two days and thereafter a milk replacer until the age of 2 months supplemented with clinoptilolite at the level of 1 gr/kg of bodyweight. Correspondingly, the calves of the second group (B) were fed colostrums and milk replacer for the same period as group A supplemented with clinoptilolite at the level of 2 gr/kg of bodyweight, while the animals of the third group (C) used as untreated controls fed with colostrum and milk replacer for the same period as groups A and B without the supplementation of clinoptilolite. The experiment started when the calves were born and lasted until the age of 2 months. Blood samples were collected in vacutainer with EDTA from each calve immediately after birth and before the consumption of colostrum, 12 and 24 hours later and every week until the age of 2 months. Blood samples were analysed in the ABC animal blood counter in order to investigate the blood profile (PCV, Hg, RBC, MCV, MCH, MCHC, WBC, PLT).

The results showed that the calves of groups A and B had a significant improvement of PCV, Hg, RBC, MCV and MCHC after the 4th week of age until the end of the trial compared to the calves of group C. Moreover, the calves of group C had a significant increase of WBC numbers relatively to the other two groups.

3.6 EFFECTS OF FEEDING HOLSTEIN CALVES ON A COLOSTRUM AND MILK REPLACER SUPPLEMENTED WITH CLINOPTILOLITE ON THE INCIDENCE OF DIARRHOEA AND BODYWEIGHT GAIN

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The objective of this experiment was to assess the effect of feeding newborn calves with clinoptilolite at two different levels in colostrum and milk replacer until the age of 8 weeks. Clinoptilolite, was used at two different levels (1 and 2 gr of clinoptilolite/kg of bodyweight daily). In this study 78 healthy newborn calves were used from the same farm for a total period of 11 months. The calves were assigned equally to one of three groups, 26 calves each one. At the calves of the first group (group A) colostrum and milk replacer supplemented with clinoptilolite at the level of 1 gr/kg of bodyweight were offered. The animals of the second group (group B) received colostrum and milk replacer supplemented with clinoptilolite at the level of 2 gr/kg of bodyweight, while the calves of the third group (group c) served as untreated controls. Each calf was weighted immediately after birth and at 7th, 14th, 21st, 28th, 35th, 42nd, 49th and 56th day of age. A clinical examination was carried out daily for each calf and in cases of diarrhea faecal samples were collected aseptically in order to perform microbiological examinations.

The results of the present study showed that the calves of groups A and C had a slight decrease of bodyweight during the first week of their life. The calves of Groups A and B had a pro rata increase of bodyweight gain for the next 7 weeks in comparison with the calves of group C. The higher incidence of diarrhea was noted at the animals of group C and A due to infectious reasons, while the period needed for recovery (from diarrheic to normal faeces) was shorter in the calves of group A relatively to group C under the same therapeutic approach.

3.7 COMPARISON OF TWO BLOODLESS CASTRATION TECHNIQUES IN BEEF SUCKLER CALVES <1 WEEK OF AGE

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Comparison of two bloodless castration techniques in beef suckler calves <1 week of age. Problem: In the U.K., the Burdizzo (B) method occupies the first, surgical castration the second and the rubber ring (R) method the third place in a ranking of the most commonly used castration techniques in young calves. Comparison of the bloodless techniques, concerning their effects on long-term animal welfare and results of long-term reliability of the Burdizzo technique are not available. Objective: To compare the R- and B techniques, in comparison to sham treatment (C), in locally anaesthetised beef suckler calves less than one week of age.

Materials and methods: Twenty healthy male beef suckler calves with a BW of 35-50 kg and < 1 week of age were selected for this study. The calves were randomly allocated to the following groups: R (n = 8), B (n = 8) or C (n = 4). Time needed for castration, plasma cortisol level during the first 24 h, and behavioural and clinical signs of pain and distress and wound healing over a period of three months were monitored. Sexual behaviour was monitored up to 10 months of age. Calves were slaughtered at a BW of about 400 kg at the age of 10 months. The testicular residues were histologically examined to investigate the long-term reliability of the B technique, and plasma testosterone concentration was measured. For comparative statistical analyses, level of significance was chosen at $P=0.05$.

Results: Burdizzo castration took significantly ($P < 0.001$) longer as compared to R castration or C treatment. Pain response during castration was highest in the B group, and the overall acute pain response, measured as plasma cortisol AUC from 10 min before till 100 min after treatment was significantly ($P = 0.0075$) higher in the B- compared with both other treatment groups. Wound healing took significantly ($P = 0.0036$) longer in the R- as compared to the B castrated calves. Differences of sexual behaviour among groups were not observed. At slaughter, signs of intact testicular tissue were found in all B calves, and B calves had significantly higher testosterone levels as compared to R calves ($P = 0.02$).

Conclusions: Neither the R technique, accompanied by delayed wound healing and long-term local inflammation, nor the technically demanding and poorly reliable B method may be recommended for routine castration of calves < 1 week of age.

3.8 DEVELOPMENT OF TIBIAL DYSCHONDROPLASIA, A METABOLIC CARTILAGE DISEASE, IN POULTRY

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Tibial dyschondroplasia (TD) is a production-related metabolic cartilage disease in meat-type poultry that causes lameness, bone breakage, and infection. Rapid growth rates of birds raised under confinement and controlled management conditions is thought to be a factor in the development of this disease. An atypical death of chondrocytes in the hypertrophic zones of proximal growth plates of tibia and tibiotarsal bones leads to the failure of cartilage remodeling and bone formation and eventually to the widening of growth plate cartilage. Dithiocarbamate pesticides such as the tetramethyl and tetraethyl thiuram disulfide (thiram and disulfiram respectively) when fed to chickens induce TD similar to the naturally occurring disease. Because many carbamate and dithiocarbamate chemicals are used as fly and rodent repellants, fungicidal, and bactericidal agents in a wide variety of agricultural and household applications, an objective of our study was to find whether different categories of these pesticides do have similar efficacy to induce TD in chickens. Secondly we sought to determine the possible mechanisms of action of thiram on the growth plate that leads to the development of TD. Young broiler chickens were fed diets containing different carbamate pesticides or some of their metabolites at concentrations of 0.43 and 0.86 mmoles/ kg for 48h between days 8 and 9 post-hatch.

The TD indices were determined scoring the disease incidence and severity of the proximal growth plates of their tibia at necropsy on day 15. For the second objective the growth plates of chickens, treated as above with thiram, were studied with respect to the expression of selective genes relating to angiogenesis and cell survival using RT-PCR at 48 and 166 h post feeding. The viability of enzyme released chondrocytes from these growth plates was examined by trypan blue exclusion.

The results from these studies show that only dithiocarbamates with more than two disulfide groups, such as thiram, disulfiram, ziram, and ferbam were potent inducers of TD as compared with carbaryl, sodium metam, potassium dithiocarbamate, or ethinythiourea which either lacked a disulfide group or had only one disulfide group. Thiram down regulated the expression of genes for vascular endothelial growth factor receptors 1 and 2, and Bcl2, a gene responsible for cell survival. These changes were evident as early as 48 h following the initial feeding.

We conclude that environmental exposure to certain dithiocarbamate pesticides even for a relatively short duration can increase the risk of birds developing TD. Also, these chemicals have a propensity to cause neuropathy and interfere with angiogenesis and cell survival precluding a normal development of the growth and to the pathogenesis of TD.

3.9 DOWN-REGULATION OF INSULIN-LIKE GROWTH FACTOR TYPE-1 RECEPTOR ASSOCIATED WITH DWARFISM IN HOLSTEIN CALVES

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Abnormal endocrine functions can reduce growth. Endocrine parameters were studied in three dwarf calves (DW) exhibiting retarded but proportionate growth and four phenotypically normal half-siblings (HS), sired by the same bull, and four unrelated control calves (CO). Plasma 3,5,3'-triiodothyronine and thyroxine concentrations in DW and HS were in the physiological range and responded normally to injected thyroid-releasing hormone (TRH; 0.4 mg/calf). Plasma glucagon concentrations were different (DW, CO > HS; $P < 0.05$). Plasma growth hormone (GH), insulin-like growth factor-1 (IGF-1) and insulin concentrations in the three groups during an 8-h period were similar, but integrated GH concentrations (areas under concentration curves) were different (DW > CO, $P < 0.02$; HS > CO, $P = 0.08$). Responses of GH to xylazine (0.2 mg/kg body weight) and to a GH-releasing-factor analogue [Ile2-Ser8-Ala15-Ser28-Hse30 bovine GRF-(1-30)-NH-ethyl acetate salt; 0.2 mg/kg body weight] were similar in DW and HS. Relative gene expression of IGF-1, IGF-2, GH receptor (GHR), insulin receptor, IGF-1 type-1 and -2 receptors (IGF-1R, IGF-2R), and IGF binding proteins were measured in liver and anconeus muscle. The GHR mRNA levels were different in liver (DW < CO, $P < 0.002$; DW < HS, $P = 0.06$; HS < CO, $P = 0.08$), but not in muscle. The IGF-1R mRNA level in liver in HS and CO was 2.4- and 2.5-fold higher ($P = 0.003$ and $P = 0.001$, respectively) and in muscle tissue was 2.3- and 1.8-fold higher ($P = 0.01$ and $P = 0.08$, respectively) than in DW. Hepatic IGF-1R protein levels (based on western blots) in muscle were 2.5-fold higher ($P < 0.05$) and in liver and muscle (based on quantitative immunohistochemistry) were higher ($P < 0.02$ and $P < 0.07$, respectively) in HS than in DW.

In conclusion, a reduced quantity of IGF-1R at the mRNA and at the protein level was associated with dwarfism in the studied calves and may have been the cause of the reduced growth rate, similar as in Laron-type dwarfism in humans.

4 METABOLIC HEALTH AND NUTRITION

4.1 THE POTENTIAL INTERACTION OF OBESITY AND INFLAMMATION IN THE METABOLIC RESPONSE OF COWS TO NEGATIVE ENERGY BALANCE

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Introduction

The role of excessive body fat as a risk factor for peripartum disease in dairy cows is well established (Dechow et al., 2004; Gearhart et al., 1990) and has been the subject of many papers in previous ICPD meetings. The general focus of physiological investigation has been on the role of fat mobilization in metabolism and the pathogenesis of metabolic disease, particularly fatty liver and ketosis. This focus on classical metabolic diseases and pathways of intermediary metabolism may, however, be too narrow and may not account completely for the overall increase in morbidity and mortality associated with excessive fatness and adipose mobilization in cows.

A general hypothesis recently has emerged that obesity may be considered a chronic inflammatory state with engorged adipocytes producing many of the same molecules of inflammatory modulation as do stimulated cells of the immune and inflammatory systems (Wellen and Hotamisligil, 2005). These include hormones, cytokines, signaling proteins, transcription factors, and bioactive lipids that can function in both metabolic and immune roles. Especially important in this role appear to be tumor necrosis factor alpha (TNF α) and interleukin 6 (IL6) (Hotamisligil et al., 1993; Sethi and Hotamisligil, 1999). In human medicine this association between adipose tissue and inflammation has led to speculation that chronic inflammatory stimuli associated with obesity sensitizes the body to other chronic inflammatory conditions, such as chronic airway disease and atherosclerosis (Hotamisligil et al. 1995; Kern, P.A., et al. 1995). This may then explain the association of obesity with several chronic inflammatory diseases of humans. The potential role that obesity-associated chronic inflammation may have in cow health is perhaps less readily apparent because chronic inflammatory diseases, other than those associated with chronic infections, are not an important problem in cattle. However, there are important ways through which molecular modulators of inflammation may interact with metabolism to affect cow health. These include the induction of insulin resistance, the creation of reactive oxygen species, and a complementary but destructive synergy between adiposity and infection.

Adiposity, insulin resistance, and adaptation to negative energy balance

Adaptation to negative energy balance (NEB) is an essential part of early lactation in dairy cows. This adaptation is achieved through metabolic checks and balances that allow the animal to mobilize stored energy to support body needs, while directing nearly all available glucose towards milk synthesis (Herdt, 2000). Paradoxically, a large adipose mass appears to interfere with adaptation to NEB, i.e. in times of energy deficiency having a large amount of reserve energy is generally detrimental to cow health. Thus, extensive adipose stores are a positive risk factor for many peripartum diseases in dairy cows. The mechanism of this association is still incompletely understood. A major factor that appears to contribute to the development of metabolic disease is inappropriately extensive adipose mobilization.

Under ideal metabolic circumstances a system of metabolic checks and balances is expected to regulate adipose mobilization. The expected sequence of events is 1) blood glucose concentrations diminish in response to high demand and insufficient feed intake, 2) insulin concentrations diminish in response lowered blood glucose, 3) declining insulin concentrations release inhibitory influences on adipose tissue, allowing net lipolysis and an increased rate of NEFA release into serum, 4) increasing serum NEFA concentrations stimulate insulin secretion, but conserve glucose by down regulating the hypoglycemic effects of insulin, 5) increasing insulin

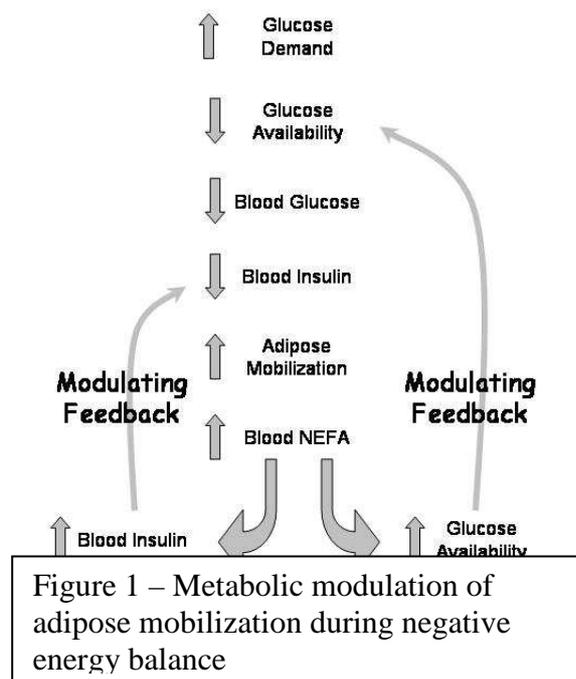


Figure 1 – Metabolic modulation of adipose mobilization during negative energy balance

concentrations down-regulate the rate of adipose NEFA release by suppressing the activation of hormone-sensitive lipase. The cumulative physiological effect is an increased set point for serum NEFA and a decreased set point for serum insulin concentrations, with relatively constant serum glucose concentrations. (Figure 1)

Ample evidence indicates that efficacy of this feedback loop is diminished as adipose mass increases. Figure 2 shows the relationship of pre-partum rump fat thickness to post-partum serum NEFA concentrations. Rump fat thickness was measured ultrasonically at a point approximately 10 cm anterior to the tuber ischi. Measurements were taken between one and two weeks pre-partum. Serum NEFA concentrations were measured one to two weeks postpartum. Rump fat thickness greater than 10 mm was associated with significantly

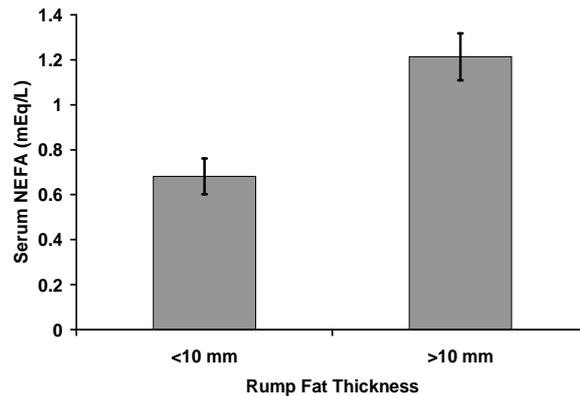


Figure 2 – Association of pre-partum rump fat thickness with post-partum serum NEFA concentrations. Joshi, unpublished data

($p < 0.05$) higher serum NEFA concentrations (Joshi, unpublished data). It is reasonable to assume that all or nearly all of these cows were in NEB, because they were in early lactation, although there is insufficient information available to quantitatively estimate the degree of NEB. The point here is that physiologically we might expect serum NEFA concentrations to be modulated at some set point consistent with delivery of energy substrate for metabolism, instead serum NEFA concentrations continued to increase with increasing adiposity.

The direct association of adiposity with serum NEFA concentration during periods of NEB suggests that as adipose mass increases it becomes less responsive to the modulating effects of insulin. Ample evidence in human medicine indicates that obesity is associated with insulin resistance, which is defined as a reduced metabolic response at a given serum insulin concentration. There is some evidence to suggest that obesity-induced insulin resistance occurs in cows as well. The mechanism of obesity-induced insulin resistance is, however, poorly understood. A recent hypothesis is that inflammatory cytokines produced by adipose tissue may contribute to insulin resistance, particularly the resistance of adipose tissue to insulin (Wellen and Hotamisligil, 2005).

Tumor necrosis factor alpha ($\text{TNF}\alpha$) is a cytokine protein produced by adipose cells, in addition to macrophages. $\text{TNF}\alpha$ is produced in cell-associated and soluble forms, with the cell-associated form predominating in adipose cells. Cleavage with solubilization of the cell-associated $\text{TNF}\alpha$ is the probable reason for increased serum $\text{TNF}\alpha$ concentrations in the blood of obese humans (Xu H., et al. 2002). $\text{TNF}\alpha$ stimulates inhibitory phosphorylation of serine residues of insulin-receptor substrate (IRS) family proteins (Hotamisligil et al., 1994), thus blocking down-stream signaling of insulin-

stimulated cellular events. These insulin-stimulated events include the inhibition of hormone-sensitive lipase, the major mediator of adipose fatty acid mobilization.

Information recently published from Michigan State University (O'Boyle et al., 2006) suggests that expression of TNF α may be increased in obese dairy cows. Two groups of eight cows each were selected from a commercial dairy herd, one group with moderate body condition score (BCS), 2.5 – 2.7, and the other with high BCS, >3.5. These cows were in mid to late lactation (150 to 200 days in milk). TNF α concentrations were measured in sera using a sandwich ELISA procedure with bovine-specific antibodies. The results are in Figure 3 and show a significantly ($p < 0.05$) higher TNF α concentration in the high-BCS cows. Because cell-associated TNF α is more strongly expressed in adipose tissue than is soluble TNF α , the proportional difference in adipose tissue TNF α concentrations in these cows may be even greater than the proportional difference in the serum concentrations. Cows at this stage of lactation can be expected to be in positive energy balance with low serum NEFA concentrations. In fact, in this study the serum NEFA concentrations of the high-BCS cows were lower than those of the low-BCS cows. The point is that obese cows with persistently high expression of TNF α may respond with insufficiently modulated and excessive adipose mobilization when challenged with NEB.

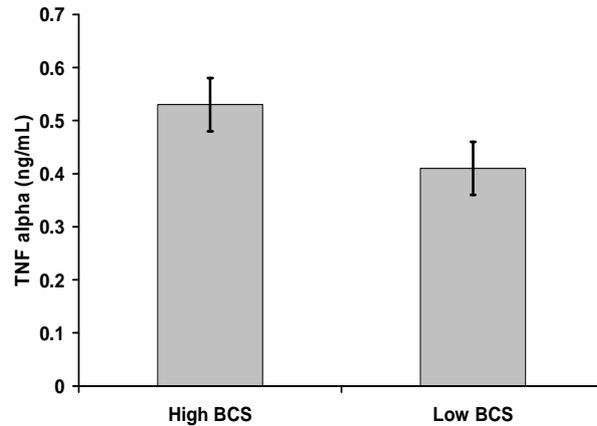


Figure 3 - Association of serum TNF α concentration with body condition score in mid-lactation dairy cows (data from O'Boyle et al., 2006).

Adiposity and Reactive Oxygen Species

Reactive oxygen species (ROS) include oxygen ions, free radicals, and lipid peroxides. They are highly reactive molecules generally recognized as capable of damaging various body tissues. ROS are the products of several metabolic pathways, and of inflammatory reactions. Numerous physiological mechanisms exist to protect the body from the harmful effects of ROS. These mechanisms include such things as vitamin E, glutathione peroxidase, ascorbic acid, and catalase. Under normal homeostatic conditions there is a preponderance of protective capacity and oxidative damage from ROS is kept in check. However, under conditions of either excessive ROS production, or insufficient protective capacity a state of oxidative stress exists and tissue damage can occur. It appears that in humans obesity can lead to oxidative stress, and recent

evidence suggests that the same is true in cattle (Bernabucci et al., 2005; Castillo et al., 2005)

In the same cattle in which serum TNF α concentrations were measured (see above, O'Boyle et al., 2006), assays were completed to assess oxidative damage and antioxidant capacity. Total lipid peroxide concentrations in serum were measured as an assessment of oxidative damage, and the total antioxidant potential of white blood cells (WBC) was determined as an indication of antioxidant capacity. In this study, there was no difference between the high- and low-BCS cows with respect to serum lipoperoxide concentrations, indicating no evidence of excessive oxidative damage. However, the antioxidant capacity of WBC was reduced in the high-BCS cows, compared to the low-BCS individuals. This suggests that creation of ROS may have been increased in the cows with more body fat, but that the increase was not enough to overcome normal body defenses. The lower WBC antioxidant capacity in the high-BCS cows suggests that antioxidant defense molecules were being consumed.

The overall implication of this for the interaction of obesity and cow health is that obesity, through chronic inflammatory stimulation may lead to oxidative stress, or at least reduction in the body's ability to neutralize ROS. In the face of infectious disease, or the intense metabolic needs of early lactation, compromise in oxidative defense mechanisms may leave the animal more susceptible to oxidative stress. In a destructive feedback loop, ROS stimulated inflammatory responses could lead to further release of inflammatory cytokines, more severe insulin resistance, and more rapid mobilization of adipose tissue.

Potential Destructive Synergy Between Adiposity and Infection

An increased incidence of all diseases, including infectious diseases, is frequently associated with obesity, fatty liver, and metabolic disease in fresh cows. It is often hypothesized that the increase in infectious disease incidence is due to immunosuppression that may result from metabolic disease, and indeed there is evidence that metabolites such as ketone bodies may adversely affect some immune functions. However, an association between the adverse health effects of adiposity and infectious disease may reflect an interaction of inflammatory infection and the inflammatory nature of obesity. The potential for complementary, albeit destructive effects of obesity and infection are illustrated in figure 4.

In the figure an additive effect of cytokines from inflammatory cell and adipose cell origin is proposed. This additive effect is manifest in unrestrained mobilization of adipose tissue, leading potentially to further immunosuppression and worsening infection. The ramification of this concept is that thin cows may recover spontaneously from a given infectious challenge, and fat cows without infection may adapt adequately to NEB, but a combination of infection and obesity may lead to a destructive spiral of events from which a cow with both conditions may not recover.

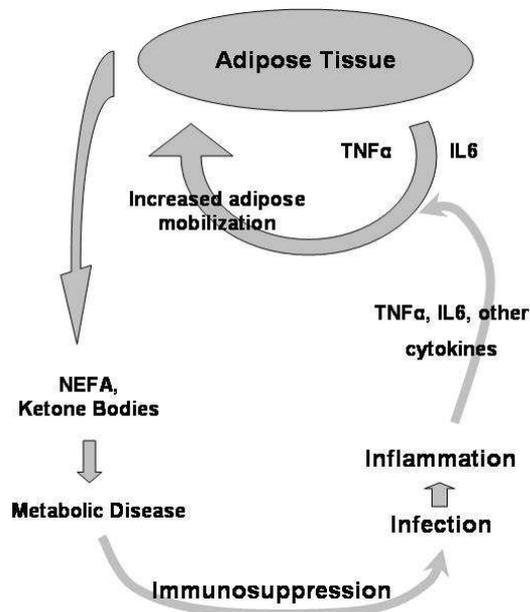


Figure 4 – A potential positive, destructive feedback loop linking adiposity and infection with the severity of metabolic disease in obese dairy cows.

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4.2 MOBILISATION OF BACK FAT AND MILK PRODUCTION IN DAIRY COWS

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Introduction: After parturition, dairy cows are unable to cover the energy requirement for maintenance and milk production by intake of the regular diet and hence, they experience a period of negative energy balance (NEB) associated with a higher risk in the incidence of a variety of diseases like fatty liver, displacement of the abomasums, ketosis and delayed onset of ovarian cyclicity (Pushpakumara et al., 2003). It was the intention of this study to investigate if a subgroup of cows shows high milk yield despite moderate mobilisation of body fat, suggesting a higher capacity for feed intake and intestinal absorption.

Methods: The experiment was performed on a commercial dairy farm and 258 cows were monitored from 56 days before expected calving through 98 days of lactation. Milk production was recorded daily and the thickness of back fat (BFT) was measured by ultra sound on days 56, 28 a.p. and on days 3, 28, 56, 98 p.p. The difference (Δ) between back fat thickness at day 3 p.p. and day 98 p.p. was used as a measure of fat mobilisation. BFT-values were expressed in percentage with day 3 p.p. as reference point (100 %) for each cow; other measurements were related to day 3 p.p. Total milk yield was obtained as the sum of the daily yields for 98 days. Blood samples for NEFA-analysis were collected on day -8 before expected calving and on days 3, 28, 56, and 98 p.p. Days open were chosen as fertility parameter.

Results: Cows with Δ values in the lower third of total range were pooled in a “Low Mob. (LM)” group (n = 86) and cows with Δ values in the highest third were assigned to a “High Mob. (HM)” group (n = 86). When the “Low Mob.” group was tested against the “High Mob.” group, a significant difference in total milk yield (3207 ± 549 and 3495 ± 566 kg/98 days, $p < 0.05$) emerged. When looking at the daily milk-output and its deviation only small differences in milk-performance were observed. LM-cows showed lower NEFA-values at day 28 p.p. ($p < 0.05$). Non-pregnant time (open days) was shorter in LM-cows (LM: 101 d vs. HM 128 d).

Conclusion: Generally HM-cows produce more milk than LM-cows, but some LM-cows produce as much milk as HM-cows, suggesting a higher feed intake. Thus, it seems to be achievable to breed cows with high milk production and low mobilisation of body fat avoiding a severe negative energy balance. Studies investigating the correlation between mobilisation of fat and health performance will follow.

4.3 INFLUENCE OF DRY PERIOD LENGTH AND FEEDING STRATEGY ON MILK YIELD, FEED INTAKE AND METABOLISM IN HIGH YIELDING DAIRY COWS

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The dry cow period is recommended to consist of two phases. During the first four to five weeks the cows are fed a ration with a low level of energy. In the two to three weeks before calving the level of energy is increased and the ration consists of most components fed in early lactation. Based on some newer studies there is the recommendation of a shortened, one-phased dry cow period lasting only 30-40 days. With extension of lactation length the milk yield will increase. Furthermore the extreme change in feed is diminished which has a positive effect on the metabolism of cows post partum. The effects of a one-phased, shortened dry cow period or a two-phased dry cow period on milk yield, feed intake, metabolism and on the overall animal health were compared in an experiment. The results on the colostrum quality and the frequency of mastitis is discussed separately. 80 Holstein Friesian cows were assigned to group 1 (two-phased period: first period 35 ± 2 days, second period 17 ± 5 days) or group 2 (one-phased, shortened period: 31 ± 5 days). The energy level of the ration fed in group 1 first period was at 5,7 MJ NEL and 125g nXP/kg DM whereas in group 1 second period and group 2 the energy level lay by 6,85 MJ NEL and 150g nXP. After calving the energy level was raised to 7,2 - 7,3 MJ NEL. Up to day 100 in milk feed intake and milk yield were measured daily. The milk composition was measured weekly. Blood samples were taken on approximately day 21 and 7 before calving and on day 1, 7, 14, 28 and 56 after calving. On these dates and on day 84 and 100 the body weight and back fat thickness were measured. Liver biopsies were taken on day 1, 10 and 21 after calving to determine the fat content. Data on the overall health and the fertility were taken from the herd management program. The cows with shortened dry period had a significantly lower milk yield (during the first 50 days in milk 41,0 and 44,1 kg ECM in group 2 and 1 respectively, during 51 to 100 days in milk 40,2 and 43,0 kg ECM in group 2 and 1 respectively). The milk loss was higher than the amount won through a longer lactation. The feed intake during the first 50 days was similar (21,1 kg DM). In the following period up to day 100 in milk group 2 tended to consume less fed than group 1 (24,1 and 25,3 kg DM in group 2 and 1 respectively). Based on the higher milk yield group 1 developed the energy deficiency after calving more significantly, but this did not show in either the back fat thickness, the body weight nor the blood or liver parameters. Concerning the frequency of illness, treatment and the data for fertility there were no differences found. This study shows no necessity or advantage in a shortened, one-phased dry cow period compared to the common two-phased.

4.4 GENE EXPRESSION OF HEPATIC ENZYMES RELATED TO THE CARBOHYDRATE AND LIPID METABOLISM DURING THE TRANSITION PERIOD IN DAIRY COWS

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The capability of cows to adapt to milk production during the transition period involves tremendous metabolic changes. However, the mechanisms underlying these differences are widely unknown.

The aim of this study was to assess changes in plasma metabolites and gene expression of hepatic enzymes related to the carbohydrate and lipid metabolism in dairy cows. The study was conducted in 28 multiparous dairy cows. Liver biopsies and blood samples were obtained during the transition period, at week 10 ante partum (a.p.), within 24 hours after parturition, at 4 weeks and between 12 and 16 weeks post partum (p.p). Liver samples were analysed for mRNA expression levels of cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (PEPCKc and PEPCKm, resp.), pyruvate carboxylase (PC), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 and 2 (HMGCS1 and 2, resp.), carnitine palmitoyltransferase 1A and 2 (CPT1A and 2, resp.), acyl-CoA synthetase long-chain (ACSL), and acyl-coenzyme A dehydrogenase very long chain (ACADVL) by real-time RT-PCR. Blood plasma was assayed for concentrations of beta-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), and glucose. Data analyses were conducted by the MIXED procedure of SAS with time-point as fixed effect and cow as repeated subject. Differences were localized by Bonferroni's t-test. Additionally, Pearson's coefficients of correlation between plasma metabolites and gene expression of the hepatic enzymes were calculated. Plasma NEFA concentration decreased from parturition onwards whereas BHBA was highest in week 4 p.p ($P < 0.001$). Plasma glucose was lowest in week 4 p.p. compared to the other time-points ($P < 0.01$). Regarding the lipid metabolism, levels of mRNA of CPT1A and CPT2 increased after calving. The mRNA expression of ACSL and ACADVL were similar at all time-points. Lowest levels of mRNA expression of HMGCS2 were measured at calving ($P < 0.001$), whereas levels of mRNA expression of HMGCS1 were lowest in week 10 a.p., increased slightly at partum and further at the other time-points p.p. ($P < 0.01$). Regarding the glucogenic enzymes, time-point affected levels PEPCKm and PEPCKc significantly ($P < 0.05$). In week 4 p.p, significant negative correlations ($P < 0.01$) were found between plasma NEFA concentration and ACADVL, PC, ACSL, HMGCS1 and CPT2.

Results from this study indicate that the metabolic adaptation during the transition period in the studied dairy cows involved changes in plasma metabolite concentrations and levels of mRNA expression of glucogenic and lipogenic liver enzymes. Furthermore, our study shows that plasma NEFA concentrations in week 4 p.p. are closely but inversely related to key enzymes in the lipid metabolism.

4.5 HEPATIC AND MUSCULAR GEN EXPRESSION OF GLUCOSE TRANSPORTER AND INSULIN RECEPTORS AFTER DEXAMETHASONE TREATMENT IN DAIRY COWS

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Introduction and objective: Ketosis and fatty liver are frequent production diseases in dairy cows and are commonly treated with glucose and glucocorticoids such as dexamethasone (Dexa). Although the clinical efficacy of Dexa was repeatedly demonstrated the mechanism of action is still not well understood. Application of Dexa leads to temporary hyperglycemia which can be due to increased gluconeogenesis or to increased insulin resistance. Thus, the objective of the study was to investigate the gen expression in muscular and hepatic tissues of glucose transporter and insulin receptors after dexamethasone application.

Material and methods: In a cross over design six German HF cows (150-250 days in milk) were treated either with dexamethasone (40µg/kg BW) or physiologic saline in an equal volume. Prior to, one day, and seven days after treatment muscular and hepatic tissue samples were obtained. Blood samples were taken on daily basis and analysed for glucose. From tissue samples RNA was extracted and Glucosetransporter-4 (Glut-4) and insulin receptors (Ins-R-A, Ins-R-B) were analysed by means of RT-PCR.

Results: Blood glucose peaked 24 h after Dexa treatment. In hepatic tissues Glut-4, Ins-R-A, and Ins-R-B were not regulated by Dexa. In muscular tissues Glut-4, Ins-R-A, and Ins-R-B were up-regulated for more than seven days after single Dexa treatment.

Conclusion: According to the preliminary results dexamethasone treatment does not lead to insulin resistance in late lactating cows. However, further studies investigating the protein expression of glucose transporter and insulin receptors are necessary.

4.6 METABOLIC AND HORMONAL RESPONSES TO GLUCOSE INJECTION IN ESTONIAN HOLSTEIN AND ESTONIAN RED COWS

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Tissues sensitivity to insulin, often studied through glucose tolerance test, is considered one of the factors controlling lipid mobilisation post partum. The objective of the experiment was to compare metabolic and hormonal responses to glucose injection in multiparous Estonian Holstein (EHF) and Estonian Red (ER) cows. Glucose tolerance was studied in six EHF and in nine ER cows, kept in tie stall barn, fed TMR and milked thrice a day. Testing time was on an average 42 (32...61) and 33 (27...54) days after calving, energy balance during the test -26 (-81...99) MJ/d and 34 (-48...132) MJ/d and ECM yield 39 (18...50) kg/d and 35 (22...41) kg/d in EHF and ER cows respectively. Cows were deprived of feed 90 min before and during the glucose tolerance test. Catheter was inserted into the jugular vein 15 min before the test and it was filled with Li-heparin until the blood sampling and between the samplings to avoid clotting. After glucose infusion the tubing and catheter were flushed with normal saline. Discarding the first portion, blood samples were collected into vacuum tubes with Li-heparin -15, -5, 5, 10, 20, 30, 40, 50 and 60 min relative to the start of infusion of 0.15 g/kg BW glucose (40% wt/vol). Plasma was separated by centrifugation immediately after sampling and kept at $-24\pm C$ until the analyses for insulin, glucose (GLC), keton bodies (KB), triglycerides (TG) and non-esterified fatty acids (NEFA) concentration. General linear model with SAS System was used to compare breeds basal concentration (BAS) of metabolites (average of pre-infusion samples); clearance rate (CR) of GLC; area under the curve (AUC) of insulin and GLC; maximum increase (MAX) of insulin, GLC and TG and maximum decrease (MIN) of KB and NEFA relative to BAS; time to MAX or MIN relative to glucose infusion (TMAX, TMIN) for TG, KB and NEFA. BAS of insulin was similar in ER and EHF groups, but MAX higher ($P=0.04$) and AUC larger ($P=0.03$) in ER group. Groups' BAS and MAX of GLC as well as CR and AUC were nearly similar. BAS of KB was higher, MIN deeper and TMIN longer; BAS and MAX of TG were higher and TMAX shorter; BAS of NEFA was higher, MIN deeper and TMIN shorter in ER group, although differences between the groups were statistically not significant. Obtained results - higher MAX and larger AUC of insulin; deeper MIN and shorter TMIN of NEFA, and shorter TMAX of TG - suggest that ER cows are more sensitive to GLC infusion and their adipose tissue might be less resistant to insulin.

4.7 A SIMULATION MODEL TO EVALUATE THE RISK OF SUBACUTE RUMINAL ACIDOSIS IN HIGH-YIELDING DAIRY COWS FED TOTAL MIXED RATIONS AD-LIBITUM

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Subacute ruminal acidosis (SARA) is a common and economically important metabolic disorder in dairy cattle, which occurs when ruminal pH drops below an optimum for fiber digestion, due to excessive consumption of fermentable carbohydrates and low dietary fiber. In this study, we defined the response of ruminal pH to chemical and physical characteristics of the diet to develop a physiologically-based, easy-to-use, simulation model to assess the risk of SARA occurrence in high-yielding dairy cows. To model the variation of ruminal pH throughout the day, a database from 77 published studies ($n = 316$ dietary treatments) was generated. Further, a second database from 58 ($n = 238$ dietary treatments) recently published studies with high-yielding dairy cows (in average: 95 days in milk and 34.9 kg milk/d) fed total mixed rations (TMR) was compiled including information on ration composition, DM intake (23.1 ± 2.1 kg DM/d) and ruminal pH measured over 24h. The average content of physically effective NDF, estimated by the proportion of diet DM retained on sieve 1.18 mm (peNDF), using dry-sieving methods, was $23.7 \pm 7.2\%$. The amount of ruminally degradable starch of concentrates (RDSC) and forages (RDSF) composing TMR averaged $14.8 \pm 4.9\%$ and $5.99 \pm 5.2\%$, respectively. The model was developed in SAS (2001) using mixed modelling procedure to account for random effect of experiment, within-study covariance and unequal variances among studies. The model simulations were performed using a stochastic Monte-Carlo algorithm with Beta-PERT probability distribution using computer software RiskAMP (Structured Data, LLC). The model revealed that high-yielding dairy cows are not at risk of SARA (0% risk), if duration of time in which ruminal pH is below 5.8 does not exceed 1h per day. This study showed that daily mean ruminal pH or time of ruminal pH < 5.8 can be accurately predicted ($R^2 > 50\%$) accounting for dietary peNDF, RDSC and total DM intake. The model suggested that to keep high-yielding dairy cows at 0% risk of SARA, their diets must contain not less than 28% peNDF, without exceeding 10% dietary RDSC and a DM intake of about 20 kg/d. Alternatively, the simulation model suggested that cows are at a relatively low risk of SARA ($< 10\%$), if their diets contain at least 24% peNDF, even when dietary RDSC increased up to 15%. Overall, increasing dietary RDSC with 4% increased the risk of SARA with 30-40%.

It can be concluded that this simulation model is a valuable tool to estimate the response of ruminal fermentation to dietary factors (i.e., peNDF, RDSC, and DM intake), and hence to evaluate the risk of SARA in high-yielding dairy cows. Acknowledgement: This study was supported by German Research Foundation (DFG, code no. DR 92/12-1).

4.8 ACCURACIES OF SERUM β -HYDROXYBUTYRATE AND GLUCOSE FOR DIAGNOSIS OF SUBCLINICAL KETOSIS

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Aim: The purpose of this study was to estimate the accuracies of serum BHB and glucose tests and their conditional dependence for diagnosis of SCK and also to estimate the true prevalence of SCK in our study region. **Materials and methods:** One hundred and two animals were randomly sampled from 4 dairy herds in Tehran Province (Tehran, Iran). Serum from each animal in the third week after parturition was subjected to both BHB and glucose diagnostic tests. Serum BHB level, measured by Ranbut (Randox Laboratories Ltd, Crumlin, UK), of more than 1.2 mmol/L were considered as a positive test result for SCK. Blood glucose of less than 35 mg/dL was also considered to be a positive test result for SCK.

Results and Discussion: We have used Bayesian method to for estimation of S_{es} and S_{ps} because it allows expert opinion or current knowledge be incorporated into the analysis, and it provides direct probability interpretation of results (Branscum, 2005). The uncertainties about the S_{e} and S_{p} were modeled using independent beta prior distribution based on the most-likely (modal) values of parameters and their upper or lower percentile. BHB and glucose accuracies (S_{e} and S_{p}) assumed to be constant across herds because they have been performed in the third week after parturition in all animals. The scientifically most likely values for various parameters of the model were constructed using expert information from Drs. Todd Duffield (University of Guelph, Ontario, Canada) and Shahabeddin Safi (Islamic Azad University, Tehran, Iran). The prior modes for S_{es} of BHB and glucose tests were 0.98 and 0.54, respectively, with corresponding 5th and 95th percentile of 0.90 and 0.70. The prior modes for S_{ps} of BHB and glucose tests were 0.90 and 0.60, respectively, with corresponding 5th and 95th percentile of 0.80 and 0.70. The parameters for the beta distribution based on the modal value and its corresponding upper or lower percentile were calculated using the BetaBuster software. Posterior inferences were based on 200,000 iterations after discarding 20,000 burn-in iterations using the WinBUGS software. The conditional correlation between serum BHB and glucose for animals with and without SCK were 0.027 (-0.221, 0.321) and 0.152 (-0.172, 0.414), respectively, which indicated almost no dependence between serum BHB and glucose tests at the selected cutoff points in diagnosis of SCK. Glucose at the selected cutoff point had low while serum BHB showed high accuracy in diagnosis of SCK.

4.9 COMPARATIVE EUROPEAN STUDY FOR THE VALIDATION OF A NOVEL COW-SIDE β -CAROTENE ASSAY IN SERUM AND BLOOD

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β -Carotene is an important dietary component positively associated with bovine fertility. Different studies point to the importance of a local conversion of β -carotene into vitamin A in target tissue. Through a highly regulated local conversion in the ovary, the development and the secretory activities of the follicle and the corpus luteum are positively modulated. To obtain a sufficient supply of the ovary with β -carotene sufficiently high plasma levels are necessary. To initiate or to optimise β -carotene supplementation in cattle, β -carotene status is determined by β -carotene plasma levels. Currently the extraction of β -carotene from blood is a time consuming and cost-intensive multi-step procedure, which needs specific equipment to perform extraction and centrifugation. Finally, β -carotene is determined by spectrophotometric or HPLC methods in qualified analytical laboratories. Major concerns assessing β -carotene status using plasma colour are due to the potential interference from haemoglobin and bilirubin especially in the diagnostically critical marginal range (1.5–3.5 $\mu\text{g/ml}$). In a total of 176 cows from different European countries (Germany, n=19; Spain, n=19; France, n=69; Israel, n=27; Ireland, n=20) β -carotene levels in plasma and blood (Germany, n = 22) were determined by the classical method of HPLC taken as golden standard and compared with the novel assay system for β -carotene, the iEx, consisting of an all-in-one extraction unit and a hand held photometer, the iCheck (www.bioanalyt.com) that enables to extract β -carotene from blood without prior separation of plasma in a single step at cow-side. β -Carotene values measured range from 0.32 mg/L to 15.30 mg/L. No differences were observed between both methods for individual herds or for all animals either measured in serum or in blood (HPLC serum vs iCheck serum: 3.46+/-2.43 vs. 3.69+/-2.46 and HPLC serum vs. iCheck blood: 3.46+/-2.07 vs. 3.33+/-2.04). Furthermore, the results show that the novel cow-side test for β -carotene correlated (Pearson correlation coefficient) very well with HPLC analysis ($r_2=0.98$ and 0.99 , serum and blood respectively, both $P<0.001$). In a subset of animals found to have plasma levels mostly in the deficient range (<1.5 mg/L, 29 out of 32 animals) from Israel, the comparison of the validation by plasma colour and HPLC showed that 24 out of 32 animals were classified wrongly into the marginal (1.5–3.5 mg/L) or optimal (>3.5 mg/L) range, while with the cow-side assay all animals were classified properly.

In conclusion, results show that with the novel test system blood levels of β -carotene can easily be assessed within a few minutes at cow-side achieving a comparable quality as with highly sophisticated time consuming and expensive laboratory methods.

4.10 RELATIONSHIP OF PRE-HARVEST SERUM ANTIOXIDANTS TO POST-HARVEST OXIDATIVE DAMAGE IN BEEF OF STEERS FINISHED ON PASTURE OR ON A HIGH CONCENTRATE DIET

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Provision of grain or forage in the ration of finishing steers alters both the fatty acid concentration and composition of the harvested beef. Increased polyunsaturated fatty acids in beef should increase the risk of post-harvest fatty acid oxidation and subsequent decline in quality. The objective of the present study was to determine if pre-harvest measures of antioxidant status and oxidative damage in serum are associated with post-harvest fatty acid oxidation in ground longissimus muscles of steers. Twelve steers were randomly selected from a larger group of steers finished on either a conventional corn grain – corn silage ration (CON), alfalfa pasture (ALF) or naturalized cool-season grass (NAT) pastures. Serum was collected approximately 1 week pre-harvest for analysis of the antioxidants beta-carotene and alpha-tocopherol, antioxidant capacity via Trolox equivalent antioxidant capacity (TEAC), and oxidative lipid damage via malondialdehyde (MDA). Vacuum packed, refrigerated rib sections (ICPS 107) were opened 7 d post harvest and a lean trim subportion was ground and kept at 4 °C for thiobarbituric acid reactive substances (TBARS) analysis on d 1, 4, and 7 after grinding. Data for vitamins, MDA, and TEAC were analyzed with the PROC GLM (SAS 9.1.3) with finishing treatment as the main effect. Post-harvest TBARS was analyzed with the PROC MIXED (SAS 9.1.3) with finishing treatment as the main effect and day as the repeated term. Means for treatment were separated with orthogonal contrasts to compare CON to pasture finishing treatments (ALF + NAT) and compare ALF or NAT. Mean for day were separated by Tukey's test. The relationship between all pre-harvest variables and TBARS was analyzed with PROC REG (SAS 9.1.3), using backwards elimination to determine the variables to retain in the model ($P < 0.10$). Serum beta-carotene ($P < 0.001$) and alpha-tocopherol ($P = 0.048$) concentrations were higher in ALF and NAT steers than CON steers. Differences in MDA ($P=0.27$) and TEAC ($P=0.98$) between finishing systems were not detected in pre-harvest serum. Post-harvest fatty acid oxidation in ground beef as measured by TBARS was higher ($P = 0.004$) in CON than in ALF or NAT. Likewise, TBARS was higher on d 4 ($P = 0.004$) and 7 ($P = 0.002$) than on d 1. Ground beef TBARS on d 1 and d 7 could best be explained with a model that only contained beta-carotene (d 1: $r^2 = 0.32$, $P=0.057$; d 4 $r^2=0.28$, $P=0.08$). Ground beef TBARS on d 7 could best be explained with a model that contained both beta-carotene and MDA ($r^2 = 0.59$, $P = 0.02$).

In conclusion, beef from steers finished on a high concentrate diet have higher lipid oxidation as measured by TBARS. Beta-carotene may protect against post-harvest oxidation of fatty acids.

4.11 VITAMIN E IN TRANSITION COWS

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Vitamin E in Transition Cows Vitamin E supplementation around calving is associated with enhanced functionality of blood macrophages and neutrophils (Politis et al., 1995), and decreased incidence of placental retention and other reproductive disorders (Allison et al., 2000). Supplementation of vitamin E can provide adequate amount of antioxidants in neutrophils that assist in killing pathogens and provide protection against mastitis. It was recommended that in transition cows, plasma concentration of α -tocopherol should exceed 3 to 3.5 mg/l at calving (Blezinger, 2004), a low plasma α -tocopherol concentration is a significant risk factor for mastitis and other diseases. Commercially available vitamin E supplements contain either the natural 100% RRR- or the synthetic all-rac- α -tocopherol (12.5% RRR). Natural source vitamin E (NSE) is derived from vegetable oils whereas synthetic vitamin E is chemically synthesized, consisting of an equimolar mixture of 8 stereo-isomers of varying bioavailability. Jensen and Lauridsen (2003) determined that RRR- α -tocopherol is the absolutely dominating form of stereoisomers retained in cows' plasma (96%) and milk (86%). Plasma and neutrophil α -tocopherol concentration is significantly improved with NSE compared to synthetic vitamin E during calving (Hidiroglou et al., 1997; Meglia et al., 2006), indicating an increased immune response for protection against mastitis. Further on, NSE significantly improved vitamin E concentration in colostrums and milk 1 d post partum, thus improving the immune status of the newborn. Increasing serum α -tocopherol concentration in calves is associated with decreased medical treatment costs (Carter et al., 2005). Practical experiences with NSE indicate reduced occurrence of placenta retention, milk cell count and mastitis. The estimated treatment cost per cow for a single case of placenta retention or mastitis is ~120 EUR or ~100 EUR, respectively, thus the use of NSE in the transition phase is promising a sound economic return.

To improve health status of transition cows and to reduce the incidence of mastitis, dietary supplementation of NSE is recommended 30 days before calving at 2000mg /cow/d and 30 days after calving, at a level of 1000mg/cow/d.

4.12 INVESTIGATIONS ON THE ANTIOXIDATIVE STATE OF DAIRY GOATS IN THE PERIPARTURIENT PERIOD

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Introduction: Investigations on cattle and sheep have shown that there are severe changes in the antioxidative state during the periparturient period. With regard to the increasing incidence of disease in this special situation, a weakened antioxidative system might bear an additional risk. For goats there are no comparable investigations yet.

Aim of the study: Characterization of the antioxidative state of dairy goats around parturition and description of possible influences.

Material and methods: Samples were collected from 40 clinically healthy dairy goats 6 times during the time span from 6-8 weeks a. p. to 6-8 weeks p.p. Whole blood and serum was taken and analyzed for the activities of glutathioneperoxidase (GPX, whole blood) and superoxidodismutase (SOD, erythrocyte pellet) as well as for Trolox Equivalent Antioxidative Capacity (TEAC, serum) and the Antioxidative Capacity of Water-soluble substances (ACW, serum), sum parameters measuring the non enzymatic components of the antioxidative system. Red and white blood cell counts including differential cell counts were done and the concentrations of bilirubin, urea, total proteins, albumin, cholesterol and betahydroxybutyrate as well as the activities of glutamatedehydrogenase, creatinekinase and aspartateaminotranferase were measured in the serum. To avoid seasonal effects, two groups were analyzed. 19 goats in the winter term (October to January) and 21 goats in the summer term (April to July). A third group (n = 13) was analyzed in summer term containing goats who didn't have any dry period.

Results: For all goats blood cell counts didn't reveal any systemic inflammation or infectious disease. Clinical chemistry didn't indicate metabolic decompensation. The activity of the SOD showed an increase within the last two weeks a.p. followed by a slight decrease p.p. reaching the initial values 3-4 weeks p.p. GPX's activity increased as the SOD did a.p. but remained on this level until the end of the study. TEAC and ACW showed a similar pattern, with an increase 3-4 wks p.p. Goats with no dry period showed higher levels of non enzymatic antioxidants and lower enzyme activity of the SOD. In the summer term goats had higher levels of non enzymatic antioxidants and GPX's activity whilst SOD was not influenced.

Conclusions: Like cattle and sheep, dairy goats show significant changes in the antioxidant system during the periparturient period. Seasonal effects and extended lactation seem to influence the system as well. Further investigations should be done, to find out whether supplementation of antioxidants could improve animal health and minimize the risk of disease.

4.13 COMPARISON OF THE METABOLIC EFFECTS OF PROPYLENE GLYCOL AND GLYCERINE IN HIGH-PRODUCING COWS IN PREVENTING KETOSIS

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Propylene glycol (1,2-Propanediol) has a positive effect on stabilizing the metabolism of cows and on reducing the occurrence of ketosis. But problems in feed-acceptance are mentioned. This is not true for Glycerine (Glycerol, 1,2,3-Propantriol). But the direct metabolic effect of glycerine is discussed controversially. Glycerine is a by-product in the biodiesel production and therefore the costs of glycerine are less than those for propylene glycol. The effects of feed additions on the metabolism were to be compared in a feeding experiment. 21 - 14 days before calving 36 cows were assigned to the propylene glycol group and 35 were assigned to the glycerine group. At this point either 150g propylene glycol or 190g glycerine were added to the TMR. In the time between calving and 100 days in milk the amount was raised to 250g propylene glycol and 310g glycerine. The content of pure glycerine was assumed to be 80 % and therefore it could be figured that with this amount both groups got the same amount of effective feed addition. Otherwise the contents of the TMR were identical. Feed intake and milk yield were measured daily from day 6 in milk and milk composition was measured weekly. Blood (β-hydroxybutyrate, bilirubin, ASAT, GLDH, CK, cholesterol, free fatty acids, urea, insulin, leptin, glucose, creatinine, Mg) and urine (pH, NABE, BAQ, NH₄, Ca, P) samples were taken on approximately day 21 and 7 before calving and on day 1, 7, 14, 28 and 56 after calving. On these dates and on day 84 and 100 the body weight and back fat thickness was measured. Liver biopsies were taken on day 1, 10 and 21 after calving to determine the fat content. Data on the overall health of the animals and the fertility were taken from the herd management program. There were no significant differences found in body weight, back fat thickness, milk yield or milk composition, blood, urine and liver parameters. The only difference that was found was the feed intake. The dry-matter intake of the cows fed glycerine tended to be higher than of the cows fed propylene glycol. In the time between the second and the eighth week in lactation the multiparous cows of the glycerine group consumed 22,2 kg DM compared to 21,0 kg DM in the propylene glycol group. In the following 6 weeks the dry-matter intake increased to 25,2 kg in the glycerine group compared to 23,8 kg in the propylene group. However the results of fertility tended to be better in the group fed propylene glycol. Concerning the overall effects both feed additions were similar. It is to consider that the group fed glycerine had a higher dry-matter intake. It can be assumed that the effectiveness of glycerine lies in the higher feed intake, whereas the effectiveness of propylene glycol lies in other metabolic paths.

4.14 METABOLIC CHANGES AND GLUCOSE TRANSPORTERS IN SMALL INTESTINAL MUCOSA OF HIGH-YIELDING DAIRY COWS FED EITHER A STARCH-BASED (SD) OR A FAT-ACCENTUATED DIET (FD)

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Feeding rumen-protected fat enhances energy density of the ration and is proposed to improve energy supply for high-yielding dairy cows. On the other hand, diets based on corn silage may improve glucose supply by increasing starch digestion and glucose absorption in the small intestine. Glucose absorption in the small intestine requires specific glucose transporters, i. e. sodium-dependent glucose co-transporter-1 (SGLT1) and transport by facilitated diffusion (GLUT2), that are usually down-regulated in the small intestine of functional ruminants, but are up-regulated when luminal glucose is available. We have tested the hypothesis that in high-yielding dairy cows expression of intestinal glucose transporters as well as metabolic traits are affected by the energy source of the diet. Starting at 98 days post partum 18 dairy cows in second lactation were fed either a SD or a FD (isoenergetic and isonitrogenic) for 4 weeks. Feed intake and milk yield were measured daily and milk composition once a week. Blood samples were taken weekly for analyses of plasma triglyceride, NEFA, glucose, lactate, urea and bilirubin. After slaughtering, tissue samples of the small intestinal mucosa (duodenum and jejunum) were taken, frozen in liquid nitrogen and stored at -80°C until analysed. Total RNA was extracted from duodenal and jejunal mucosa and mRNA abundances for SGLT1 and GLUT2 were quantified by real-time RT-PCR relative to a housekeeping gene (beta-actin). Protein expression of GLUT2 in crude mucosal membranes and of SGLT1 in BBMV was quantified by SDS PAGE and immunoblot. The Mixed Model of SAS was used to examine feeding effects and time-related changes on metabolic traits and the General Linear Model of SAS was used to examine feeding effects and intestinal site on gene and protein expression of glucose transporters as well as zootechnical data. Dry matter intake was higher ($P < 0.05$) in cows fed SD than FD, but the decrease of milk yield during the experimental period tended to be less ($P < 0.1$) and milk protein content was lower ($P < 0.05$) in FD than SD. Plasma concentrations of triglycerides and NEFA were higher ($P < 0.05$) in cows fed FD than SD. Concerning glucose transporters, GLUT2 mRNA levels were higher ($P < 0.05$) in jejunal than duodenal mucosa, but mRNA levels of SGLT1 and GLUT2 as well as SGLT1 and GLUT2 protein expression did not differ with regard to SD or FD feeding.

In conclusion, intake of increased amounts of fat in high-yielding dairy cows affected dry matter intake and milk production as well as metabolic traits related to fat metabolism. However, expression of glucose transporters in the small intestine did not differ between SD and FD feeding. This study was partly supported by a grant of the Deutsche Forschungsgemeinschaft.

4.15 EVALUATION OF FRUCTOSAMINE AS A TOOL TO ASSESS GLUCOSE LEVELS IN DAIRY COWS

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Inadequate glucose metabolism may negatively affect dairy cow health and performance. Many factors, including feeding strategies and different stressors, may have an acute influence on the plasma glucose level, which reduces its value as an indicator of the glucose metabolism. Fructosamines are complexes produced by an irreversible, nonenzymatic glycosylation of proteins. Serum fructosamine concentrations depend on glucose and protein concentrations and provide a retrospective record of blood glucose levels during the previous one to three weeks. It has been proven useful in monitoring glucose levels in humans and in monogastric animals with diabetes, but there is limited knowledge about its usefulness in dairy cows.

The aim of this study was thus to investigate if serum fructosamine might serve as a predictor of previous plasma glucose levels in dairy cows. Five Swedish dairy herds with 150 to 300 cows and a milk production over 9500 kg ECM per cow and year were studied. Dry cows within four weeks ante partum (ap) were clinically examined and blood sampled. The cows were examined and sampled again at 0-3, 4-6, and 7-9 weeks post partum (pp). In total 88 clinically healthy cows (11-21/ herd), sampled on at least 2 occasions 12-30 days apart (n=215) were included in the study. Glucose, fructosamine and total protein were analysed with standard laboratory methods. Glucose had a mean of 3.2 mmol/L (SD 0.45, range 2.0-4.5), fructosamine had a mean of 235 micromol/L (SD 23.5, range 177-297) and total protein had a mean of 76.4 g/L (SD 7.5, range 60-100). A mixed linear regression model was used to assess how the preceding glucose level affected the fructosamine level at day of sampling. Explanatory variables were, in addition to glucose, number of days between glucose and fructosamine sampling (classified as 12-17, 18-23 and 24-30 days), week in relation to parturition at fructosamine sampling (4 w ap to 9 w pp), total protein at glucose sampling and the interaction between glucose and days between sampling. The repeated tests within animals and herds were accounted for. The fructosamine level was significantly associated with the preceding glucose level, where an increase in glucose with 1.0 mmol/L corresponded to an increase in fructosamine with 8.4 micromol/L (p=0.01). However, the interaction between glucose and days between samples was not significant, i.e. the association between glucose and fructosamine was the same irrespective of days between samples. Furthermore, total protein did not significantly affect the concentration of fructosamine. More research is needed to assess the usefulness of fructosamine to determine glucose levels in practice.

4.16 THE EFFECTS OF PER ORAL ADMINISTRATION OF DIFFERENT FORM OF GLYCEROL ADDITIVES ON SELECTED METABOLIC PARAMETERS AND MILK PRODUCTION OF DAIRY COWS

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Glycerol as a glucoplastic substance is used for the prevention of nutritional deficiencies in dairy cows, predominantly during transition period till the top of lactation. The prepartum depression of intake and slow rising of appetite result in negative energetic balance of dairy cows. This period is characterized by decreased concentration of glucose in blood and mobilization of body fat reserves which are reduced to NEFA (nonesterified fatty acids) and glycerol. Also there are changes in the levels of acetic acid as a precursor for fat synthesis, levels of propionic acid as glucogenic precursor and the changes in acetate vs. propionate ratio in the rumen fluid of dairy cows. The experiment was performed on two farms with dairy cows during transition period. We studied the effects of long-term feeding of different forms of glycerol additive on prevention of lipomobilisation syndrome of dairy cows during transition period. The selected metabolic parameters in blood serum and rumen fluid, on milk production and feed intake were evaluated. The liquid form of glycerol additive was poured into the feed directly whereas the glycerol supplement in powder form was admixed with diet. We found antilipolytic, hepatoprotective, glucoplastic properties and the benefit effects on health of cows (significantly reduced negative energetic balance, increased feed intake) as well as on quantitative and qualitative parameters of milk production.

In conclusion, the both forms of glycerol additive had similar positive effect on the selected metabolic parameters in blood serum and rumen fluid, on milk production and feed intake.

Key words: glycerol, dairy cows, prevention of lipomobilisation syndrome

4.17 PERFORMANCE OF PERIPARTURIENT DAIRY COWS FED EITHER ALFALFA HAY OR PEANUT HAY IN TOTAL MIXED RATION

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Forty Holstein Friesian cows in the dry period were used to evaluate alfalfa hay-base (ALF) and peanut hay-base (PNT) total mixed rations on performance in periparturient period. Twenty cows were fed ALF diet (16.5% CP) and 20 cows were fed PNT diet (19% CP). All cows were drenched once daily with propylene glycol as early as 1 wk prior to anticipated calving date until 7 d after calving. At - 2, 1, 2, 3 and 4 wk from parturition, blood samples were collected for determination of serum glucose, non-esterified fatty acid, 3-hydroxybutyrate, and urea nitrogen concentrations. Milk yields were recorded daily, and milk samples were collected twice a week to determine urea nitrogen concentration and milk compositions. During the 4 wk postpartum, daily dry matter intake of cows in both groups did not differ. Serum glucose, non-esterified fatty acid, 3-hydroxybutyrate, and urea nitrogen concentrations did not differ between the two groups at any sampling times. After calving, decreased glucose, increased non-esterified fatty acid and increased 3-hydroxybutyrate concentrations in the blood indicated cows in both groups entered some degrees of negative energy balance. Serum urea nitrogen concentrations did not change during the sampling period. Cows fed PNT diet seemed to have higher urea nitrogen concentrations in the milk than cows fed ALF diet. Average milk production during the 30 days postpartum was greater for cows fed ALF diet than for cows fed PNT diet. Milk compositions did not differ between groups. Although average days from calving to first service did not differ between groups, cows fed ALF diet had better conception rate at first service than cows fed PNT diet.

In conclusion, propylene glycol could improve negative energy balance in cows fed ALF diet (16.5% CP) and in cows fed PNT diet (19% CP). Cows fed ALF diet improved milk yield and conception rate. In Thailand, replacing PNT hay with ALF hay in total mixed ration would, however, depend on the economic analysis due to the cost of importation of ALF hay into the country.

4.18 THERMAL BARLEY PROCESSING MINIMISES THE RISK OF INTESTINAL STARCH FERMENTATION, BUT INCREASES BLOOD GLUCOSE AND INSULIN RESPONSES IN HEALTHY HORSES

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In humans it is postulated that the intake of carbohydrates which produce high glycaemic responses and consequently pronounced insulin responses are associated with a higher risk for obesity and diabetes type 2. However, in horses there is considerable interest to increase starch availability in the small intestine to avoid starch overflow in the hindgut as amylolytic capacity of starch degradation is very limited. In that context a starch overload in the hindgut is supposed to be one of the main trigger for laminitis and other gastrointestinal disturbances.

The objective of the present study was to evaluate the effects of thermal barley processing on the glycaemic and insulinaemic responses of horses to predict pre-caecal starch digestibility; postprandial breath hydrogen and methane excretion were measured as an aid to detecting microbial fermentation of starch. Four horses were randomly fed a diet containing rolled, micronised or extruded barley; the barley intake was adjusted to supply 2 g starch/kgM/day. During a ten day acclimatization period the horses were also fed 1 kg grass hay/100 kgM/day; blood and breath were collected at the end of each period after the test meal of barley was fed following a 12h overnight fast. The extruded barley caused the highest serum glucose and serum insulin peaks and AUCs, a reduced response was measured for the micronised barley and the lowest serum glucose and serum insulin values were measured after the meal of rolled barley (Treatment $p < 0.05$). Breath hydrogen increased within 240 min of feeding all barley diets (Time $p < 0.05$). The micronised and extruded barley produced the numerically lowest hydrogen peaks and AUCs in comparison to rolled barley (Treatment NS). These changes in breath hydrogen showed that there was some pre-caecal fermentation of starch. Breath methane values were very variable and, although there were no significant differences, there was a trend for higher methane values following the feeding of rolled barley. Thermal processing of barley, especially extrusion, appeared to increase glucose availability from starch in the small intestine of the horses. However, based on changes in breath hydrogen and methane measurements, it would seem that some starch was still fermented in spite of the barley having been processed. Although starch intake was moderately high, an excessive thermal disruption of starch does not completely exclude a starch flow into the large intestine hereby initiating bacterial starch breakdown, predisposing to the risk for gastrointestinal disturbances. The approach to avoid a starch overflow in the large intestine demand those grain processing techniques which minimises the risk to a maximum, taking higher blood glucose and insulin responses into account.

4.19 RELATIONSHIP BETWEEN NDF:STARCH RATIO OF DIET AND RUMEN FLUID COMPOSITION IN BEEF STEERS

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The relationship between management and feed intake and their influence on ruminal fluid characteristics in beef steers (in particular on the onset of ruminal acidosis) remains unclear.

The objective of this experiment was to determine whether increasing the dietary neutral detergent fiber (NDF):starch ratio would affect ruminal fermentation in beef steers. 4 groups of Charolaise beef steers (500 Kg of B.W.) with different diets were studied; each group was composed of 12 animals. All diets had 41.0% dry matter (DM) but the concentration of starch varied from 22.0 to 29.0% and the NDF:starch ratios were 1.0, 1.25, 1.45 and 1.75. Ruminal fluid was obtained by rumenocentesis without sedation, using a 13G 105 mm needle. Rumenocentesis was chosen because it is the most used technique providing the most accurate results. Ruminal pH was determined immediately after sampling using a portable pHmeter, concentrations of volatile fatty acids (VFA) in ruminal fluid were determined on samples after storage by high performance liquid chromatography (HPLC).

Results were subject to ANOVA and correlation analysis using SIGMA STAT 2.03. Feed intake tended to increase as the NDF:starch ratio increased. Ruminal pH, increased linearly (5.72, 6.14, 6.32, 6.41) as the NDF:starch ratio increased, while the concentration of total VFA decreased (140.80, 130.46, 123.46, 111.54) and the VFA profile was altered by diet. In particular, absolute values of propionic acid decreased and absolute values of acetic acid as the NDF:starch ratio increased. Statistical difference were also recorded on absolute values of valerate acid, that linearly increased linearly (3.38, 3.77, 4.54, 5.20) as the NDF: starch ratio (and ruminal pH) decreased.

Therefore, while on dairy cows ruminal pH could be influenced by many factors (feed intake but also pregnancy, lactation, dry period, management), this study suggests that, on beef steers, there is a better linear correlation between feed intake and ruminal fluid characteristics and particularly regarding the onset of ruminal acidosis.

4.20 THE COMPARISON OF FEEDING PROPYLENE GLYCOL AND GLYCEROL TO FRESH COWS: EFFECTS ON BLOOD METABOLITES AND RUMEN FERMENTATION

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The aim of our study was to make comparison of feeding propylene glycol and glycerol to fresh high producing dairy cows. We follow the effect on blood and rumen metabolites, health condition and efficiency.

Material and methods: The experiment was conducted in a herd of Holstein cattle with a mean milk yield of 11 000 l. Twenty four dairy cows were divided into control (P, n=12) and experimental (G; n = 12) groups. Daily ration was the same for all cows. Dairy cows from group P received immediately after the delivery 300 g of propylene glycol as a drench and than every day 300 g of propylene glycol divided in two doses with which TMR was watered. The group G received 500 g of glycerol in the same manner. Blood samples were collected at the end of 1st, 2nd and 3rd week of lactation. Ruminal fluid was collected at the end of 3rd week by rumenocentesis. The following parameters were measured in blood serum: glucose, beta-hydroxybutyrate, nonesterified fatty acids, triacylglycerols, cholesterol, whole protein, urea, aspartate aminotransferase, gama-glutamyl transferase, total bilirubin, Na, K, Ca, P and Mg. Ruminal fluid was analysed for pH, total acidity, volatile fatty acids (acetic, propionic, butyric, valeric), lactic acid and ammonium. The clinical state of the cows was monitored daily by the veterinarian in charge of the herd.

Results: The results of the experiment showed higher occurrence of subclinical ketosis in group G than in group P (1st week 6 vs. 2 cows; 2nd week 9 vs. 6; 3rd week 7 vs. 3 cows respectively). Mean concentration of beta-hydroxybutyrate in 1st and 3rd weeks was significantly higher in group G than in group P (1st week 1.18 ± 0.49 vs. 0.80 ± 0.35 mmol/l, $p < 0.05$; 3rd week 1.07 ± 0.45 vs. 0.71 ± 0.15 mmol/l, $p < 0.05$). Mean concentration of glucose was in G and P groups lower than 3 mmol/l (1st week 2.86 vs. 2.98 mmol/l; 2nd week 2.70 vs. 2.79 mmol/l; 3rd week 2.74 vs. 2.84 mmol/l, respectively), but the differences as well as at other monitored parameters were not statistically significant. Contrary of the published dates, that glycerol increased the amount of butyric and propionic acid in rumen juice, in our experiment we didnt' found statistically significant differences. The mean concentration of volatile fatty acid in G and P groups were for total volatile fatty acid 111.33 vs. 111.04 mmol/l, acetic acid 68.80 vs. 69.08 mmol/l, propionic acid 25.64 vs. 26.21 mmol/l, butyric acid 13.63 vs. 12.89 mmol/l and valeric acid 3.25 vs. 3.27 mmol/l.

Conclusion: On the basis of our result was application of 300 g of propylene glycol more effective in prevention of subclinical ketosis than 500 g of glycerol. The work was realized with the support of MSM Project No. 6215712402.

4.21 EFFECT OF DIFFERENT DIETARY FAT SUPPLEMENTATIONS ON LIVER LIPID AND GLYCOGEN OF HIGH YIELDING DAIRY COWS AROUND CALVING

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Effects of two types of protected fat were studied on the liver lipids and glycogen of high yielding dairy cows. Cows were allocated into three homogeneous groups considering the age, body condition, number of previous calving and milk production. Cows of each group (n=10) were fed on a corn silage base diet either without fat supplementation (control) or with 11.75 MJ NEL calcium soaps of palm oil fatty acids (CAS) or hydrogenated triglyceride (HTG). Experimental diets were individually calculated for the cows from 21±3 day (d) prior to expected calving up to d 100±5 postpartum. At d 14 prepartum and d 5 and 25 postpartum liver samples were collected by percutaneous biopsy. At d 14 prepartum and d 5, 25 and 100 postpartum blood samples also were taken. Total lipid (TL), fatty acid composition and glycogen of liver tissues were determined. Blood was analyzed for triglyceride, free fatty acid, cholesterol, AST, beta-OH butyrate, insulin and ammonia. No significant ($P > 0.05$) differences were detected in the liver fat content among the groups at d 14 prepartum or d 25 postpartum. However, at d 5 postpartum both control and CAS cows had higher liver lipid and lower glycogen concentrations ($P < 0.05$) than cows in the HTG group. Glycogen concentration slightly decreased in the liver of each treatment from d 14 prepartum to d 5 postpartum, however, this decrease was more intensive in both control and CAS groups than in the HTG group. The variations in the liver lipid concentrations accompanied by significant changes in the proportions of C16:0, C16:1n-7, C18:0, C18:1n-9, C18:2n-6 and C20:4n-6 fatty acids in the liver lipids. At d 14 prepartum CAS group had higher blood ammonia concentration than cows in the HTG group. Triglyceride concentrations of the CAS and control groups decreased in blood from d 14 prepartum to d 5 postpartum, while increased in the HTG group during this period. At d 25 postpartum cows in the control and HTG group had significantly lower ($P < 0.05$) blood cholesterol concentration than cows in the CAS group. At the same sampling time both groups fed any of the fat supplemented diets had lower beta-OH butyrate and glucose concentrations than the control group ($P < 0.05$). At d 100 postpartum both fat supplemented groups had lower ($P < 0.05$) AST activity and free fatty acid concentration than the control group, while the cholesterol concentration of the HTG and the control groups was shown significantly ($P < 0.05$) lower level than that of the CAS-treated cows at this sampling time. No significant differences were found in the insulin concentrations among the groups or sampling times.

These results show that HTG supplementation provided more advantageous effects on liver lipid and glycogen metabolism than CAS.

4.22 EFFECT OF RUMEN PROTECTED CHOLINE SUPPLEMENTATION ON LIVER METABOLISM IN PERIPARTURIENT DAIRY COWS

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Remarkable changes take place in the metabolism of dairy cows during the periparturient period. The metabolism of liver is disturbed around calving, which leads to fat accumulation in the liver and development of various metabolic disorders.

The aim of this experiment was to study the effect of rumen protected choline (RPC) supplementation on metabolism during the periparturient period. Thirty high producing multiparous Holstein cows were paired by parity, body condition score and previous lactation performance and randomly assigned to one of two groups. Cows were fed 0 (control) or 100 g/d RPC (RPC group, equivalent to 25 g/d choline chloride) from an average of 21 d prepartum and 0 or 200 g/d RPC (equivalent to 50 g/d choline chloride) from calving to 60 d postpartum. RPC was a fat encapsulated product therefore hydrogenated palm oil was used to equalize fat intake in the control diet. All cows were offered a TMR of identical ingredient composition (corn silage 300 g/kg DM, alfalfa hay 160 g/kg DM, wet brewers grain 50 g/kg DM, corn, soybean meal based concentrate 490 g/kg DM). Body condition (BCS) was scored and liver biopsies were taken at -21, 7, 35 and 60 d relative to calving and milk samples were collected on d 7, 35 and 60 postpartum for measurement of choline concentration. BCS and BCS change did not differ between treatments. Total lipid content of the liver varied considerably between cows but was significantly ($P < 0.05$) lower in RPC group (control vs. RPC, g/kg wet weight: 145.8 vs 81.7 (day 7 after parturition), 84.9 vs. 49.6 (day 35)). Glycogen concentration differed significantly only on 35 d sampling; it was higher in the RPC group (control 27.9 vs. RPC 35.8 g/kg wet weight). Milk total choline concentration was significantly ($P < 0.01$) higher in RPC group (control vs. RPC, mg/kg milk: 86.2, 109.4; 97.1, 143.0; 115.3, 137.2; for days 7, 35 and 60, respectively), which proves the better choline supply of RPC supplemented cows. Milk choline yield was significantly ($P < 0.01$) higher in RPC group (control vs. RPC, g/d: 2.36, 3.86; 3.87, 6.45; 4.65, 6.14 for days 7, 35 and 60, respectively) as a consequence of higher choline concentration and higher milk yield of choline supplemented cows.

Feeding RPC during the periparturient period had a positive effect on liver metabolism as evidenced by lower total lipid and higher glycogen content.

4.23 THE INFLUENCE OF TRANSPORTATION STRESS ON THE HAEMATOLOGICAL AND BIOCHEMICAL RESULTS IN PREGNANT HEIFERS

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The aim of the study was to investigate the influence of a 24-hour-transportation (without a rest period, with limited possibilities of feeding and watering) on the physiological parameters of blood and duration of stress reaction of cows. The experiment was carried out in November 2006, in a group of 7 imported pregnant Holstein-Friesian heifers. Clinical examination of cattle and blood samples were collected 4 times: just before loading off the track (D0); 84 hours (D4), 164 hours (D7) and 28 days (D28) after arrival in loose system cattle-shed. Blood was analysed for haematological and biochemical results. Data were statistically analysed by SPSS 11.5. The most important results of clinical examinations were light diarrhoea in one to three heifers all the time of experiment, and increase of the body temperature in all 7 cows 39.0C-39.7C in the period D0-D7. On D28 the body temperature was 38.4C-39.0C. Stress reaction of heifers manifested on D0 by neutrophilia, lymphopenia, monocytosis and with high concentration of glucose and cortisol. Signs of dehydration were detected (D0-D7) - an enhanced level of haematocrit (Hct), haemoglobin (Hb), red blood cell count (RBC), which started to fall significantly ($p < 0.05$) after D4, but high concentration of total protein (TP) and albumin (Alb) started to lower after D0 ($p < 0.001$). There was high correlation between these parameters on D0 and D4 ($r > 0.750$, $p < 0.05$). Simultaneously there was signs of haemolysis because AST had a high correlation ($r > 0.800$, $p < 0.05$) with concentrations of Hct, Hb, potassium (K), TP on D0 and D4. At the state of underfeeding the highest concentration of total bilirubin (TB) and direct bilirubin (DB) was on D0, but the lowest level of urea nitrogen was on D4. The stress provoked unappetite continued till D4, when indirect bilirubin had high correlation with cortisol concentration ($r = 0.770$, $p = 0.04$), and TB with DB ($r = 0.886$, $p = 0.008$). Catabolism of muscle tissue associated with the increase of creatine kinase (CK) 2-8 times on D0 over basic level of CK on D7. The increase of CK on D4 was only for 7-70 U/l more than on D7. There was high correlation between CK and AST on D28 ($r = 0.837$, $p = 0.02$), only. The biggest changes in concentrations in electrolytes detected on D4 (Na, Cl, Mg - the lowest and K, Ca, P - the highest level in 28 days. Only the concentration of Na fell out of reference value on D4 (131.14 ± 0.99 mmol/l) and continued to go down till D28. There was an inadequate supply of NaCl in feed. Finally, a 24-hour-transportation of pregnant heifers caused physiological stress reaction to 7 days after arrival in a new place of loose system shed.

4.24 USE OF SILYMARINE FROM SEEDS OF ST. MARY'S THISTLE (SILYBUM MARIANUM, L. GAERTN.) TO PREVENT HEPATIC STEATOSIS IN POSTPARTURIENT DAIRY COWS

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The aim of this experiment was to evaluate the effect of native substances pressed from seeds of St. Mary's thistle to prevent hepatic steatosis. A total of 24 dairy cows were used for the experiment from parturition up to 100 days post partum. Cows were divided into one control (C) and two experimental (E1, E2) groups of 8 animals and provided with a standard feeding ration (SFR) and a production feeding ration (PFR). Control cows were given extra 0.6 kg of PFR for 60 days following parturition, E1 cows received 0.3 kg of PFR plus 0.3 kg of native substances pressed from seeds of St. Mary's thistle (PSMT) and E2 cows were given 0.6 kg of (PSMT). SFR was supplied ad libitum, while PFR was dosed for the production of 10 l of milk and over as 0.5 kg/l. There were 41 g of silymarine per 1 kg of PSMT. Repeated examinations of biochemistry profiles of cows on days 10, 26 and 58 post partum resulted in finding lower levels of acetone, albumin, total cholesterol (TCH), free fatty acids(FFA) and aspartate aminotransferase (AST) in E1 and E2 cows as compared with control ones. Considering control cows on day 10 after parturition, the ratio of FFA/triglycerides (TG) was equal to 2.0 and the ratio of FFA/TCH was nearly 0.2, i.e., results characteristic for cows with higher fat content in the liver during this period. The above ratios were lower in E1 and E2 cows. There was a significant difference in FFA/TCH of control and E2 cows ($P < 0.05$). The difference in FFA/TG of control and E1 as well as E2 cows was even more pronounced ($P < 0.01$). There were higher milk yields in experimental cows E1 and E2 in comparison with control ones. The difference between control and E2 groups on days 58 and 98 of lactation was of statistical significance ($P < 0.05$). E1 and E2 cows produced milk with higher fat content influencing thus the production when converted for FCM(Fat corrected milk). Differences were of statistical significance on days 26, 58 and 98 of lactation comparing control and E1 groups ($P < 0.01$) and on day 58 only comparing E1 and E2 groups ($P < 0.05$). No changes in the milk protein content were found. The content of fat in the liver tissue determined on a semiquantitative basis from histological specimens on day 10 after parturition was by 38.8 and 44.4% lower in E1 and E2 cows, respectively, when compared with control cows. The study was supported by the Research project of Ministry of Education, Youth and Sports No. MSM6215712403.

4.25 ENERGY SUPPLY OF 10 HERDS OF HIGH YIELDING DAIRY CATTLE IN EARLY LACTATION PERIOD

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Problem: Against the background of increasing milk yield the situation is not satisfying regarding fertility and health of dairy cattle. This problem is exemplified by reproduction rate >40% and an average 'inter calving period' of 412 days.

Material: 10 herds of Holstein cattle have been investigated within a 3-year-period. These herds had an annual calving rate of approx. 8000 and a herd milk yield between 9000-11 000 kg. Beside other parameters the energy supply status of dairy cattle was determined in the early lactation period as follows. For period of four weeks the back fat thickness (BFT) and milk parameters were measured once a week. On the basis of blood and urine samples of ante partum, post partum and lactating cows the metabolism parameters were analyzed by random test methods as well.

Results: In the month before calving the mean average of BFT was between 13.4 mm and 22.7 mm. Four month after calving BFT decreased to a value between 9.4 mm - 6.1 mm. Thus the BFT of the average cow decreased up to a max. 0.25 mm per day. To estimate the required amount of energy the results of the monthly MLP were considered. If milk protein content is below 3.2% one can speak of an undersupply of energy. A surplus of energy is indicated by milk protein content over 3.8%. In the second month of lactation period 46-90% of heifers and 54-82% of cows (more than one lactation period) had an undersupply of energy. In the 6th month of lactation period 71-89% of heifers and 58-77% of cows were supplied according to their need of energy. Based on the results of the MLP during the 10th month of lactation period 22-49% of heifers and 28-66% of cows had a surplus of energy. Quarterly 15 cows (5 ante partum, 5 post partum and 5 lactating cows) of each herd were selected over a period of two years. Blood and urine samples were taken from these cows and tested for various metabolic parameters. To evaluate these parameters limits of LUA Sachsen were used.

Conclusion: It can be pointed out that the vast majority of selected cows show clear signs of a nutrition deficiency during the early lactation period. Negative effects on fertility and health have to be expected. BFT= back fat thickness

4.26 THE EFFECT OF ANIONIC SALTS FEEDING DURING DRY PERIOD ON HEALTH AND METABOLIC PARAMETERS OF DAIRY COWS

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Milk fever is one of the most important metabolic diseases in dairy cows that sustain a great cost on the dairy cattle industry and has a lot of secondary complications even in treated cows because of this, investigators struggle to find the way of prevention of milk fever. Using anionic salts in pre partum ration of dairy cows is the most customary and useful method, but the effect of these salts on metabolic parameters and other production diseases remain a matter of debate. The objective of this study was to estimate the effect of pre partum feeding anionic-supplemented ration on occurrence of milk fever and other calving related diseases, and also to determine the effect of anionic salts on blood metabolites. Sixty cows were selected and randomly divided into two groups during a 9 month period in a commercial dairy farm in suburb of Mashhad. Thirty cows were received anionic ration for 21 days to parturition and the other group (no=30) were fed usual dry period ration. Blood and urine samples were collected from each cow three times at 21 days before parturition and on postpartum days of 3 and 21. Calcium, inorganic phosphorus, magnesium, chloride, cholesterol, urea, creatinine, total protein, albumin, sodium, potassium, NEFA, BHB, glucose, and AST were measured in the serum using automated biochemical analyzer. Blood pH and bicarbonate were measured using blood gas analyzer within 1 hour after sampling. The results indicated that the serum calcium, albumin concentrations were higher in treatment than control group and AST activity were lower in treatment than control group at day 3 post-partum. At day 21 after calving creatinine concentrations and blood pH were lower in cows received anionic salts than control group. There was not significant difference of BHB, NEFA and glucose between 2 groups. Significant time related changes (time of sampling) were observed on blood calcium, magnesium, sodium, chloride, cholesterol, urea, creatinine, total protein, albumin, glucose, BHB, NEFA, AST, pH. In this study, the probability of culling was significantly ($P < 0.05$) increased in control cows in comparison to treatment group and the risk of milk fever tended to be higher in anionic supplemented cows ($P = 0.08$).

The results of this study showed, with using anionic salts pre-partum, calcium balance and metabolism could be improved and the probability of milk fever occurrence and culling rate were decreased.

4.27 ANTIOXIDANT AND SELENIUM STATUS OF SHEEP FED DIET SUPPLEMENTED WITH SODIUM SELENITE

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In this experiment was investigated the effect of supplementation of sodium selenite on superoxide dismutase (SOD) in erythrocytes, glutathione peroxidase (GPx), malondialdehyde (MDA) and Se concentration in plasma, blood and tissues. Ten animals were randomly divided into two groups and were fed with experimental or basal diet (BD) for 5 months. Control group of sheeps received BD providing a daily intake of Se 42,4 µg only. The diet for experimental group consisted of BD enriched with Se 0,3 mg.kg⁻¹ of DM in the form of Na₂SeO₃ and provided total daily intake 270,4 µg of Se per head. Elevation of Se concentrations in whole blood and tissues as well as increases of the activities of GPx in blood and tissues were highly significant in group of sheep fed diet supplemented with sodium selenite. Interestingly, the concentration of Se in muscle was significantly increased in this group of animals too. Activity of SOD in erythrocytes as well as the contents of MDA in tissues were significantly reduced in animals fed diet supplemented with Se. The concentration of Se was significantly increased in bacterial and protozoal fraction of rumen fluid. The results suggest on the important role of rumen microflora in the creation of selenomethionine from sodium selenite which is the only form of Se able to build significant body deposits. The benefit effects of sodium selenite supplementation on the maintainance of antioxidant and selenium status of sheep were demonstrated.

4.28 MYCOTOXIN SCREENING IN HEALTHY COWS AND COWS WITH ABOMASAL DISPLACEMENT

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The aim of this study was to examine samples of fodder, blood, milk and bile for concentration of deoxynivalenol (DON), Zearalenone (ZON) and their metabolites de-epoxy-DON, a-zearalenol(ZOL), β -ZOL, zearalanon (ZAN), α -Zearalanol(ZAL) sowie β -ZAL. A second aim was to study possible relations between the Toxin arrears and diseases in 61 ill and 13 healthy milk cows. All examined cows had abomasal displacement. A high number of accompanying diseases (endometritis, laminitis, mastitis, peritonitis) was determined. Of the patients without mycotoxins 28 (77,8%) were cured and 8 died. From those with mycotoxins 18 (72%) were cured without clinical reactions; 7 had to be euthanized due to severe accompanying diseases and severe haematological or clinical chemical deviations in the blood being typical for abomasal displacement. The physical examination of the patients with mycotoxins did not show specific effects on health; except reduced rumen contractions. ZON/ZOL associated changes in the ovaries and uterus could not be macroscopically determined. The transabdominal collection of bile by aspiration can be easily performed in sick cows because of bile accumulation. In healthy cows this is much more difficult. In healthy cows mycotoxins were not found. In the slightly contaminated fodder samples the DON concentration was 0.161 g/kg (0.086-0.191) and ZON concentration was 6.35 μ g/kg (4.88-7.85). None of the milk samples contained mycotoxins. DON (0,002 μ g/ml) was found in one of 61 serum samples, de-epoxy-DON in four sampels (8%). In bile de-epoxy-DON (37.6 μ g/mL) was found only once. DON was not present.. 39% of the examined bile samples were contaminated with ZON and/or its metabolites thereby were: ZON 9.85 ng/g (8.10-16.33), α -ZOL: 59.9 ng/g (5-78) and β -ZOL: 37.6 ng/g. In all cases concentrations were within the range of the detection limit and reference values. The metabolic parameters protein, albumin, urea, kreatinin and the parameter of oxidative status TEAC did not show differences between cases with or without mycotoxins. Mg, Ca, Na, K, Cl, pH, PCO₂, bilirubin, glucose, FFS and cholesterol concentrations were not in physiological ranges in all cases independed on mycotoxins. Erythrocyte, thrombocyte, and leukocyte numbers as well as the differential blood count, hematocrit and hemoglobin of all patients with or without mycotoxin did not show deviations. Serum enzyme activities (AP, GGT and CK) of all patients were within the normal ranges. However mean AST and GLDH activities of all mycotoxin positive patients were slightly increased compared to mycotoxin negative cows. We concluded that no specific clinical, hematology or clinical chemical changes for mycotoxin influences (DON, ZON) could be determined.

4.29 EFFECT OF HONEY AND PROPOLIS ON PERFORMANCE AND IMMUNOLOGICAL PARAMETERS OF BROILER CHICKS

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Public demand for safe and healthful food is at an all time high for poultry producers and consumers however, food safety issues present a challenge, pairing healthy birds without drugs and remaining economically viable requires finding new ways to manage disease. Three studies were conducted to investigate the effect of some honey bee products such as (honey and propolis) on immune response of broilers. The first study involved one day broiler chicks received honey in their water at different levels (0, 2, 4 and 6 %). The second study involved one day broiler chicks received propolis at four levels (0, 0.5, 1.0 and 1.5) in their diet. The third study was conducted to investigate the interaction effect between honey and propolis at the same levels. The performance and immunological measurements included, the growth, F.E., mortality, F. I., the lymphoid organs weights relative to live body weight, white blood cell count and titration of humeral immunity (antibodies) against vaccines.

The results of the present study have shown that propolis and honey levels in seed and water of broiler chicks had a beneficial influence on immune system response. Propolis and honey did not improve the productive performance but shown a better health improvement to the broiler chicks.

4.30 UTILIZATION OF BIOCHEMICAL ANALYSES FOR DETERMINATION OF METABOLIC PROFILE OF FARMED ELAND (TAUROTRAGUS ORYX) ON EXPERIMENTAL FARM OF CULS PRAGUE IN LÁNY

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The Institute of Tropics and Subtropics of the University of Life Sciences in Prague (CULS) in cooperation with the school farm at Lány established an experimental farm rearing eland (*Taurotragus oryx*).

The aim of the projects is to use modern methods of determining metabolic profiles of farm and wild animals for the monitoring of their health and effects of nutrition and of external conditions on their welfare. 19 samples of eland (*Taurotragus oryx*) blood were collected by a veterinary surgeon on a farm at Březová. Blood samples were taken as part of health checks before the elands were transported to their new school farm paddocks at Lány. When they were caught and immobilized, the elands were tagged and zootechnical and veterinary registrations were performed. Blood samples were processed and analyzed by using IDEXX Laboratoires mobile machines. Blood serum samples (collected in Lithium Heparin) were analyzed using VetTest analyzer for 13 biochemical parameters; QBC VetAutoread – 10 haematological parameters and VetStat – 8 blood gasses and electrolytes were analyzed. The results obtained were compared with the horse and the cow reference ranges. Biochemical parameters, glucose and creatine kinase values were generally higher than their physiological levels (GLU averaged 10.2 mmol/l and CK 791 mmol/l), calcium values were lower (Ca averaged 2 mmol/l), while levels of albumin, alkaline phosphatase, creatinine, magnesium and urea were within their standard ranges. Haematological tests of samples returned varying values within the reference ranges. The hematocrit, haemoglobin and the lymphocytes/monocytes ratios were generally near the low limit of the standard. Blood gasses and electrolytes, pH and K⁺ values were generally much higher than their physiological levels (pH averaged 7.9 and K⁺ 6.7 mmol/l), pCO₂ values were much lower (pCO₂ averaged 16.8 mm Hg), while levels of HCO₃⁻, Na⁻ and Cl⁻ were within their standard ranges. Also we analysed the effect of eland's age and sex on all parameters values by General Linear Mixed Model using the SAS System V 9.1. We found only the effect of age on creatinine to be lower in young animals (F(4,13)= 5.1, P < 0.01; range= 100 – 165 μmol/l, mean= 142 μmol/l). It follows from the results obtained that the elands were under severe stress before immobilisation – run breathing deeply as demonstrated by high levels of creatine kinase, pH, K⁺ or glucose and, on the other hand, very low pCO₂ levels. Breeders should provide higher calcium intake for the monitored animals. Based on the values found, the elands can be considered healthy.

There were preliminary priority results and the other investigations are running. This project was supported by grant No. 51120/1161/1603 FRVŠ Czech Republic.

4.31 VARIATIONS IN BLOOD ACID-BASE STATUS IN FINISHING BULL CALVES FED DIFFERENT HIGH-GRAIN DIETS

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Introduction: Cereal grains are the principal energy source in feedlot cattle diets. Nevertheless, these diets often induce unstable fermentation conditions that favour the accumulation of acids in the rumen and lead to ruminal dysfunction.

The aim of the present study was to evaluate the effects of high-grain diets on acid-base balance and productive performance in cattle maintained in a commercial feedlot system, taking into account the finishing period.

Material and methods: Thirty bull calves were utilized for an 80-day feedlot study. Animals were allotted randomly to one of three experimental groups: 1) corn-based diet (n=10), 2) an equal mixture of corn and barley (n=10), and 3) barley-based diet (n=10). Blood pH, pCO₂, HCO₃⁻ and serum L-lactate were determined. Productive data (average daily gain, feed intake and feed:gain ratio) were also evaluated as complementary evaluation.

Results: Significant differences were detected in performance data depending on the type of ration: although animals fed a corn-based diet reached the highest final weight, a barley-based diet may have important advantages. Animals fed a mixed grains (barley and corn) can be considered as an intermediate group. Only significant effects of time were found on blood pCO₂ and HCO₃⁻ but not of treatment or T*TR interaction. Blood pH was not significantly affected by time, treatment or T*TR interaction throughout the study. In addition, serum L-lactate were under significant effects of time and treatment, but not T*TR interaction.

We conclude: that under the conditions of the present study none of the three diets used significantly increases the risk of acid overload associated with high grain consumption, probably because of the buffering action of the crude protein content of the ration. The equal mixture of corn and barley gave worse results, both in terms of productivity and metabolic indicators, than the corn-based and barley-based diets. Additionally, diets mainly composed of corn and with high crude protein content, supplementation with bicarbonate may cause blood alkalization.

4.32 ENERGY BALANCE OF PRIMIPAROUS ESTONIAN HOLSTEIN, ESTONIAN RED HOLSTEIN AND ESTONIAN RED COWS

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High producing dairy cows tend to be in negative energy balance during their first weeks of lactation whereby severe and prolonged negative energy balance (NEB) leads to compromised animal health and production.

The objective of the study was to compare energy balance parameters during the first 150 days in milk of primiparous Estonian Holstein (EH), Estonian Red Holstein (ERH) and Estonian Red (ER) cows. EH (n=86), ERH (n=19) and ER (n=31) cows kept from 2000 till 2005 in the 80-head tie-stall Põlula Experimental farm were milked three times and fed the same total mixed ration (TMR) ad libitum twice a day. One kg of TMR dry matter contained metabolizable energy and crude protein from 2 weeks before calving till the 13th day of lactation 11.3...11.5 MJ and 160...170 g, from the 14th till the 150th day of lactation 11.5...12.0 MJ and 170...180 g respectively. Milk recording together with composition analyses was performed and feed intake measured twice per month, the cows were weighed once a month. Metabolizable energy intake was calculated based on every cow's intake and feed analyses data. Legendre polynomial of 3rd order with random coefficients was used to model everyday energy intake, body weight and ECM production data for each cow. Based on these predicted data the energy balance for 150 days in milk was calculated and NEB nadir, duration, and total energy deficit recorded. General linear model was used to study the effect of breed and the effect of breeding value within the breed on the recorded negative energy balance characteristics considering the year of experiment as confounding factor. Energy balance of none EH and ERH but three ER (9.7%) cows was positive during the 150 days in milk; on average the cows returned to positive energy balance at day 45, NEB nadir was 71 MJ and total energy deficit 1400 MJ. Breed was related to NEB nadir and total energy deficit: in EHF cows the nadir was deepest and deficit largest. Breeding value had correlation with NEB duration: in all the breeds the cows with higher breeding value had longer NEB period. The year of the experiment was related to all the studied NEB characteristics.

In conclusion, the energy balance pattern differs between EH and ER dairy cows, in addition to breed and breeding value environmental factors are important contributors to energy balance characteristics. The study was supported by Estonian Science Foundation (Grant 5422)

5 TRANSITION COW BIOLOGY AND MANAGEMENT

5.1 HIGH FORAGE OR HIGH GRAIN FOR DRY COWS: WHAT IS BEST FOR ANIMAL HEALTH AND PRODUCTION?

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Introduction

For the past decade, there has been considerable controversy in the US regarding how dry cows should be fed. For several decades, it was recommended to feed far-off dry cows a high-fiber, low-energy diet and increase concentrate (grain) feeding during the final three weeks prior to calving. In the US, we often say that we are “steaming up” the cow prior to calving or that we should be feeding a “steam up” diet prior to calving. The origin of the term is attributed to Robert Boutflour who at the World Dairy Congress (1928) first proposed the “steam up” ration as a way to circumvent “the neglect of the preparation of the cows for her lactation period”. The term was meant to be an analogy to the preparation of a steam thresher. Essentially, the logic behind this feeding strategy was to adapt rumen microorganisms to higher grain diets that would be encountered by the cow following parturition. By following this practice, it was believed that fresh cows would be less likely to go off feed or experience ruminal acidosis. Over the next decades, other reasons were put forth for steaming cows up prior to calving. These included: maximization of dry matter intake (DMI), provision of more propionate to support gluconeogenesis and decrease fat mobilization from adipose tissue, and increasing rumen papillae length to increase volatile fatty acid absorption from the rumen. However, today, many nutrition consultants and some scientists are suggesting to continue feeding high-fiber diets during the pre-fresh transition period. This manuscript will examine the data to support or refute the feeding of additional concentrate during the final weeks prior to freshening. Briefly, nutrition of the far-off dry cow will be discussed. Recently, a high-straw, low-energy “Goldilocks” diet which is “just right” for far-off dry cows has been promoted (Drackley and Janovick Guretzky, 2007a,b). We will discuss that concept in relationship to a recent study we conducted.

Pre-fresh Transition Cows-Justifications for Increasing Grain Feeding

Microbial Adaptation?

Amazingly, there is no research that describes the changes in microbial populations in the rumen as a cow progresses through the transition period or how those changes may

be influenced by diet. This information is desperately needed. Therefore, the concept of feeding additional grain prior to calving to facilitate microbial adaptation to high starch diets can be challenged merely because of the lack of data to support doing it. There is data from feedlot beef cattle and to a lesser degree from lactating dairy cattle to suggest that three weeks are needed for microbial populations to reach steady state after abrupt diet changes. But is it essential to facilitate adaptation to high concentrate diets prior to calving? There are several lines of argument against it being necessary. First, when the concept of steaming up cows was introduced, cows were fed concentrate separate from forage. Today, most dairy producers feed a totally mixed ration (TMR). When concentrates are fed separately, the cow is allowed to consume large amounts of grain all at once. This is not the case, when feeding a TMR. Feeding a TMR results in smaller amounts of grain being consumed over a longer period of time. Hence, there is less likelihood of “upsetting” the rumen microorganisms. Second, feed intake of transition cows, is inherently low. Because feed intake is low, cows are less likely to consume excessive amounts of grain and have large bursts of acid production in the rumen. Lastly, feed intake gradually increases postpartum. So in effect, cows are gradually introduced to greater grain feeding when being fed a TMR post-calving. In other words, the adaptation can take place after calving when a lactation diet is introduced. Rabelo et al., (2003) fed diets containing 1.58 or 1.70 Mcal NEI/kg DM for the final four weeks prior to calving. After calving, half of the cows in each treatment group were fed a diet containing 1.57 or 1.63 Mcal NEI/kg DM. Energy density of diets was calculated using the NRC (2001) and are influenced by level of DMI. For cows that consumed the 1.63 Mcal NEI/kg DM diet after calving, there was no benefit (as measured by ruminal pH or volatile fatty acids, udder edema, or hoof health) in consuming the 1.70 Mcal NEI/kg DM diet (vs. 1.57 Mcal NEI/kg DM) prior to calving.

Maximizing Dry Matter Intake?

For years, our research group strongly promoted high DMI by transition cows prior to calving. Numerous studies have indicated that feeding additional concentrate (higher non-fiber carbohydrate [NFC]) prior to calving increases DMI (See Table 1). There is little debate on this in the scientific community. The premise for feeding more grain prior to calving was that cows would avoid negative energy balance prior to calving and there would be less fat mobilization from adipose tissue. Less fat mobilization would result in fewer cases of fatty liver and ketosis. Although a comparison of cows that experienced feed intake depression prior to calving to those that were force fed prior to calving supported this recommendation (Bertics et al., 1992), other more traditional studies have not. Recently, we have identified two major lines of evidence that do not fit with the notion of maximizing feed intake. First, heifers consume less DMI than mature cows when expressed as a percentage of body weight, yet they have lower fat in the liver post-calving (Rabelo et al., 2005). Second, pre-fresh transition cows that are feed restricted, either by offering less feed or by decreasing grain feeding (increasing fiber content of diet) do not have greater occurrence of fatty liver or higher blood ketones (Grum et al., 1996, Rabelo et al., 2005). Since heifers and feed

restricted cows have flatter DMI curves prior to calving, we speculated that the magnitude of drop in feed intake would be more highly associated with plasma non-esterified fatty acids (NEFA) and liver triglyceride (TG) than the absolute amount of DMI. This hypothesis would fit the results of Bertics et al., (1992), because in that study not only was feed intake maximized, feed intake depression was minimized. Grummer et al. (2004) pooled data from three studies and confirmed that our hypothesis was probably correct. Consequently, the objective of dairy managers should be to prevent large declines in feed intake prior to calving. While increased grain feeding does not cause greater prepartum feed intake depression per se, it does promote greater feed intake and hence greater potential for a larger drop in feed intake. Management practices that predispose cows to go off-feed are more likely to result in greater harm to cows that are aggressively being fed grain prior to calving. These management practices include diet or pen changes close to calving, overcrowding of pre-fresh pens, or absence of heat abatement for pre-fresh cows.

Suppressing Fatty Acid Mobilization?

The major premise for maximizing DMI prior to calving is that it will improve energy balance and consequently reduce fatty acid mobilization from adipose tissue. Presumably, this occurs because greater glucose precursors (propionate) are formed during rumen fermentation and this leads to an insulin response by the cow. Insulin is antilipolytic, i.e., it suppresses fatty mobilization from adipose tissue. If cows do enter negative energy prior to calving, it is usually not extensive and only for a couple of days. Therefore, some have argued that it is not worthwhile to formulate a transition diet that is much higher in energy than is needed except for the last few days prior to calving.

In fact, two arguments have been forwarded to suggest that from a metabolic viewpoint, higher concentrate diets should be avoided. One argument states that lipid mobilization is a natural phenomenon common to all mammals and is part of an orchestrated pattern of body weight change to support lactation (Friggens et al., 2005). Furthermore, it was suggested that feeding additional concentrate will not reduce the “normal” amount of lipid that is mobilized. Considerable evidence does suggest that increasing energy density prepartum will not reduce fatty liver (see Table 1). (The counter argument is that providing supplemental glucose precursor, e.g. propylene glycol, as an oral drench does reduce fat accumulation in the liver at calving (Studer et al., 1993)).

Table 1. A summary of recent trials examining the feeding low or high NFC diets beginning at dry-off (Grum et al., 1996, Douglas et al., 2004) or 3 to 4 weeks prior to parturition (all other studies listed below). Values with a * indicate a significant difference ($P < .05$) between low and high NFC diets.

Trial	NFC, % DM	NDF, % DM	Prepartum DMI, kg/d	Postpartum DMI, kg/d	Milk Yield, kg/d	Liver TG, units vary
Grum et al., 1996	18	60	9.7	17.9	35.1	5.9
	28	50	11.6*	18.7	35.5	7.3
Minor et al., 1998	35	49	10.2			6.2
	44	30	13*			5.6
Mashek and Beede, 2000	35	39			37.4	
	38	35			37.4	
Keady et al., 2001	13	61	9.28	15.2	27.4	
	28	47	11.03*	15.1	28	
Holcomb et al., 2001	25	44	10.7	21.2	35.8	
	30	39	14.1*	20.5	29.9	
Doepel et al., 2001	24	52	13.9	13.3	30.9	9.8
	30	44	12.8	14.4	33.8	7.1*
Rabelo et al., 2003, 2005	38	40	11.3	16.2	41.4	9.2
	45	32	13.0*	16.7	39.4	8.7
Douglas et al., 2004	24	47	15.1	21.1	40.2	5.4
	31	41	13.9	20.9	40.0	7.6
Smith et al., 2005	34	44	13.8	19.2	41.8	
	40	37	13.7	18.7	40.6	

The argument that lipid mobilization is normal and good has been taken one step further with the suggestion that promotion of a moderate degree of fat mobilization prior calving is beneficial because it will “prime” the liver for the dramatic increase in plasma NEFA and liver uptake of NEFA that occurs at parturition. The support for this was a study from the University of Illinois (Grum et al., 1996) that showed feeding a diet which dramatically reduced feed intake for almost the entire dry period (except one week prior to calving) led to a moderate increase in plasma NEFA during the dry period and lower liver TG at calving. Their data suggested that this feeding regime primed the liver to cope with increased mobilization by increasing rates of fatty acid oxidation and decreasing rates of fatty acid esterification (e.g. TG synthesis) in the liver. We tested this hypothesis using a fatty liver induction model in far-off dry cows (Rich et al., 2003). Cows were fed 80 or 160% of energy requirements from day 61 to 40 prepartum. Then they were feed restricted to 30% of energy requirements from day 39 to 32 prepartum. Prior energy status did not affect the amount of TG that was accumulated in the liver during feed restriction. The concept of priming the liver by feeding high fiber diets during the dry period needs further evaluation.

A second argument against increased concentrate feeding during the pre-fresh transition period is the potential for the cow to develop insulin resistance. First

proposed by Holtenius et al. (1993), this hypothesis is based on high concentrate feeding leading to prolonged elevation of blood insulin. Prolonged elevation of blood insulin can lead to insulin resistance. If adipose tissue is more resistant to the actions of insulin (an antilipolytic hormone), then fatty acid mobilization associated with parturition will be increased and the cow will be more susceptible to fatty liver. The data to support this is based on high concentrate feeding for the entire dry period and may be related to the development of over-conditioned cows. It is not known if this occurs when feeding high concentrate for shorter periods of time (e.g. three weeks) when cows are unlikely to become obese.

Adaptation of Rumen Epithelium?

It is often suggested that grain should be supplemented during the pre-fresh transition period so that the rumen epithelium will be stimulated to elongate. In theory, elongation of rumen epithelium should lead to more absorptive capacity for volatile fatty acids (VFA) produced from rumen fermentation. After calving, as feed and grain intake increases, VFA production increases. If they are not efficiently cleared from the rumen, pH will drop and the cow can become susceptible to subclinical or clinical acidosis. There is one study that is classically cited to support feeding more grain during the pre-fresh transition period to promote elongation of rumen papillae (Dirksen et al., 1985). However, these experiments were conducted on two cows that went through drastic changes in dietary fiber to elicit the response in papillae growth (approximately 32 to 11% crude fiber). Cows went from a high straw diet to a lactating cow diet. Additionally, DMI was never reported for the trial. It is very likely that changes in energy intake, rather than changes in forage:concentrate ratio, were responsible for the changes in papillae length. Subsequent studies that have looked at changes more typical of those experienced by cows on commercial dairy farms have not supported substituting concentrates for forages for the purpose of elongating papillae and increasing the absorptive capacity of the rumen (Anderson et al., 1999; Reynolds et al., 2004). For example, Reynolds et al. (2004) did not observe a change in papillae when dry cows that were fed a diet containing 25% chopped barley straw, 15% corn silage, 45% grass silage, and 15% concentrate were switched at calving to a diet containing 43% corn silage, 14% grass silage, and 43% concentrate. Anderson et al. (1999) fed the same energy level for the final four weeks of gestation as either primarily grass silage or as a small amount of grass silage (in the afternoon) and approximately 4 kg/d barley in the morning and saw no differences in rumen epithelium. Therefore, it appears that feeding additional concentrate to promote development of rumen epithelium is only warranted if drastic changes in diet take place during the transition from a dry cow to a lactating cow.

Greater Milk Yield?

If increasing concentrates during the pre-fresh transition period would lead to reductions in fatty liver by suppressing fatty acid mobilization, microbial

adaptation to higher NFC diets, and greater absorption of VFA from the rumen and subsequently less acidosis, then one would expect that lactation performance would be enhanced the following lactation. Table 1 summarizes milk yield responses to increased concentrate feeding in trials conducted during the past 10 years. In all nine of the studies, there were no significant effects on milk production. Although each of the studies was conducted with limited cow numbers, collectively they strongly indicate that NFC content of dry cow diets does not affect subsequent milk yield.

Pre-fresh Diets-Conclusions

There is essentially no evidence that suggests that pre-fresh transition diets should contain elevated concentrate, e.g. between the level fed to the far-off dry cow and the fresh lactating cow. There is some evidence that suggests that large drops in feed intake prior to calving should be avoided. However, that is more likely to be caused by problems with management (abrupt changes in diet immediately before calving, overcrowding of pens, inadequate bunk space, not feeding ad libitum, absence of heat abatement, etc) rather than from feeding a diet with an inappropriate NFC:NDF ratio. Extremely poor quality diets should be avoided (e.g. lower energy density than recommended by 2001 NRC for far-off dry cows) in the event that the change in diet at calving becomes too dramatic and causes cows to experience off-feed problems.

Far-off Dry Cows

Although very little research has examined feeding strategies of far-off dry cows, a large body of literature exists that indicates excessive overfeeding of grain during the *entire* dry period should be avoided to minimize the likelihood of over-conditioned cows, reduced feed intake, or metabolic disorders. Consequently, suggesting that far-off dry cows should be fed a diet that meets energy requirements is neither novel nor something that should be discouraged. However, it may be premature to imply that far-off dry cow diets must be formulated “just right”, contain straw, or be a bulky diet that will lead the cow to only meet requirements when fed ad libitum (Drackley and Janovick-Guretzky, 2007a,b). Consider results from Dann et al. (2006; the study that was instrumental in crystallizing the concept of the “Goldilocks diet” for far-off dry cows [Drackley and Janovick-Guretzky, 2007a,b]) versus those of a recent study conducted in our laboratory (Silva-del-Río et al., 2007; Table 2). Dann et al. (2006) conducted an experiment with a 3 x 2 factorial arrangement of treatments: three energy feeding strategies during the far-off dry period and two different energy feeding strategies during the close-up dry period. There

were no effects of close-up diet and no interactions of far-off and close-up dry periods, so only the main effects of far-off dry period will be reported. Far-off dry period treatments were feeding a diet containing 1.59 Mcal NEI/kg DM at ad libitum (150% of NRC requirements) or restricted (80% of NRC requirements) feed intake or a low-energy diet containing straw fed ad libitum (1.30 Mcal NEI/kg DM, 100% of NRC requirements). Silva-del-Río et al. (2007) conducted an experiment with a 2 x 2 factorial arrangement of treatments: cows pregnant with singletons or twins and a “close-up” diet with moderate energy for 3 or 8 wk prepartum. The “close-up” diet contained 1.54 Mcal NEI/kg DM and the far-off dry cow diet contained 1.32 Mcal NEI/kg DM. Therefore, treatments were 1.32 or 1.54 Mcal NEI/kg DM during the first 5 weeks of the dry period and were very similar to two of the far-off treatments employed by Dann et al. (2006). There were few interactions between pregnancy status and diet, so only the main effects of diet are shown (Table 2).

Table 2. A comparison of two trials that compared feeding strategies for far-off dry cows.

Far-off dry cow treatment/parameters	Dann et al., 2006 ^{1,2}			Silva-del-Río et al., 2007 ^{1,3}	
	1.30 Mcal NEI/kg ad libitum	1.59 Mcal NEI/kg ad libitum	1.59 Mcal NEI/kg restricted	1.32 Mcal NEI/kg ad libitum	1.54 Mcal NEI/kg ad libitum
Prepartum body condition, scale 1-5	3.04	3.16	2.94	3.25	3.25
Milk, kg/d	39.4	36.9	37.0	43.3	48.5
Fat, %	3.59	3.77	3.58	3.65	3.62
Liver TG, % or µg/µg DNA	2.5	2.6	1.4	3.6	3.2
NEFA, µEq/L	786	792	627	393	461
BHBA, mg/dL	8.1	9.0	6.6	6.4	7.8
Total health disorders	29	51	37	57	52

¹Dann et al. (2006): wk 1-8 postpartum for milk parameters and health disorders and d 1-10 for blood and liver measurements. Silva-del-Río et al. (2007): wk 1-15 for milk parameters and health disorders, wk 1-10 for blood measurements, and d 1 and 35 postpartum for liver TG.

²Prepartum body condition, $P = 0.003$; Liver TG, $P = 0.14$; BHBA, $P = 0.03$; other parameters $P \geq 0.15$ or insufficient animals for statistical analysis (health disorders).

³Milk, $P = 0.04$; NEFA, $P = 0.06$; BHBA, $P = 0.07$; other parameters $P \geq 0.15$ or insufficient animals for statistical analysis (health disorders).

The results of the two trials are strikingly different and there are no apparent reasons for the differences in results. What conclusions can be drawn? Based on our results, should we be recommending that producers feed transition diets during the entire dry period? The answer is probably not. There are two logical conclusions. One might conclude that diets for far-off dry cows do not have to be formulated to be “just right” and there is substantial variability in diets that can be tolerated. Another conclusion might be that these trials were under-replicated ($n = 20-25$ cows/treatment for these two

studies), making it difficult to draw firm conclusions. Both conclusions probably have some merit.

Overall Conclusions

Approximately 15 years ago, our research group strongly recommended that producers feed moderate-energy diets to pre-fresh transition cows. Today, after many research trials, it has become obvious that we were in error and there was not sufficient evidence to make such recommendations. More recently, nutrition of the far-off dry cow has started to gain attention. We see a similar scenario developing, in which rather specific feeding recommendations are being made with insufficient supportive evidence. Perhaps down the road, following numerous additional trials, we will have sufficient evidence to make more firm recommendations for far-off dry cows. One area of neglect has been nutrition of the immediate post-fresh cow. Data from Rabelo et al. (2003, 2005) indicated that energy density of diets immediately postpartum are more critical than energy density of diets immediately prepartum. Perhaps this is not surprising because the most difficult challenge in meeting nutrient requirements occurs during the first weeks after calving. Once again, feeding strategies during this time period need considerably more research. However, it is unlikely this will occur soon. Researchers avoid doing studies on immediate post-fresh cows because of enormous cow variability during that time and the associated difficulty of designing experiments with sufficient replication.

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5.2 METABOLIC AND ENDOCRINE CHANGES AND HEPATIC EXPRESSION OF GLUCONEOGENIC ENZYMES IN HIGH-YIELDING DAIRY COWS WITH LOW AND HIGH LIVER FAT CONTENT DURING THE TRANSITION PERIOD

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Negative energy balance after parturition in high-yielding dairy cows goes along with mobilization of non-esterified fatty acids (NEFA) and elevated liver fat content, when fatty acids not oxidized in liver were stored. Hepatic fat storage may impair liver function that leads to imbalanced energy metabolism. We have tested the hypothesis that metabolic and endocrine changes and hepatic gluconeogenic enzyme expression are affected by liver fat content in high-yielding dairy cows not suffering from clinical ketosis or fatty liver disease. Cows (> 10,000 kg milk/305 days) with low (LF; 82 ± 4.6 mg total fat/kg wt; n=10) and high (HF; 213 ± 9.6 mg total fat/kg wt; n=10) hepatic fat content on d 10 post partum were investigated during the transition period. Cows were fed TMR ad libitum with 5,7 MJ NEL/kg DM and 120 g/kg DM utilizable protein (nXP) during dry period (8-4 wk before parturition), 6.7 MJ NEL/kg DM and 152 g/kg DM nXP during transition to lactation (3-0 wk before parturition) and 7 MJ NEL/kg DM and 175 g/kg DM nXP during lactation. Blood samples were taken on d -20, -7, 0, 7, 14, 28 and 56 relative to parturition for measuring of plasma glucose, NEFA, beta-OH-butyrate (BHB), insulin, glucagon, IGF-I and leptin. Liver samples on d 1, 10 and 21 relative to parturition were taken to measure glycogen content and mRNA levels of glucose-6-phosphatase (G6-Pase; EC 3.1.3.9), pyruvate carboxylase (PC; EC 6.4.1.1) and cytosolic (-C) and mitochondrial phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32). Data were analysed using the Mixed Model of SAS with liver fat content and time as fixed effects. Back fat thickness decreased and DMI and energy intake increased with onset of lactation, and back fat thickness was higher ($P < 0.05$), but DMI and energy intake were lower ($P < 0.05$) in HF than LF. Milk yield (ECM) did not differ between groups, but milk fat content was higher ($P < 0.05$) and lactose content was lower ($P < 0.05$) in HF than LF at begin of lactation. Energy balance was more negative ($P < 0.05$) in HF than LF. Plasma NEFA and BHB increased ($P < 0.01$) and plasma glucose tended to decrease ($P < 0.1$) more in HF than LF with onset of lactation. Glucagon/insulin ratios (mol/mol) increased more ($P < 0.05$) in HF than LF with onset of lactation. Hepatic glycogen content was higher ($P < 0.05$) in LF than HF, whereas mRNA levels of G6-Pase and PC were higher ($P < 0.05$) in HF than LF. PEPCK-C mRNA increased ($P < 0.05$) after parturition in both groups but showed no group differences.

In conclusion, high-yielding dairy cows with high liver fat content during the transition period indicated a reduced DMI, a more adverse negative energy balance, greater body fat mobilisation and elevated expression of gluconeogenic key-enzymes when compared to cows with low hepatic fat content.

5.3 ACUTE METABOLIC RESPONSES OF POSTPARTAL DAIRY COWS TO SUBCUTANEOUS GLUCAGON INJECTION, ORAL GLYCEROL, OR BOTH

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This study examined the effects of subcutaneous glucagon injection with or without co-administration of oral glycerol on blood metabolites and hormones of Holstein dairy cows in the early postpartal period. Twenty multiparous cows were fed a dry cow ration supplemented with 6 kg of cracked corn during the dry period to increase the likelihood of developing postpartal fatty liver disease. Cows with a body condition score of ≥ 3.5 points (1-5 point scale) were assigned randomly to one of four treatment groups: saline, glucagon, glucagon plus glycerol, and glycerol. Following treatment, serial blood samples were collected over an 8-hour period to determine the effects of glucagon and/or glycerol on blood metabolites and hormones. Treatment effects were determined by comparing the concentration of metabolites and hormones during the 8-hour period after treatment administration (time 0) with the concentration of the same compounds at time zero on d 1, d 7, and d 13 postpartum. Glucagon alone tended to increased postpartal plasma glucose on d 7 and d 13 postpartum ($P = 0.07$ and $P = 0.06$, respectively), increased postpartal plasma glucagon on d 1, d 7, and d 13 ($P=0.01$, $P = 0.03$, and $P = 0.03$, respectively) and insulin on d 1 and d 7 ($P < 0.04$ and $P = 0.01$, respectively), and decreased postpartal plasma nonesterified fatty acids (NEFA) on d 13 postpartum ($P = 0.04$) relative to the control. Administration of glucagon plus glycerol increased and sustained postpartal plasma glucose on d 1, d 7, and d 13 ($P = 0.006$, $P = 0.0008$, and $P = 0.02$, respectively), glucagon on d 1 and d 7 ($P = 0.03$ and $P = 0.05$), respectively), insulin on d 1, d 7, and d 13 ($P = 0.002$, $P = 0.0004$, and $P = 0.01$, respectively) and decreased plasma NEFA on d 13 ($P = 0.01$) and β -hydroxybutyrate (BHBA) on d 1 and d 7 ($P = 0.05$, and $P = 0.03$, respectively). Interestingly, administration of glycerol alone decreased postpartal plasma NEFA on d 1, d 7, and d 13 ($P = 0.03$, $P = 0.05$, and $P = 0.0002$, respectively) and BHBA on d 1 ($P = 0.02$) and increased TAG on d 1 and d 13 ($P = 0.03$ and $P = 0.3$). Early postpartal treatment of dairy cows with glucagon or glucagon plus glycerol increased plasma glucose and insulin, decreased plasma NEFA and BHBA, and increased secretion of liver NEFA as plasma TAG. These responses suggest that glucagon and glycerol act additively when co-administered to decrease the likelihood of fatty liver disease development in dairy cows. Research was supported, in part, by the USDA grants number 416-43-44-21-6605 and 411-40-06-21-6648.

5.4 PANCREATIC INSULIN SECRETION IN HIGH-YIELDING DAIRY COWS: CAUSES AND EFFECTS.

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High-yielding dairy cows are more susceptible to metabolic and reproductive disorders. The metabolic profile during negative energy balance (NEB), characterized by low glucose and insulin and elevated non-esterified fatty acid (NEFA) concentrations, is a major risk factor. In human medicine, NEFA are also known to suppress pancreatic beta-cell function. Therefore, a decreased pancreatic insulin release in dairy cows may contribute to the metabolic challenges during the transition period. Pancreatic function remains, however, poorly documented in cows.

In this study, we aimed to assess the pancreatic function of dairy cows on different moments before and after calving. Furthermore, we aimed to obtain a metabolic profile and the time of first ovulation, in order to elucidate possible causes and effects of a decreased pancreatic function. We studied 23 healthy, multiparous high-yielding cows from 14d pre partum to 42d post partum (pp). Intravenous Glucose Tolerance Tests (IVGTT) were performed on -14, 14 and 42d relative to calving to assess insulin release, estimated by the Area Under the Curve (AUC), and glucose clearance. Blood samples were obtained at 3d intervals for glucose, insulin and NEFA analysis. The time of first ovulation was defined by ultrasound examination and progesterone analysis. Compared to the dry period, insulin AUC and peak concentration in the IVGTT decreased and glucose clearance increased during lactation ($P < 0,05$). Insulin AUC was negatively correlated to simultaneous NEFA levels. Thirteen cows ovulated within 42d pp; the remaining 10 cows suffered from delayed ovarian resumption. Mean glucose levels (g/l) declined from 0,59 pre partum to 0,48 on 3d pp and remained significantly lower ($P < 0,05$) during the entire pp period. Mean insulin levels ($\mu\text{U/ml}$) decreased from 1,19 pre partum to 0,35 on 3d pp and remained significantly lower ($P < 0,05$) until 22d pp. Mean NEFA levels (mmol/l) increased from 0,18 pre partum to 0,60 on 3d pp and remained elevated ($P < 0,05$) until 28d pp. Prepartum NEFA levels tended to be higher in non-ovulating cows (0,25 vs 0,13 mmol/l; $P=0,051$).

Our results suggest a decreased pancreatic function pp. The negative correlation between NEFA and insulin AUC suggests that NEFA might impair pancreatic function in dairy cows. However, this correlation does not necessarily imply a causal relationship; further research is needed in order to elucidate the exact mechanism. In addition, our results confirm the high incidence of ovarian disturbances in the transition period; an early onset of NEB during the dry period was identified as a risk factor.

5.5 THE EFFECT OF LIVER FAT ACCUMULATION ON HEPATOCELLULAR PHOSPHORUS CONTENT IN EARLY LACTATING DAIRY COWS

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Hypophosphatemia is a common finding in lactating dairy cows with ketosis or hepatic lipidosis. Low plasma phosphorus (P) concentrations have been attributed to decreased feed intake but recent studies have suggested that liver dysfunction may also act as a causative factor. To our knowledge, the effect of hepatic lipidosis on liver P content has not been studied.

We therefore investigated the effect of liver fat accumulation on hepatocellular P content in early lactation dairy cows. Liver biopsy samples of 33 lactating dairy cows with a broad range of hepatic triacylglycerol concentrations (TAG, 0.2-19.9%) were obtained on day 14 of lactation. Amounts of P, magnesium (Mg), and potassium (K) in the sample was determined using inductively coupled plasma-mass spectrometry and expressed as $\mu\text{g/g}$ of dry weight. The amount of DNA was also determined in order to explore the potential confounding effect of changes in cell volume on liver P content. Blood samples were obtained immediately before biopsy and assayed for indices of hepatic function and injury. The amount of P in wet weight (PW) liver, non fat wet weight (PNFW) liver, and per cell (indexed to DNA; PDNA) was calculated. Water content as % of cell mass (W) was estimated as $100 - \% \text{ dry matter}$. Pearson correlation and multiple stepwise regression analyses were performed. Liver P and PW were negatively correlated with the log to the base 10 of TAG (LTAG, $r=-0.79$, $p < 0.0001$; $r=-0.49$, $p=0.004$, respectively). Liver P was positively correlated with W ($r=+0.87$, $p < 0.0001$). Strong positive associations were found for liver P with Mg ($r=+0.98$, $p < 0.0001$) and K ($r=+0.92$, $p < 0.0001$). The value for PDNA tended to decrease with increasing TAG. Stepwise regression analysis revealed the strongest associations of P with W (part. $R^2=0.74$, $p < 0.0001$) and LTAG (part. $R^2=0.05$, $p=0.03$) and of PW with LTAG (part. $R^2=0.30$, $p=0.004$). Strongest associations for PDNA were with β -hydroxybutyrate concentration (BHBA, part. $R^2=0.40$, $p=0.005$) and log to the base 10 of plasma aspartate aminotransferase (logAST) activity (part. $R^2=0.18$, $p=0.02$).

Our findings indicate that hepatic P content is inversely and linearly related with hepatic TAG content but suggest that cytosolic P concentration remains constant despite large changes in TAG. The findings also indicate that an increase of TAG primarily results in a decrease in cell water with a relatively smaller increase in cell volume. Hepatocellular fat accumulation in lactating dairy cows therefore appears to be associated with constant cytosolic electrolyte concentrations but decreased hepatocellular electrolyte mass. Negative associations of PDNA with BHBA and logAST suggest that hepatocellular damage may be associated with a decreased amount of P in hepatocytes.

5.6 CLINICALLY RELEVANT BIOCHEMICAL ABNORMALITIES IN KETONE BODY-RESPONSIVE COWS WITH ABOMASAL DISLOCATION TO THE LEFT

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Problem: Every disease of high yielding dairy cows is a risk factor for the development of secondary ketosis. This is particularly true for diseases with an implicit decrease in feed intake, such as parturient paresis and abomasal dislocation. The increase in circulating ketone bodies, such as beta-hydroxybutyrate (BHB), acetone, or acetoacetate, indicates a negative energetic balance and is therefore often accompanied by signs of high fat metabolism and liver damage. Ketosis, however, is also able to trigger abomasal dislocation by decreasing appetite, resulting in a vicious cycle. **Aim:** The scope of this retrospective analysis was to assess potential changes in selected clinically relevant biochemical parameters of energy metabolism in a subgroup of cows diagnosed with abomasal dislocation to the left and screened positive for ketone bodies in urine.

Material and methods: Cows of different breeds admitted to four veterinary clinics in Germany for surgical repositioning of their abomasum, displaced to the left, were tested for hyperketonuria, using the Ketur Test (Roche Diagnostics GmbH, Germany). Pre-operative blood samples of 139 animals tested positive with a readout of ++ on the stick were analyzed for serum levels of BHB, aspartate aminotransferase (ASAT) activity, glutamate dehydrogenase (GLDH) activity, free fatty acids (FFA), total bilirubin, cholesterol, creatine kinase (CK) activity, and cortisol. The analysis was performed in one single central laboratory per parameter to avoid a methodological bias.

Results: Laboratory blood analysis confirmed hyperketonemia (BHB levels >90 mg/l) in 133 out of the 139 patients (95.7%), indicating very reliable results of the screening with just 4.3% false positive hits. Regarding the serum markers of impaired liver function, a high percentage of animals showed levels exceeding the respective upper reference limits for ASAT activity (>105 U/l; 94.2%), GLDH activity (>25 U/l; 81.3%), and FFA ($\geq 700 \mu\text{mol/l}$; 98.5%). Elevated levels of bilirubin ($\geq 1.0 \text{ mg/dl}$) and CK activity ($\geq 500 \text{ U/l}$) were found in 37.4% and 29.5% of the animals, respectively. Three animals (2.2%) showed elevated serum levels of cholesterol, and 81 (58.3%) had cholesterol levels beyond the lower limit of the reference range (75-175 mg/dl). Serum cortisol levels in the patient collective were below the reference range (0.72-18 $\mu\text{g/dl}$) in 26 cases (18.7%) and normal in the remaining cases.

Conclusions: In view of the high impact of ketosis even in its subclinical presentation on performance and health, the results of the current study underline the importance to screen cows with abomasal dislocation for hyperketonemia and to appropriately treat the metabolic disorder in addition to repositioning the abomasum.

5.7 HOMEOPATHIC MEDICINE'S IMPACT TO THE MORPHOLOGIC BLOOD PARAMETERS DURING THE TRANSIT PERIOD

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The biggest high productivity cows' problem is a deteriorating health after the calving. When system's homeostasis balance is disordered in this period, the productivity decreases, the cows hardly recover and the farms suffer big economic loss.

The aim of this research is to determine the homeopathic medicine's impact to the morphologic blood parameters and reduce the cows' morbidity. The research has been carried out in high productivity Lithuanian cow herd in the period from the 22nd of January, 2007 till the 1st of April, 2007. During the research 40 dried out cows have been divided into two groups - the exploratory group (n=20) and control group (n=20). The research has been carried out in four stages. During the first stage (30 days till the prospective calving) the cows were injected in the muscle with 5 ml Traumeel and Lachesis comp. and under the skin with 5 ml Coenzyme comp. Whereas the cows in control group were injected with 15 ml 0,9 % saline. During the second stage the first stage process was repeated after an interval of 10 days after the first stage. During the third stage (just after the calving) the process was repeated again, except for Lachesis comp., which was changed to 5 ml Carduus comp. into the muscles. The blood has been obtained from the jugular vein to vacuum proofs (BD Vacutiner, England) with the preservative EDTA.K3 before the research and 30 days after the calving. The data was processed using the statistical R package. The clinical tests made after the calving shows that 22 % cows in the exploratory group suffered from clinical ketosis. The average milk productivity in the exploratory group was 1.5 kg bigger than in the control group. Whereas 56 % cows in the control group suffered from clinical ketosis and 22 % - from the left-sided abomasum dislocation. The morphological blood parameters were measured with hematological analyzer Abacus junior vet (Diatron, Austria) and revealed that the parameters changed equally in both groups.

Except from MCV that remained stable statistically reliably ($p>0,05$) for 30 days after the calving, whereas it decreased in the control group. MCHC, PDWc, PCT and MPV in the exploratory group statistically reliably ($p>0,05$) increased. The homeopathic prophylaxis reduced the risk of ketosis, left-sided abomasum dislocation, improved the metabolism processes and positively influenced the blood-producing organs during the dry out and calving period. Because of the homeopathic therapy, the cow morbidity decreased and the milk production increased, therefore medical expenses reduced and the profitability increased.

5.8 LIPID PEROXIDATION PRODUCT MODIFIED PROTEINS FORMED DURING THE PERIPARTURIENT PERIOD OF HIGH-YIELDING DAIRY COWS

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High-yielding dairy cows mobilize large amounts of body fat to meet the high energy requirement for milk production at the onset of lactation. As a consequence, cows show increased plasma concentrations of lipids, non-esterified fatty acids (NEFAs) and ketone bodies (KBs) within the first weeks after calving. Under these conditions, lipid peroxidation products (LPPs) can be formed in a non-enzymatic reaction between lipids, NEFAs or KBs and reactive oxygen species, yielding carbonyl compounds such as glyoxal, hydroxynonenal or acrolein which in turn are able to react with lysine residues of proteins. It is hypothesized that those modified proteins lost their original physiological functionality, and thus contribute to metabolic disturbances, and delay the return to stable metabolic conditions.

Therefore, we aimed to examine the formation of LPP modified proteins over the periparturient period. To this end, plasma samples of 5 high-yielding dairy cows were taken 3 weeks before and 2 and 4 weeks after parturition. Samples were analysed for NEFA and beta-hydroxybutyrate (BHBA) concentrations and subjected to Western blot experiments. LPP modified proteins were detected by use of a monoclonal antibody directed against carboxymethyllysine residues (a glyoxal derived product) and with polyclonal IgGs raised against hydroxynonenal mediated modifications. For both types, main immunoreactivity was observed around 60–65 kDa, while further distinct bands were detected at 170, 120 and 28 kDa. In comparison to the ante parturient period, we found increasing levels of LPP modified proteins within the first four weeks after parturition. In addition, elevated LPP levels are accompanied with increasing NEFA and KB concentrations peaking around 10 days after parturition.

In conclusion, increasing concentrations of NEFAs and KBs might be one reason for the increased formation of LPP modified proteins and their impaired functionality after parturition.

5.9 FEED INTAKE, MILK PRODUCTION AND METABOLIC CHANGES IN HIGH-YIELDING DAIRY COWS FED EITHER A STARCH-BASED OR A FAT-ACCENTUATED DIET DURING THE DRY PERIOD

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The transition period from 3 wk before to 3 wk after parturition is the most important part of the lactation cycle in high-yielding dairy cows. This time period is coined by dramatic metabolic changes and adaptations for milk production. As nutrient intake does not meet energy demands at the beginning of lactation, the cows mobilize fat reserves to meet energy requirements. Feeding strategies during the dry period aim to facilitate adequate energy supply after onset of lactation by making stored energy easily available, and to rapidly increase dry matter intake after parturition. We have tested the hypothesis that feeding rumen-protected fat during the dry period may improve the energy status of high-yielding dairy cows during early lactation by facilitating fat mobilisation. Dairy cows (18 half sib, > 9000 kg milk yield during first lactation) were divided into 2 feeding groups 12 wk before expected parturition. Cows received either a starch-based (SD) or a fat-accentuated diet (FD) during late lactation (wk -12 to -9 relative to parturition), dry period (wk -8 to -4) and transition period to onset of lactation (wk -3 to -1). Diets were calculated to be isonitrogenous and isoenergetic. After parturition, all cows received SD up to 98 d in milk (DIM). Individual feed intake and milk yield were recorded daily. Plasma concentrations of triglycerides, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), glucose, lactate, urea and bilirubin were measured in blood samples taken at 55, 40, 30, 20, 10, 5 d before calving and 1, 5, 10, 15, 20, 28, 40, 55, 70, 85, and 95 DIM. Data were analysed by the Mixed Model of SAS with diet (SD versus FD before parturition) and time as fixed effects. Dry matter intake (DMI) was higher ($P < 0.05$) during the dry period in SD- than FD-fed cows, but not during late first lactation, transition period and after parturition. Milk yield was higher ($P < 0.05$) in SD- than FD-fed cows. Plasma triglyceride concentrations were higher ($P < 0.01$) before parturition in FD- than SD-fed cows, decreased after parturition in both groups and showed no differences after parturition. Plasma NEFA and BHBA concentrations increased around parturition in both groups, but showed no differences with regard to different feeding. Plasma glucose decreased with onset of lactation in both groups, but there was no significant feeding effect.

Our results suggest an impaired lactation performance in cows fed FD before parturition, but no effect on DMI after parturition. Preliminary results also indicate that different nutrient composition before parturition does not affect metabolic traits during subsequent lactation. This study was supported by the Deutsche Forschungsgemeinschaft, Bonn, Germany.

5.10 BIOCHEMICAL CHECK OF THE PERIPARTAL LOAD OF HIGH-PRODUCING DAIRY COWS

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The peripartal period determines the development of the health status and milk yield in the lactation. High prevalence of diseases in this period is determined by the fact that the peripartal period represents a major metabolic turning point and load of the organism.

It was our aim to evaluate the peripartal load using selected biochemical parameters. Blood of 326 cows on the second and higher lactation from 23 farms was examined. Blood was collected up to 24 h post parturition (p.p.) and then 5-12 days p.p. Parameters NEFA, BHB, total bilirubin, urea, AST, GMT, Ca, P and Mg were examined. According to the levels of NEFA, samples from the first and second collection were divided into three groups. Group 1: NEFA \leq 0.6 mmol/l (n=139, resp. n=61), group 2: NEFA 0.61-1.0 mmol/l (n=117, resp. n=50) and group 3: NEFA $>$ 1.0 mmol/l (n=70, resp. n=44). Analyses were performed using the automatic analyser DCP Konelab 20i and Cobas Mira Basic. On the first blood sampling comparing group 1 and groups 2 and 3 we found concentrations of BHB 0.53 vs. 0.68, resp. 0.94 mmol/l (both $p\leq$ 0.001), bilirubin 3.96 vs. 7.74, resp. 11.50 μ mol/l (both $p\leq$ 0.001), urea 4.91 vs. 5.39 ($p\leq$ 0.05), resp. 5.76 mmol/l ($p\leq$ 0.01), and calcium 2.04 vs. 1.94 ($p\leq$ 0.05), resp. 1.86 mmol/l ($p\leq$ 0.01). There were no differences in parameters AST, GMT, P and Mg. Comparing group 1 and groups 2 and 3 regarding blood sampling 5-12 days p.p. we found the concentration of BHB 0.95 vs. 1.23 ($p\leq$ 0.05), resp. 2.13 mmol/l ($p\leq$ 0.001), bilirubin 4.96 vs. 7.38, resp. 10.01 μ mol/l (both $p\leq$ 0.001), AST 1.70 vs. 1.97 ($p\leq$ 0.05), resp. 2.26 μ kat/l ($p\leq$ 0.01) and urea 4.37 vs. 4.89 mmol/l ($p\leq$ 0.05 only in group 3). Levels of calcium were 2.53 vs. 2.35, resp. 2.24 mmol/l ($p\leq$ 0.001). No significant differences concerned GMT, P and Mg. NEFA up to the selected level of 0.6 mmol/l may be considered as levels expressing the tolerated load, because other biochemical parameters both from the first and second blood collection did not show metabolic disorders.

There is no direct documentation of ketosis by levels of NEFA, because similar levels of NEFA were accompanied by BHB values which were nearly doubled on the second blood sampling. Thus to study the degree of lipomobilization and risk of ketosis both parameters are to be recommended. Higher NEFA levels were accompanied by significantly higher values of total bilirubin, but not AST on the first blood sampling. Bilirubin thus may be another sensitive parameter of negative energy balance around the time of parturition. Calcium drop (to 2.0 mmol/l) is a characteristic finding during the first 24 h p.p and caused still no problems. This study was supported by Grant MSM6215712403.

5.11 CHANGES IN THE INTERNAL ENVIRONMENT OF COWS IN THE PERIPARTUM PERIOD

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Aim: The study objective was to determine serum concentrations of calcium, inorganic phosphorus, magnesium, glucose, FFA, BHB, total bilirubin and AST in cows with different body condition scores (BCS) in the peripartum period.

Material and methods: In Holstein cows with average yield of 10,650 kg milk per lactation, selected blood metabolites were monitored 15 to 20 days prepartum, 2 to 3 days prepartum, 0.5 to 3 hours postpartum, 24 hours postpartum, 3 days postpartum, 7 days postpartum, 10 days postpartum and 15 days postpartum. The monitoring included 20 cows from 4 to 7 years of age. The cows were divided into two groups based on their prepartal BCS (Body Condition Score). The Group A included 10 cows with BCS ranging from 3.25 to 3.75, Group B included 10 cows with BCS 4.5 to 5. Blood samples from the cows under study were collected in the above-mentioned periods and the selected serum parameters were measured by the automatic analyser HITACHI 909.

Results: In the period from 15 to 20 days prepartum, no differences in the concentrations of the measured parameters between the groups were found. In the period 2 to 3 days prepartum, significantly higher concentrations of FFA, BHB and AST were found in the Group B. During the peripartum period, significantly lower concentrations of calcium and phosphorus as well as significantly higher concentrations of FFA, BHB, bilirubin and AST were found in the cows of Group B. In the next period the difference in calcium concentrations persisted till 10th day postpartum, and significant differences in FFA, BHB and AST concentrations persisted till 15th day postpartum.

Conclusion: The cows with high BCS in the prepartum period showed rather compromised internal environment, mainly due to decreased calcium concentrations, and increased FFA, BHB and AST concentrations. The cows of Group A showed faster elimination of changes in the internal environment. The study was part of the project no. 1G46086 on the National Agency for Agricultural Research (NAZV).

6 METABOLISM AND IMMUNOLOGY

6.1 METABOLIC SYNDROME IN HUMANS

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Abstract

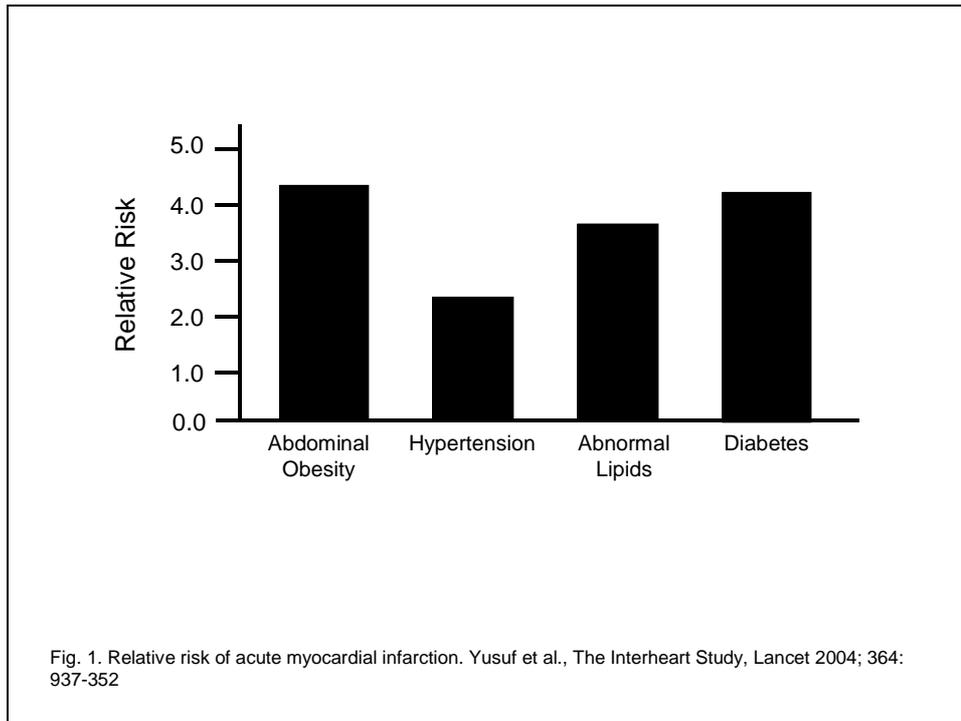
Excess body fat mass and, in particular, intra-abdominal adiposity is the strongest predictor of the metabolic syndrome and its vascular co-morbidities. Free fatty acids (FFAs) have long been recognized to induce insulin resistance in muscle and liver. Due to receptor distribution and other factors visceral fat cells have a higher lipolytic activity and excess visceral fat exposes the liver to increased concentrations and flux rates of FFAs. This results in hepatic insulin resistance and increased endogenous glucose production. In addition, visceral adiposity results in a metabolically disadvantageous pattern of release of adipokines (=adipocytes derived peptide hormones). Currently, the most interesting adipokine appears to be adiponectin, an endogenous insulin sensitizer, which is strongly associated with improved insulin sensitivity, and reduced risk of diabetes and atherosclerosis. Its beneficial metabolic effects are largely due to improvement in hepatic insulin resistance. Others, such as Vaspin, are currently under investigation. Moreover, cells other than adipocytes may have a specific role in the pathogenesis of the metabolic syndrome. For example, more macrophages are present in visceral compared to subcutaneous adipose tissue. This finding may be causatively related to the observation that low-grade inflammation accompanies visceral obesity. While it is undisputed that the specific biology of visceral fat underlies the cardiovascular risk association, it remains unclear why excessive calories are deposited in one compartment and not the other in some people and not in others.

Abdominal Obesity (waist circumference)	
Men	>102 cm
Women	>88 cm
Triglycerides	> 150 mg/dL (1.7 mM)
HDL cholesterol	
Men	<40 mg/dL (0.9 mM)
Women	<50 mg/dL (1.0 mM)
Blood pressure	> 130/85 mm Hg
Fasting glucose	> 110 mg/dL
Albumin i. U.	> 30 mg/min

Table 1. Metabolic Syndrome, WHO Definition

Introduction

In humans the constellation of metabolic abnormalities includes glucose intolerance (type 2 diabetes, impaired glucose tolerance, or impaired fasting glycaemia), insulin resistance, central obesity, dyslipidaemia, and hypertension, all well documented risk factors for cardiovascular disease. These conditions co-occur in an individual more often than might be expected by chance. When grouped together, they are associated with increased risk of cardiovascular disease. Three of the components shown in table 1 must be fulfilled to make the diagnosis of the metabolic syndrome (table 1). Of particular note, abdominal adiposity is a strong risk factor in itself (fig. 1) (Eckel et al., 2005; Stumvoll et al., 2005)



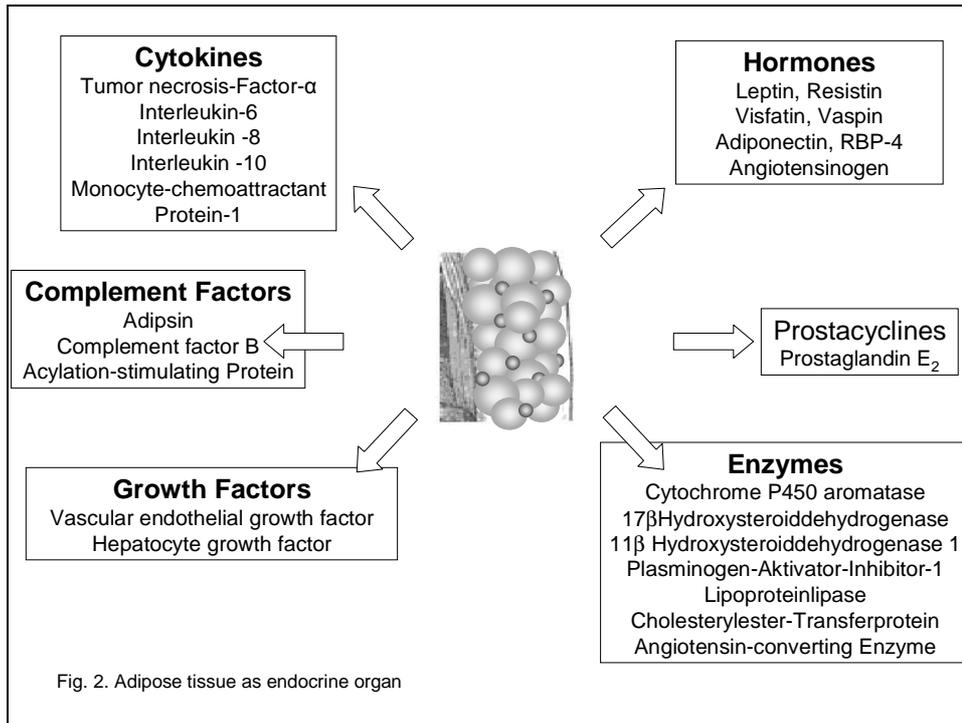
Secretory products of adipose tissue

The traditional view of adipose tissue as an inert fuel storage depot that releases fatty acids and glycerol in times of food deprivation is being challenged by recent research. Triggered by the discovery of leptin a decade ago, it has emerged that adipose tissue is not merely a passive energy store, but an active endocrine organ that synthesizes and secretes a variety of bioactive molecules. These secretagogues act locally and systemically to influence a number of biological processes, such as food intake and metabolism, neuroendocrine pathways and inflammation. It has also become clear in recent years that, far from being a simple collection of adipocytes, adipose tissue contains a matrix of connective tissue and nerve cells, in addition to stroma-vascular and immune cells – particularly macrophages. Excessive accumulation of adipose tissue in the abdominal area is an essential component of the metabolic syndrome. The hallmark of these components is visceral adiposity as measured by waist circumference.

Adipose tissue secretes a wide selection of proteins and peptides with a variety of endocrine, paracrine and autocrine functions. Their ultimate actions include central, immunological, cardiovascular, and metabolic effects. Many of these factors are expressed and released from adipocytes; however, some originate

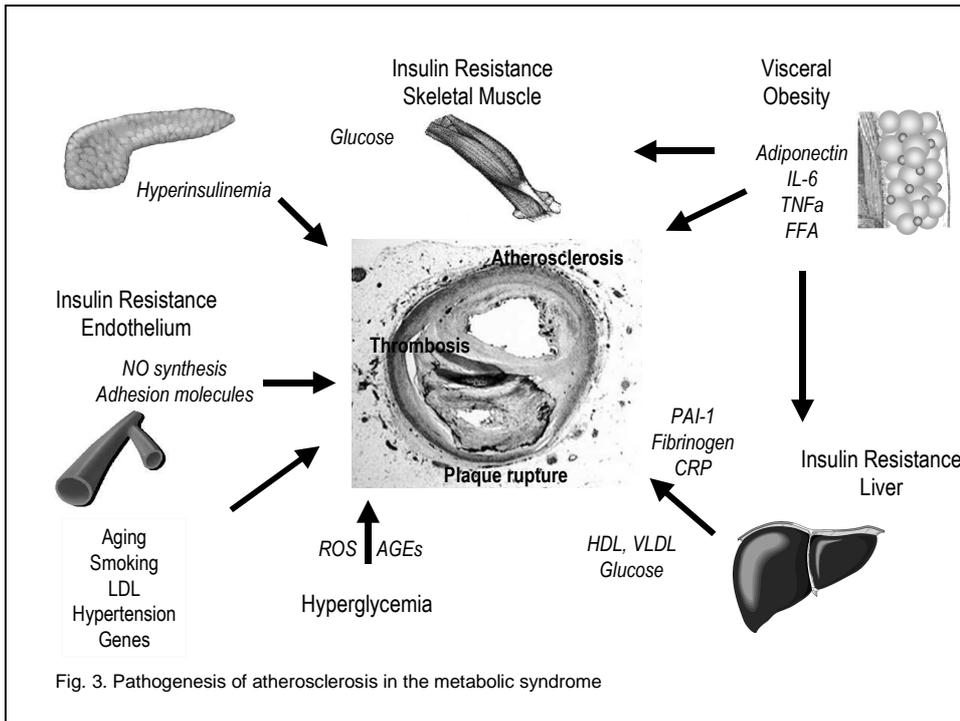
from immune and stromal cells within the adipose tissue matrix. Obesity is associated with altered serum concentrations of a number of these factors (Figure 2).

Increased levels of NEFA and inflammatory cytokines (e.g., tumor necrosis factor α [TNF α] and interleukin 6 [IL-6]) released by expanded visceral adipose tissue adversely influence the insulin signaling cascade. NEFA inhibit insulin-stimulated glucose metabolism in skeletal muscle and suppress glycogenolysis in liver. NEFA activate cellular kinases, including atypical protein kinase C isoforms by increasing cellular diacylglycerol levels, which can activate the inflammatory kinases inhibitor κ B kinase (IKK) and c-jun N-terminal kinase (JNK), increasing serine/threonine phosphorylation of IRS-1 and reducing downstream IRS-1 signaling, as described above (Kovacs and Stumvoll, 2005). TNF α enhances adipocyte lipolysis, which further increased NEFA levels, and also elicits its own direct negative effects on insulin signaling pathways. Neutralization of TNF- α dramatically reverses insulin resistance in rodent models; however, the magnitude of its involvement in human insulin resistance is not entirely clear. The proinflammatory IL-6 inhibits the insulin signal by augmenting the expression of SOCS proteins. Adiponectin is currently considered the most promising among the adipokines. While circulating NEFA and several adipokines are increased in visceral obesity, the levels of the adipose-specific protein adiponectin are decreased, reducing its insulin-sensitizing effects in liver and muscle. Adiponectin signals via AMP kinase, a stress-activated signaling enzyme implicated in a variety of metabolic responses, including suppression of hepatic gluconeogenesis, glucose uptake in exercising skeletal muscle, fatty acid oxidation, and inhibition of lipolysis, which may explain its beneficial metabolic effects. Others, such as vaspin, visfatin, retinol-binding protein 4 and apelin are being studied (Fasshauer et al., 2004; Kralisch et al., 2007).



Visceral obesity is an independent risk factor for the development of cardiovascular diseases and type 2 diabetes. This is likely to be due to biological characteristics of visceral tissue, which are different from those of subcutaneous adipose tissue in terms of decreased insulin sensitivity and increased lipolytic activity. In addition, the anatomical site of visceral fat could be one potential reason for the increased cardio-metabolic risk associated with visceral obesity. Visceral adipose tissue drains into the portal vein and therefore the liver is exposed to the undiluted metabolites and adipokines released from visceral fat. Moreover, there are profound differences between visceral and subcutaneous adipocytes in the metabolism, expression of specific receptors and secretion of a specific adipokine pattern (Kloting et al., 2007).

In patients with visceral obesity and the metabolic syndrome all of these factors contribute to a varying extent to the much feared proatherogenic milieu (fig 4) (Eckel et al., 2005). In the absence of any truly causal therapy guidelines suggest to treat the intermediary abnormalities to reduce cardiovascular mortality and morbidity (table 2).



1. Appropriate and aggressive therapy is essential for reducing patient risk of cardiovascular disease
2. Lifestyle measures should be the first action
3. Pharmacotherapy should have beneficial effects on
 1. Glucose intolerance / diabetes
 2. Obesity
 3. Hypertension
 4. Dyslipidemia
4. Ideally, treatment should address all of the components of the syndrome and not the individual components

Table 2. Management of the metabolic syndrome . International Diabetes Federation, 1st International Congress on "Prediabetes" and Metabolic Syndrome (2005)

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6.2 LINKAGES BETWEEN METABOLISM AND THE IMMUNE SYSTEM

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Introduction

Classically, the immune, endocrine, neural, and metabolic systems were usually studied in isolation with the assumption that these systems operated more or less independently of the others. In recent years, however, the interconnected nature of all these control systems has become well established (Kelley, 2001; Drackley, 2006). Thus, these systems work together to maintain homeostasis under the wide range of physical, pathological, and physiological states through which animals pass during their lifetimes. Given the current understanding of the interdependency of all the detection and response systems in the body, it does not seem surprising that molecules traditionally thought of as products of the immune system, such as the cytokines, may be produced by “metabolic” tissues such as adipose and muscle, and that they also can affect metabolic pathways traditionally thought to be only controlled via substrate and hormonal regulation of enzyme activity. However, the complexity of this interrelated homeostatic regulatory system is only beginning to be unraveled.

Malnutrition severely impairs the ability of animals and humans to ward off infectious diseases. In the light of this well-known fact, there has been considerable interest in discovering nutritional or pharmacologic factors that might boost or suppress activity of the immune system in farm animals, depending on the nature of the particular application. For example, any compound that could stimulate earlier and more vigorous immunity in young calves would be of substantial practical importance. Likewise, dairy cows during the periparturient period are more highly susceptible to infectious agents that cause metritis and mastitis. Any nutrient or compound that could provide cows with greater protection against these infectious agents would be of enormous benefit to both animal well-being and producer income. On the other hand, agents that dampen the immune system during growth of pigs or poultry may decrease nutrients needed for those “maintenance” functions and thereby improve efficiency of growth.

In the context of production diseases, however, there are critical time periods when the effects of the immune system on metabolism, or vice versa, assume particular importance. One of these is the periparturient period in dairy cows, during which time most of the production diseases occur with much greater frequency (Drackley et al., 2005). Our objective here is to provide a brief framework for the types of interactions that can occur, and then describe some of our most recent findings that may provide

new clues to both the etiology of production disease as well as mechanisms for their pathology. We will also discuss some of the evidence that nutritional status may influence these regulatory interactions.

Cross-talk between metabolism and the immune system

Many examples could be cited for interactions between the immune system and metabolism, but we will only focus briefly on two: 1) calcium metabolism and immune function, and 2) obesity as an inflammatory disease. We then discuss in more depth the potential role of inflammatory responses with development of fatty liver.

Calcium and immunity

Dairy cows undergo marked immune suppression during the period around parturition as functions of both mononuclear cells and neutrophils decline as cows approach calving (Kehrli et al., 1989a,b). During this same time, blood calcium concentrations decline as a result of the calcium demand for the initiation of lactation, with problems resulting from hypocalcemia being common (Goff and Horst, 1997). Might these two phenomena be related?

A suggestion that they might be interdependent was provided by Goff and Kimura (2002) and Nonnecke et al. (2003), who found that mastectomy of pregnant cows prevented the sharp drop in blood calcium but also lessened the loss of blood immune cell function compared with intact cows. Because immune cell activation is dependent on increased intracellular concentrations of calcium ions as a second messenger for signal transduction (Lewis, 2001), Kimura et al. (2006) postulated that alterations in blood calcium status in periparturient cows might at least be a factor in the degree of immune suppression observed. Indeed, they found that intracellular calcium stores in peripheral mononuclear cells were decreased around parturition, and that less intracellular calcium was mobilized in response to an activation signal. Furthermore, decreases of immune cell calcium and calcium mobilization were more severe in cows with milk fever, and these changes were reversed after intravenous calcium infusion.

In addition to the significance of these findings to milk fever and infectious diseases, there are broader implications for the periparturient production disease complex. Waldron et al. (2003) observed that intramammary administration of lipopolysaccharide to dairy cows resulted in decreased blood calcium concentrations. There is evidence that impaired intestinal cell barrier function around parturition leads to varying degrees of transmigration of endotoxins from the gastrointestinal tract to the portal blood (Andersen et al., 1994, 1996). Production and release of endotoxins in the rumen may be greater on higher-concentrate diets fed during the transition period (Ametaj, 2005). Endotoxins from this source or from gram-negative bacterial infection therefore might lead to periparturient hypocalcemia, which in turn might lead to metabolic disorders such as secondary ketosis and retained placenta, as well as to

infectious diseases such as metritis and mastitis. The implications of this newly elaborated interrelationship between calcium and immune function for the etiology of production diseases are extensive and profound.

Obesity and inflammation

Research in the last decade has established that obesity can be considered as a chronic inflammatory disease (Wellen and Hotamisligil, 2003). As adipocytes accumulate lipids, production of various signaling molecules by the cells increases. Many of these molecules affect both immune and metabolic processes. A well-described example discussed elsewhere in these proceedings is that adipocytes produce the pro-inflammatory cytokine tumor necrosis factor- α (TNF α), which can disrupt insulin signal transduction and cause insulin resistance that in turn results in increased lipolysis (Gasic et al., 1998).

Recent research has established that cows with greater body condition score have greater circulating concentrations of TNF α (O'Boyle et al., 2006). Such cows also have indicators of greater oxidative stress (Bernabucci et al., 2005), thus making it likely that effects characterized to date in biomedical research also apply to dairy cows with greater fat stores. Of particular interest are the internal fat depots such as the mesenteric and omental adipose that drain directly into the portal system. If some cows accumulate lipid preferentially in internal depots as some humans do, then it is reasonable to propose that the inflammatory influences of lipid accumulation on hepatic function and metabolism would be greater in those cows. This possibility is discussed in more detail in a later section. Unfortunately it is difficult to access these internal depots for the time-sequence studies that are needed to test these hypotheses.

Does the immune system play a role in development of fatty liver and ketosis?

Since proposed by Bertoni's research group (Calamari et al., 1994), the idea that fatty liver may be caused or at least aggravated by non-specific inflammatory responses has received considerable attention. Bertoni et al. (1997) reported that the rate of culling of cows due to health problems in the transition period was increased when cows had plasma haptoglobin (an acute-phase protein produced in the liver) concentration greater than 1 g/L in the first 15 days postpartum. These cows also had lower albumin, bilirubin, and vitamin A in plasma. Katoh (2002) reviewed evidence for relationships between molecules of the immune system and inflammatory response with incidence of production diseases. More recently, Ametaj et al. (2005) and Bionaz et al. (2007) have presented evidence for the presence of acute-phase responses in periparturient dairy cows. Ametaj et al. (2005) showed that concentrations in plasma of TNF α , cortisol, and NEFA were greater before parturition in cows that developed fatty liver after parturition in response to overfeeding during the dry period. Postpartum, cows with greater hepatic fat accumulation had greater concentrations of the positive acute-phase proteins serum amyloid A and haptoglobin, and a lower concentration of the

negative acute-phase protein calcitonin gene-related peptide than did healthy cows. In cows sampled from two farms under field conditions, Bionaz et al. (2007) found that cows with greater incidence of periparturient health problems had lower concentrations in blood of the protein paraoxonase, a negative acute-phase protein, than healthy cows. Diseased cows also presented greater concentrations of haptoglobin and reactive oxygen metabolites compared with healthy cows, consistent with the results of Ametaj et al. (2005).

Many of the products induced during the acute phase response are believed to help bind endotoxins in blood and deliver them to the liver for excretion into bile. Based on the changes that occur in cows in response to activation of the acute-phase response (Kato, 2002), Ametaj (2005) proposed that fatty liver developed in response to non-specific inflammation around parturition. Specifically, endotoxins bound to lipoproteins would be cleared from circulation by the liver via endocytosis. The endotoxins would be removed from the rest of the lipoprotein complex and excreted into the bile. As triacylglycerols (TAG) were taken up into the liver as a component of lipoproteins, fatty liver would result as the fatty acids arising from uptake of TAG-containing lipoproteins were reesterified.

Although a novel hypothesis, there are few data available to provide direct support. Moreover, there are several difficulties with the hypothesis. First, given the low concentration of TAG in blood of cows, it seems unlikely that endocytotic uptake of lipoproteins could occur at large enough rates to account for the rapid development of hepatic TG after calving. This is particularly problematic given the fact that lipoproteins are not cleared by the liver until the bulk of the TAG have been lipolyzed in peripheral tissues, making the load of TAG available to the liver much less. In contrast, estimates of the rate of NEFA delivery and accumulation as TG in liver are entirely consistent with known biology (Drackley et al., 2001). Moreover, if uptake of lipoprotein-TAG and other lipids were responsible for hepatic lipidosis, one would expect differences in plasma TG between cows that develop and do not develop fatty liver. However, data from Ametaj et al. (2005) show no difference in postpartal plasma TG concentration between cows without and with fatty liver.

Recent data from our laboratory also seem to contradict the Ametaj hypothesis. In an experiment designed to study the development of an induced ketosis, multiparous cows were classified as healthy or not based on a thorough physical examination at d 4 postpartum (Dann et al., 2005). Healthy cows were divided into a group that remained on ad libitum consumption of TMR (controls) and another group that had the amount of TMR offered decreased by 50% beginning on d 5 and continuing until development of clinical ketosis signs or until d 14. Cows that were classified as not healthy at d 4 mostly had retained placenta uncomplicated by severe metritis. Some pertinent results are presented in Table 1. At 1 d postpartum, healthy cows and diseased cows did not differ in total lipid concentration in liver (Table 1). In addition, serum concentrations of NEFA, cholesterol, ceruloplasmin (a negative acute-phase protein), haptoglobin, or bilirubin did not differ between healthy and diseased cows. Cows with disease had higher serum activity of glutamate-oxaloacetate transaminase (aspartate

aminotransferase, GOT/AST) and lower Vitamin A, indicating some impairment of liver function. At diagnosis of clinical ketosis or d 14 postpartum, cows with induced ketosis has significantly greater total lipid concentration in liver, whereas the healthy controls and peripartal disease cows had similar and lower total lipid (Table 1). At d 7 postpartum, which was 3 d after starting feed restriction for ketosis induction, serum NEFA was greater for ketotic cows but similar for healthy and diseased cows. Surprisingly, d 7 values for cholesterol, GOT/AST, ceruloplasmin, haptoglobin, and vitamin A were similar for healthy and ketotic cows but were indicative of acute-phase responses and liver impairment for diseased cows. Thus, the blood indicators of acute-phase response and decreased liver function revealed alterations in diseased cows, but liver lipid was not elevated. Alternately, cows with greatly elevated total lipid concentration in liver (i.e., those with induced ketosis) did not display altered blood chemistry during development of the fatty liver, with the exception of increased bilirubin.

Although the direct involvement of the acute phase response in development of fatty liver does not have strong support, there is still ample evidence that the immune system and metabolism have extensive interactions during the peripartal period. It seems more likely that cytokines and acute phase responses somehow potentiate the well-described processes of NEFA mobilization and hepatic esterification rather than result in large increases in lipoprotein TAG uptake. Indeed, there is some evidence that cytokines may increase delivery of NEFA to the liver. Administration of recombinant (rb) TNF α to heifers resulted in increased circulating NEFA in plasma (Kushibiki et al., 2000; Kushibiki et al., 2002), despite periods of greater blood glucose concentration in the hours immediately following infusion. In another study by the same group, large increases in haptoglobin, NEFA, and cortisol were observed in response to intravenous infusion of rbTNF in lactating dairy cows compared to lactating cows infused with saline (Kushibiki et al., 2003). Although these responses might only represent acute responses to an endotoxin challenge, TNF α produced as a result of chronic inflammation or by adipose tissues might produce similar effects. In sheep, obese ewes had greater circulating TNF α than thin ewes, which was found to be highly related to backfat thickness (Daniel et al., 2003). Fasting increased TNF α in circulation for thin ewes, but decreased it in fat ewes. Lactating cows fed *ad libitum* intake tended to have decreased TNF α in serum compared with cows fed a restricted amount of feed to induce NEB (Capuco et al., 2001).

Table 1. Liver total lipid and serum components in healthy cows, cows with induced ketosis, and cows with periparturient diseases (from Dann et al., 2005 and unpublished)¹

Variable	Group			SE
	Healthy		Disease	
Day 1 postpartum				
Liver total lipid, %	6.4		7.3	0.5
Serum				
NEFA, μM	1118		1091	104
Cholesterol, mM	2.18		2.08	0.12
GOT/AST, U/L	73.3		89.6 ²	11.5
Ceruloplasmin, μM	2.03		1.93	0.12
Haptoglobin, g/L	0.98		0.75	0.15
Bilirubin, μM	5.15		6.69	1.36
Vitamin A, mg/dL	24.3		19.7 ³	2.0
Day 7 postpartum	Healthy	Induced ketosis	Disease	SE
Serum				
NEFA, μM	710 ^b	1569 ^a	725 ^b	140
Cholesterol, mM	2.32 ^a	2.32 ^a	1.92 ^b	0.12
GOT/AST, U/L	77.1 ^b	84.6 ^b	113.4 ^a	13.0
Ceruloplasmin, μM	2.36 ^a	2.06 ^a	1.64 ^b	0.12
Haptoglobin, g/L	0.81 ^b	0.78 ^b	1.62 ^s	0.35
Bilirubin, μM	1.93 ^b	4.16 ^{ab}	5.88 ^a	1.22
Vitamin A, mg/dL	26.4 ^a	24.6 ^a	17.9 ^b	1.5
Day 14 or ketosis				
Liver total lipid, %	5.5 ^b	13.5 ^a	6.2 ^b	1.3

¹ Cows were classified at d 4 as either healthy or diseased based on a physical examination. From d 5 to clinical ketosis was detected, or d14, healthy cows either continued to be fed ad libitum (healthy controls) or had feed offered restricted to 50% of mean for d 1 to 4 postpartum (ketosis induction). The diseased cows comprised almost entirely cows with uncomplicated retained placenta. Except for NEFA, blood measurements were performed by G. Bertoni and E. Trevisi, Piacenza, Italy.

² $P = 0.16$.

³ $P < 0.05$.

^{ab} Means within a row without common superscripts differ ($P < 0.05$).

Synthesis of the acute-phase proteins is induced by glucocorticoids and cytokines, which normally aid the immune system in recovering from insults or injury. In rodents, accumulation of fatty acids in hepatocytes and fatty acid oxidation result in oxidative stress that can cause the release of cytokines and interleukins from Kupffer cells in the liver (Reddy and Sambasiva Rao, 2006). In cows, serum activity of TNF α increased as deposition of TG in the liver increased in a study designed to retrospectively evaluate non-experimentally induced fatty liver (Ohtsuka et al., 2001). Insulin-stimulated glucose disposal rate was also associated with degree of TG

deposition in liver; those with more TG had lower rates of glucose disposal (Ohtsuka et al., 2001). The glucose disposal rate and serum TNF- α activity were negatively correlated ($r = -0.56$), supporting the induction of insulin resistance in cows similar to the well-documented situation in non-ruminant species.

Other proinflammatory cytokines also have been implicated in periparturient dysfunction. Cows that developed metritis postpartum had a greater concentration of interleukin-6 (IL-6) prepartum and lower IL-6 was observed in cows that developed retained placenta (Ishikawa et al., 2004). The cytokines IL-6 and IL-1 β , as well as TNF α , increased the production of haptoglobin and reduced production of albumin in bovine hepatocytes cultured in vitro (Yoshioka et al., 2002). Haptoglobin production was maximal when hepatocytes were cultured with the combination of IL-6 and TNF- α .

Cytokines and adipose tissues

Adipose tissue produces a variety of adipocytokines that have been linked to insulin resistance in humans (Wellen and Hotamisligil, 2003). Of these, TNF- α is one of the most studied (Gasic et al., 1998; Hotamisligil et al., 1993; Ruan et al., 2002). The production of other acute phase reactants and complement components also has been linked to adipose tissue depots in humans (Gabrielsson et al., 2003; Lin et al., 2001). Large depots of visceral adipose have most often been linked to insulin resistance and inflammation in humans (Bertin et al., 2000; Kabir et al., 2005; Yang et al., 2003); however, subcutaneous abdominal adiposity has been linked even more strongly to insulin resistance in humans (Kelley et al., 2000). The profile of adipocytokines differs among adipose tissue depots in both humans (Ruan et al., 2002; Yang et al., 2003) and dairy cows (Lor et al., 2004).

Little is known about the profile of products produced in adipose tissue of periparturient dairy cows, and whether adipose depots differ in cytokine products. Cows that die as a result of 'fat cow syndrome' (Morrow, 1976) often have large depots of internal adipose (heart, kidney, pelvic canal, omentum) even though body condition score may be "normal". A study characterizing the adipose depots in thin, normal, and fat cows demonstrated that even thin cows may have internal depots of adipose (mesenteric and omental) that are not different from normal cows (Shemeis et al., 1994). This study also showed that fat cows start to accumulate a great deal of adipose around the internal organs. Whether rates of lipolysis under basal and catecholamine-stimulated conditions differ between subcutaneous and internal adipose depots remains controversial. Regardless, the venous drainage from omental and mesenteric depots enters the portal circulation and therefore has a large potential to affect metabolism and function of the liver.

Recent models of interactions of the immune system with metabolism in liver

As described above, the exact nature of interactions between immune products and metabolism remains uncertain. We have used molecular approaches to characterize some of the interrelationships at the level of gene expression.

We have used a bovine cDNA microarray to profile transcript changes across the transition period in cows fed a standard dry period diet (Loor et al., 2005). Changes were consistent with involvement of the immune system in affecting metabolism around parturition, as well as changes that might increase oxidative stress in liver. Although expression of *TNFA* was only slightly increased around parturition, *SAA1* was markedly upregulated at 1 d after calving. In a follow-up study using the same techniques, we found differences in response of hepatic gene expression due to over- or undernutrition during the dry period. From changes we observed, we proposed a model to explain differences due to prepartal nutrition that accounted for both immune and metabolic observations (Figure 1).

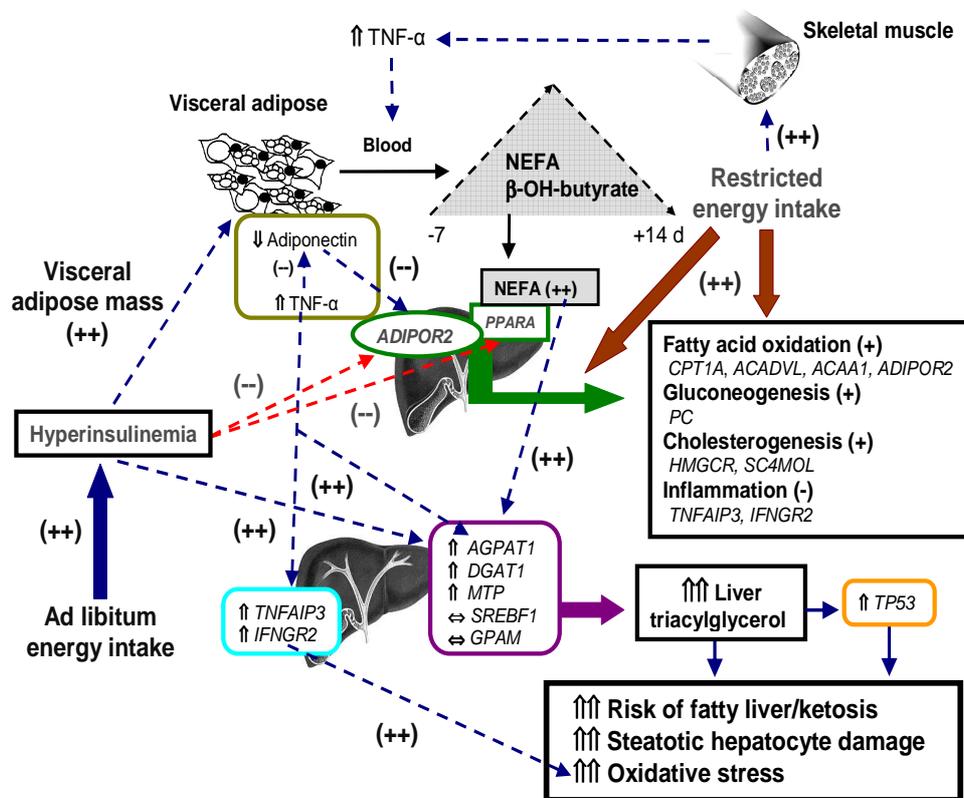


Figure 1. Schematic representation of interactions among gene expression patterns in liver and physiological events in visceral adipose, skeletal muscle, and liver tissue during the periparturient period in response to ad libitum or restricted feeding of

moderate energy diets. Data indicate that hyperinsulinemia induced by overfeeding a moderate energy diet pre-partum has a direct role in promoting 1) visceral adipose mass accumulation leading to up-regulation of TNF- α and concomitant down-regulation of adiponectin in adipose tissue, 2) selective up-regulation of hepatic lipogenic genes, and 3) selective down-regulation of hepatic fatty acid oxidation genes. Both lower serum adiponectin, due to excessive visceral adipose tissue accumulation, along with hyperinsulinemia could signal a reduction of or hamper *ADIPOR2* expression in liver, whereas higher circulating serum TNF- α in portal blood is a positive signal for up-regulation of hepatic pro-inflammatory cytokines (e.g., IFN- γ). Up-regulation of *TNFAIP3* in overfed cows likely is a necessary response to dampen effects of TNF- α and NF- κ B-induced signaling. Another crucial effect of excessive visceral adipose tissue mass accumulation is higher serum NEFA early post-partum. Overfed cows appear to have lower capacity to completely oxidize NEFA in liver (e.g., higher serum BHBA) as shown by reduced expression of *ADIPOR2*, *ACADVL*, *ACAA1*, and *CPT1A* mRNA post-partum, but have higher capacity to store TAG as shown by up-regulation of *AGPAT1*, *DGAT1*, and *MTP*. Overfeeding pre-partum had little effect on *GPAM* and *SREBF1* pre- and post-partum, suggesting they do not play a crucial role in liver lipidosis early post-partum. However, these data reflect mRNA levels and do not discount effects of nutrition on protein expression. Restricted energy intake allows for up-regulation of fatty acid oxidation, gluconeogenesis, and cholesterol synthesis while dampening pro-inflammatory responses in liver. It is possible, however, that caloric restriction might lead to excessive skeletal muscle tissue breakdown close to parturition and induce an inflammatory response in this tissue that could result in greater systemic TNF- α . Uncontrolled TAG accumulation in liver and oxidative stress increases the risk of periparturient health disorders by predisposing the cow to fatty liver and ketosis, but also by up-regulating *TP53* expression, which could increase steatotic hepatocyte damage. (From Loores et al., 2006).

Most recently, we have used an expanded 13,000-sequence oligonucleotide microarray to characterize gene expression profiles in control cows and those with induced ketosis early postpartum (Loores et al., 2005). We have used a pathway analysis software package (Ingenuity Pathway Analysis) to form related changes into networks that describe relationships within and between pathways. An interesting finding was the potential central importance of increased IL-6 in liver of ketotic cows. This cytokine interacted with several other transcription factors and metabolic pathways.

In our studies of transition metabolism and ketosis, we have accumulated evidence for a central role of the nuclear transcription factor, peroxisomal proliferator-activated receptor α (PPAR α). This isoform is most abundantly expressed in tissues with high rates of fatty acid catabolism such as liver (Feige et al., 2006). Activation of PPAR α by binding of its ligands (NEFA) leads to increased transcription and translation of enzymes involved in mitochondrial and peroxisomal β -oxidation, which are termed PPAR-responsive genes. Upregulation of fatty acid oxidation machinery during periods of NEFA mobilization is necessary to avoid extensive accumulation of TAG in liver (Drackley and Andersen, 2006). Glucocorticoids regulate the expression of PPAR- α in the liver to induce the expression of β -oxidation genes such as CPT-1

(Desvergne et al., 2006; Engeli et al., 2003). Expression of HMG-CoA synthase is regulated by both hepatocyte nuclear factor-4 (HNF4; another transcription factor of importance in the liver) and PPAR α (Hegardt, 1997). Although the networks generated from pathway analysis are much more complex and not shown here, the key features of the potential role of PPAR α are shown in Figure 2. The mRNA for PPAR-responsive oxidation genes was increased as expected. At the same time, PPAR α activity suppresses the inflammatory response via inhibition of IL-6 and C-reactive protein synthesis, a result of a PPAR-responsive protein interfering with IL-1 signaling through NF κ B (Feige et al., 2006; Wellen and Hotamisligil, 2005). Consequently, factors such as periparturient nutrition and environmental stressors that regulate the expression and activation state of PPAR α may be important in development of production diseases. A plausible hypothesis is that environmental and physiological influences that result in suboptimal activity mediated by PPAR α is a major component mechanism that leads to periparturient production diseases in dairy cattle. These working hypotheses are under continued investigation in our laboratories.

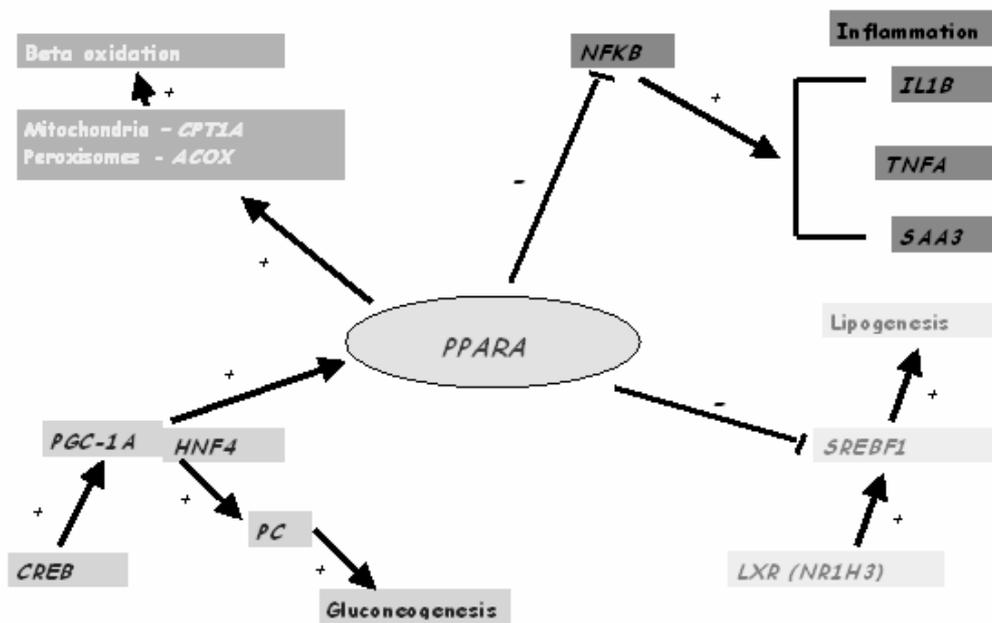


Figure 2. Schematic of the potential interactions of peroxisome proliferator-activated receptor α (PPARA) with other nuclear receptors and downstream genes involved with beta oxidation, gluconeogenesis, lipogenesis, and inflammation. Activation of PPAR α leads to increased transcription of genes encoding fatty acid oxidation enzymes in mitochondria and peroxisomes. The transcription factors PGC-1A and HNF4 can stimulate transcription of PPARA. At the same time, PPAR α inhibits lipogenesis by suppressing transcription of SREBF1, and also modulates inflammation by inhibiting NF κ B, thereby decreasing IL1, IL6, and TNF α . Abbreviations for gene names: ACOX1 (acyl-coenzyme A oxidase 1, palmitoyl), CPT1A (carnitine palmitoyltransferase 1a), HNF4A (hepatic nuclear factor 4, alpha), IL1B (interleukin 1, beta), NF κ B (nuclear

factor, kappa B), *NRIH3* (nuclear receptor subfamily 1, group H, member 3), *PC* (pyruvate carboxylase), *PGCIA* (peroxisome proliferative activated receptor, gamma, coactivator 1, alpha), *PPARA* (peroxisome proliferator activated receptor, alpha), *SAA3* (serum amyloid A 3), *SREBF1* (sterol regulatory element binding transcription factor 1), and *TNFA* (tumor necrosis factor, alpha). From Janovick-Guretzky, 2007, unpublished.

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6.3 METABOLIC CHANGES IN DAIRY COWS INDUCED BY ORAL INTERFERON-ALPHA TREATMENT

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Parturition in dairy cows is characterized by inflammatory-like conditions, as shown by the rise of positive acute phase proteins in both healthy and sick cows and, conversely, by the reduction of negative acute phase serum proteins (albumin, lipoproteins). Owing to the above, oral interferon alpha treatment in the last 10-15 days of pregnancy was investigated as a possible control strategy of such inflammatory response, because of the favourable results obtained in other animal models. The trial was realized in a commercial farm. Dry and lactating cows were kept in loose-housing stalls and fed total mixed ration once a day. Twenty-one multiparous Friesian dairy cows were allocated to 2 homogeneous groups: 10 cows received 10 IU/kg/day human interferon alpha per os in the last 10-15 days of pregnancy (IFN), and 11 cows were used as control (CTR). Blood samples were collected from the jugular vein before feeding: weekly, during the last month of pregnancy and the first of lactation, and 1 and 3 days from calving (DFC). Beside a wide metabolic profile, positive (haptoglobin) and negative (albumin, paraoxonase, vitamin A) acute phase proteins, interleukin-6 (IL-6) and Tumor Necrosis Factor alpha (TNF-alpha) were determined, as well as milk yield, body condition score (BCS), health status and reproduction traits. Data were statistically processed using a repeated-measure procedure, including treatment, DFC and interaction in the model. During the first month of lactation, IFN-treated cows showed higher weight loss (-0.47 vs -0.33 BCS points) and lower milk yield (about 2.5 kg/cow/day less). Plasma glucose was slightly lower in the IFN group after the beginning of treatment and showed a marked reduction at DFC 3. Free fatty acids and beta-OH-butyrate were also higher in the IFN group around parturition. In several cows of both groups IL-6 and TNF-alpha were increased before and around calving. TNF-alpha rose in about 50% of cows of each group, while the incidence of cows with IL-6 response was higher in the IFN group (40 vs 18%). After calving, the IFN group showed a significant higher and longer haptoglobin (vs CTR from DFC 3 to 10) and ceruloplasmin responses. The IFN group also showed a slower rise of negative acute phase proteins, in particular retinol binding protein and lipoproteins (significantly from DFC 10 to 30), measured as vitamin A and total cholesterol respectively.

In conclusion, cows around calving show an increased inflammatory response following low-dose interferon alpha treatment as opposed to monogastric animals.

6.4 EFFECTS OF ACETYLSALICYLATE TREATMENT IN DAIRY COWS AROUND CALVING ON METABOLISM AND PERFORMANCE

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Dairy cows at calving are characterized by inflammatory conditions which can contribute to their increased susceptibility to periparturient diseases and health disorders and to the lowered performance. In the attempt to reduce inflammations, we have previously explored the use of a non steroidal anti-inflammatory drugs (e.g. acetylsalicylate) immediately after calving. The results were generally positive, but often the blood inflammatory indices were not significantly modified, likely because inflammatory events started before parturition.

Therefore, to verify the possibility to minimize the inflammation effects around calving on metabolism and on performance in dairy cows, the anti-inflammatory treatments have been anticipated some days before calving. Nineteen Friesian multiparous dairy cows, kept in a loose-housing stall and fed with total mixed ration once a day, were divided into 2 homogeneous groups: 9 cows received 30 g of acetylsalicylic acid per os (AS), from 7 days before to 3 days after calving; 10 cows were used as control (CTR). Each cow was weekly bled from the jugular vein before feeding, during the month before and after calving. Blood samples were also taken at -3, 1, 3 days from calving (DFC). Besides a large metabolic profile, milk yield, body condition score (BCS), body temperature, health status and reproduction traits were monitored. Data were statistically processed using a repeated-measures procedure, including treatment, DFC and their interaction in the model. The farm was characterized by a quite high frequency of diseases around calving and the AS treatment did not modify the incidence of clinical cases. Body temperature was not affected by treatment, but one cow of CTR without clinical symptoms had a fever event 3 DFC. During the first 30 DFC, AS cows showed slightly lower body losses (-0.27 vs -0.32 BCS points) and a higher milk yield ($P < 0.09$), confirming the results of previous researches. AS cows did not show difference on plasma glucose and beta-OH-butyrate, but they had a higher level of free fatty acids ($P < 0.05$). Despite haptoglobin showed in both groups the typical post calving rise, any cow of AS showed a rise before calving; moreover the recovery after the peak was quicker. Between negative acute phase proteins, albumin of AS group showed a shorter reduction after calving, while vitamin A showed a quicker rise during the first 30 DFC ($P < 0.05$ vs CTR).

To conclude, the acetylsalicylic acid treatment - also when administered before calving - has confirmed his positive effect on milk yield and has attenuate the inflammatory processes around calving. This latter, besides better liver functions, has likely reduced the energy losses due to clinical and subclinical diseases, saving more nutrients for mammary.

6.5 ORAL LIPOPOLYSACCHARIDE PREVENTS DISTURBANCES OF PLASMA METABOLITES IN TRANSITION DAIRY COWS

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Endotoxin released in the ruminal fluid of transition dairy cows fed high proportions of grain has been implicated in multiple metabolic disorders like laminitis, fatty liver, liver abscesses, and displaced abomasum. Prevention of endotoxin translocation into the blood circulation might avoid harmful effects of endotoxin. It is known that repeated exposure of mucosal epithelium to bacterial antigens is associated with induction of tolerance to that antigen.

Therefore, the objective of this research was to study the effects of repeated oral administration of lipopolysaccharide (LPS) on plasma metabolites in transition dairy cows. Sixteen pregnant multiparous Holstein dairy cows were assigned to 2 treatment groups 2 wk before the expected day of parturition. Cows were administered orally, twice per week, for 3 consecutive wk starting 2 wk before parturition the following treatments: 2 mL of saline (control), or 2 mL of saline containing LPS from *E. coli* 0111:B4. The amount of LPS administered each wk was 0.01, 0.05, or 0.1 micg/kg BW (~650 kg cow). Blood samples were obtained from the jugular vein twice per wk before administration of the treatments as well as once per week during wk 2, 3, and 4 after parturition and plasma non-esterified fatty acids (NEFA), beta-hydroxy butyric acid (BHBA), glucose, lactate, cholesterol, and insulin were measured. Records of all clinical metabolic diseases were obtained during 2 wk before and up to 8 wk after parturition. Plasma concentrations of NEFA and BHBA increased linearly in control cows during transition period reaching peak values 2-5 wk postpartum, respectively; however, both NEFA and BHBA changed only slightly and remained within normal ranges in cows treated with oral LPS. Furthermore, plasma insulin decreased linearly in control cows until 6 wk after parturition, whereas plasma insulin remained unchanged around parturition in cows treated with oral LPS. Concentration of glucose in plasma was higher in cows administered LPS compared to those of control cows during the whole experimental period. Plasma lactate also was higher in treated cows during the 2 wk following parturition. No effect of treatment was obtained for plasma cholesterol. Clinical data showed significantly lower incidence of metabolic disorders in cows treated with oral LPS.

Taken together results of this study indicate that repeated oral administration of LPS from *E. coli* 0111:B4 during peripartum prevented metabolite disturbances typically observed in dairy cows around parturition. This suggests a causal role for endotoxin in the etiology and pathogenesis of metabolic disorders in dairy cows and the potential utilization of oral treatment against LPS for prevention of endotoxin translocation and its harmful effects.

6.6 COMPARISON OF THE MRNA EXPRESSION OF ADIPONECTIN, ADIPONECTIN RECEPTOR 1, ADIPONECTIN RECEPTOR 2, AND LEPTIN IN DIFFERENT ADIPOSE DEPOTS IN SHEEP

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The adipokines adiponectin and leptin are important in lipid metabolism and glucose homeostasis. Adiponectin is inversely related to leptin secretion and is associated with insulin resistance. Data on the expression of adiponectin and its receptors AdipoR1 and AdipoR2 are mainly limited to monogastric species, whereas for ruminants corresponding data are scarce. To understand the influence of these adipokines and their receptors on metabolism and health in ruminants, basic data are needed for these species.

We therefore aimed to characterize the mRNA expression for adiponectin, AdipoR1, AdipoR2 and leptin mRNA in different ovine fat depots. Subcutaneous (sc) and visceral (vc) fat was dissected from carcasses of 10 intact male crossbred sheep (40 kg live weight at slaughter; Blackheaded Mutton x East Frisian Milk sheep). Sc fat was sampled at 3 different sites: close to sternum (S), withers (W) and base of the tail (T); vc fat was sampled from perirenal (P) and mesenteric (M) depots. The mRNAs of adiponectin, AdipoR1, AdipoR2 and leptin were quantified by real-time RT-PCR. Data were analysed by ANOVA and Spearman's rank order correlation. No differences between depots were found for adiponectin ($p=0.36$); whereas AdipoR1 mRNA concentrations were higher ($p<0.05$) in P compared to W, T and M. Equally, AdipoR2 mRNA concentrations were higher ($p<0.01$) in P compared to W, T and M. Leptin mRNA concentrations were higher in S than in both vc depots ($p<0.05$). Sc leptin mRNA values were correlated with the total sc fat fraction of the carcass ($r=0.41$, $p<0.05$) but not with vc fat. In contrast, vc adiponectin tended to be correlated with the vc carcass fat ($p=0.1$) but not with sc. In addition, adiponectin and AdipoR2 were correlated with each other in sc depots ($r=0.52$, $p<0.05$), but not in vc. In both, sc and vc fat, AdipoR1 and AdipoR2 were correlated (sc: $r=0.48$, $p<0.05$; vc: $r=0.78$, $p<0.01$). A trend for a correlation was found between AdipoR1 and adiponectin ($p=0.08$; vc: $r=0.41$, $p=0.09$) in both adipose depots.

In contrast to reports from monogastric species, we observed a constant expression of adiponectin mRNA in different ovine fat depots. AdipoR1, AdipoR2 as well as leptin mRNA seems to be differentially expressed in different adipose depots of sheep.

6.7 INFLUENCE OF 17BETA-OESTRADIOL, NORTESTOSTERONE AND DEXAMETHASONE ON THE ADAPTIVE IMMUNE RESPONSE IN VEAL CALVES

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Problem: In veal calf production steroid hormones like androgens, estrogens and glucocorticoids are used to stimulate growth. Sexhormones and glucocorticoids however, also influence the function of the immune system. From studies in humans and mice, androgens are known to be immunosuppressive, while estrogens stimulate the production of antibodies and glucocorticoids also enhance the T-helper 2 response.

Aim: The aim of the study was to investigate whether the adaptive immune system is influenced by steroid hormone administration. **Material & methods:** Calves were treated twice with a hormone cocktail containing androgens and estrogens twice and once with glucocorticoids. The immune system was triggered thrice by vaccination against *Mycobacterium avium* spp. paratuberculosis. The activity of the adaptive immune system was measured by using an antigen specific elispot assay (ES), lymphocyte stimulation test (LST) and an enzyme-linked immuno sorbent assay (ELISA). At post-mortem examination macroscopic changes in the lungs, the organs of the immune- and reproductive system were recorded by observation, palpation and slicing.

Results: The stimulation index (SI) of the heat shock protein 70 (HSP70) response showed a vaccination effect after the third treatment in all vaccinated animals. The ES does not show a significant increase of interferon- γ production in the vaccinated group. There is a significant increase in antibody production in the vaccinated groups. The post-mortem findings showed no specific pattern of distribution among the treatment groups.

Conclusion: The hormone treatment did not result in significant differences in the function of the adaptive immune system between the hormone treated and the not hormone treated group while growth was stimulated in the hormone treated group.

6.8 IMMUNOGLOBULIN DYNAMICS IN CALVES FROM TWO HERDS IN AGE FROM 1 TO 24 WEEKS

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The aim of the research was to investigate, how the concentration of immunoglobulin classes IgG, IgM and IgA in calves, alters with age and to investigate the differences between herds. The research was performed on dairy calves from two herds with different feeding system for calves. 37 calves were from one herd (group 1) and 34 calves from the other herd (group 2). The blood samples from calves were taken from 1 to the 24 weeks of age. The concentration of IgG, IgM and IgA was measured in serum samples with quantitative ELISA. The average concentration of IgG, in the first week of life was 28.021 ± 15.416 g/L in group 1 and 24.111 ± 14.876 g/L in group 2. The level of IgG decreased from the 1st to the 3rd week and come to 24.327 ± 13.674 g/L in group 1 and 14.608 ± 8.293 g/L in group 2. At the age of 24 weeks it reached the highest value in the investigated period in group 1 (46.346 ± 12.307 g/L). In group 2 the highest average concentration of IgG was established at the age of 20 weeks (35.067 ± 14.572 g/L). In all bleedings except in the 1st week of age, the differences between herds were statistically significant. The IgM level in the group 1 decreased from the first week (median (M) = 1.738 g/L; 1st quartile (q) = 0.601 g/L; 3rd q = 2.562 g/L) to the 4th week of age. In group 2 the IgM level was higher in 2nd week (M = 1.645 g/L; 1st q = 0.716 g/L; 3rd q = 1.948 g/L) and decreased to the 3rd week of age. In both groups the IgM level was the highest in the 24th week of age; in group 1 comes to M = 6.566 g/L (1st q = 4.341 g/L; 3rd q = 10.431 g/L) and in the group 2 was M = 5.270 g/L (1st q = 4.422 g/L; 3rd q = 8.735 g/L). In the group 1, the IgA level was higher in the first week of life (M = 0.914 g/L; 1st q = 0.388 g/L; 3rd q = 1.896 g/L), then decreased to the 3rd week. In group 2 the level of IgA decreased from the 1st week (M = 0.278 g/L; 1st q = 0.111 g/L; 3rd q = 0.886 g/L) to the 4th week of age. At the age of 24 weeks, the IgA level was lower in the group 1 (M = 0.504 g/L; 1st q = 0.254 g/L; 3rd q = 0.786 g/L) in comparison to group 2 (M = 1.234 g/L; 1st q = 0.983 g/L; 3rd q = 1.540 g/L). Regarding to the IgA level, statistically significant differences between groups were established by all bleedings except in 3rd and 4th week. The concentrations of all immunoglobulin classes increased after the 3rd or the 4th week which indicates that the activity of own immune system increased with age. The differences between groups were the most significant regarding to the IgG level and were increasing with age.

These results indicate that colostrum supply is important for the health status of the calves in first weeks of life. But other factors (feeding, environment..) have an important influence on later development of the immune system.

6.9 A NOVEL PORCINE NUCLEAR COFACTOR IN METABOLIC DISEASE

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Problem: 400 million adults worldwide are obese and more than 190 million people presently suffer from diabetes mellitus. These diseases depend on lifestyle and on genetic predisposition. In human, Baumgartner et al isolated a novel nuclear cofactor, named DOR, whose expression varies in tissues between normal and obese subjects. A polymorphism in the human DOR promoter, which influences expression levels, is associated with both, obesity and type 2 diabetes (T2D).

The aim of the present study is to identify polymorphisms in the porcine DOR gene and to assess whether they have an effect on DOR expression or function. In order to develop an animal model for the role of DOR on the pathophysiology of obesity and diabetes, functional SNPs are being assessed for association to fat and diabetes parameters in population studies.

Materials and methods: A porcine genomic PAC library was screened and a clone containing the DOR gene was isolated and sequenced. Genomic DNA samples from seven races, including meaty and fat races, were used for single nucleotide polymorphism (SNP) detection. DNA was isolated using the QiAMP Kit (Qiagen) according to the manufacturers protocol. The DOR gene was amplified by PCR in at least 8 animals of each race and the PCR products were directly sequenced using the ExoSAP-IT System (GE Healthcare). For luciferase assays, mouse skeletal muscle C2C12 cells were used for transfection with Rotifect (Roth). The luciferase activity was measured using the Dual-Luciferase Assay System (Promega). Furthermore, by RH-panel-assay we determined the chromosomal localisation of the DOR gene in pig and screened the literature for reports on QTLs in the respective genomic region.

Results: In the present study we detected several SNPs in the porcine DOR gene. One is localized in the promoter region (SNP1), where two alleles are found, A or G. In meaty European races we only detected the G form. In the extreme fat Chinese race Erhualian we found both alleles, whereas in the Göttinger Minipig, which has a strong predisposition for diabetes, the A genotype was conserved. In preliminary luciferase assays the A and G alleles showed different transcription activity.

Conclusions: SNP1 seems to have an influence on DOR gene expression. Interestingly, the A allele was found either in a fat race or in a T2D prone race. Based on this observation, animals of additional races showing traits relevant for the present study are being genotyped. It remains to be confirmed by in vivo expression studies and population studies, whether SNP1 influences steady state RNA levels. In this case, the respective breeds will be highly valuable animal models for the role of DOR in metabolic diseases, both for elucidating molecular mechanisms and therapy.

6.10 ENERGETIC PROFILE AND ACUTE PHASE PROTEINS IN DAIRY COWS

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The aim of this study was to evaluate the concentrations of selected acute phase proteins – haptoglobin (Hp) and serum amyloid A (SAA) – in blood serum of dairy cows in relation to different phases of reproduction cycle, as well as to some variables of energetic metabolism (glucose, total cholesterol, triglycerides, total lipids, nonesterified fatty acids, β -hydroxybutyrate). The analysis were performed in dairy cows of a Slovak spotted breed on farm with modernized technology of breeding with loose housing and feeding by feeding ration according to the phase of lactation and milk production. The animals were divided into groups according to the reproduction period – from 4 weeks before parturition to 10 weeks after parturition. Hp and SAA were assessed by method of enzyme linked immunoassay using ELISA sets of company Tridelata Development. Glucose, total cholesterol, triglycerides and total lipids were assessed using automatic analyser Alizé. In case of Hp and SAA we found significant differences in average values of their cocentrations in several groups during the monitored period ($P < 0.05$ and $P < 0.001$, respectively). The Hp and SAA concentrations in cows during early postparturient period were significantly higher compared with later postparturient period. We found significant differences of average values throughout the monitored time in total cholesterol ($P < 0.01$), triglycerides ($P < 0.001$), total lipids ($P < 0.05$), and nonesterified fatty acids ($P < 0.01$). In assessment of correlation relations between monitored variables in the mentioned period we found significant correlations of between Hp and SAA ($r=0.916$; $P < 0.001$), Hp and nonesterified fatty acids ($r=0.766$; $P < 0.05$), SAA and nonesterified fatty acids ($r=0.775$; $P < 0.05$), glucose and total lipids ($r=0.733$; $P < 0.05$), total lipids and total cholesterol ($r=0.817$; $P < 0.01$), and between total lipids and nonesterified fatty acids ($r=-0.701$; $P < 0.05$).

Following abovementioned results we can summarize: in time around parturition there are significant changes in concentrations of acute phase proteins, as well as in complete energetic metabolism of dairy cows. We suggest that between concentrations of acute phase proteins and variables of energetic metabolism shortly before and after calving have been close relations. These mentioned facts may be attribute to important changes in metabolism of dairy cows after parturition. Key words: acute phase proteins, energetic metabolism, dairy cows

6.11 PROTEIN PROFILE AND ACUTE PHASE PROTEINS IN DAIRY COWS.

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In our study we aimed at comparison of concentrations of acute phase proteins and selected variables of protein metabolism in dairy cows of a Slovak spotted breed from 4 weeks before parturition to 10 weeks after parturition. The average utility on given farm within last years ranged about 6000 l milk per dairy cow. The cows were kept in loose housing and fed according to phases of reproduction and milk lactation. Acute phase proteins - haptoglobin (Hp) and serum amyloid A (SAA) - were assessed by method of enzyme linked immunoassay using ELISA sets of company Tridelta Development. Variables of protein metabolism (total proteins, albumin, urea, creatinin) were assessed using automatic analyser Alizé. We found significant differences in average values of the Hp and SAA concentrations in several groups during the monitored period ($P < 0.05$ and $P < 0.001$, respectively). The Hp and SAA concentrations in cows during early postparturient period were significantly higher compared with later postparturient period. We found significant differences of average values throughout the monitored time in total proteins ($P < 0.001$), urea ($P < 0.001$), and total immunoglobulins ($P < 0.05$). The concentrations of albumin, like one of representant of negative acute phase proteins, decreased progressively in postparturient period until 4 weeks after parturition. In assessment of correlation relations between monitored variables in mentioned period we found significant correlations between Hp and SAA ($r=0.916$; $P < 0.001$), total proteins and urea ($r=0.668$; $P < 0.05$), total proteins and total immunoglobulins ($r=0.827$; $P < 0.01$), and between total immunoglobulins and urea ($r=0.899$; $P < 0.001$).

Our results indicate that in time around parturition there are significant changes in concentrations of acute phase proteins, as well as in complete protein metabolism of dairy cows. These facts suggest, that the postparturient period is a critical biological phase, throughout which there is a highest incidence of metabolic disorders. Troublefree encompassment of this period is important regarding the development of health state of animals and the production all along the lactation period.

6.12 ASSESSMENT OF IMMUNOMODULATORY QUALITIES OF DIFFERENT PROBIOTICS IN LOCAL AND SYSTEMIC IMMUNITY OF PIGS

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We present investigations which aim at a mechanistic characterisation of the influence of two different probiotic bacteria on the immune system of piglets under standardized conditions and during infection with *Salmonella typhimurium*. Study 1: *Enterococcus faecium* NCIMB 10415 as probiotic bacteria - IgG and IgA were measured in the serum and faeces from sows and piglets using ELISA, and lymphocyte subpopulations from gut tissue were measured by flow cytometry. No significant differences in serum and faeces from sows for IgG and sIgA were recorded. However, probiotic treated piglets after weaning (28 d p.p.) had lower values for serum-IgG than control piglets. The amount of sIgA in faeces did not differ up to 56 d, but showed lower values from 70 d. Only a few CD8+ cells were seen in jejunum epithelium for the probiotic treated piglets (up to 8 weeks of life). No difference could be observed for CD4 + and CD8 + cells in Peyer's patches. The occurrence of pathogenic bacteria as *E.coli* O141 and *Chlamydia* spp. was decreases in probiotic-treated animals. Study 2: *Bacillus cereus* var. *toyoi* as probiotic bacteria - In consecutive cryostat sections from the proximal jejunum, absolute numbers of CD45, CD3, CD4, CD8, $\gamma\delta$ -T-lymphocytes, CD25, CD21 and CD11R1 cells were determined after labeling with fluorochrome conjugated antibodies. Significant differences were seen between treated and untreated animals with respect to the number of intraepithelial CD8 lymphocytes, CD25cells and $\gamma\delta$ -T-lymphocytes in the Lamina propria mucosae. Significantly more CD8, CD25 and $\gamma\delta$ -T-cells were found in the probiotic group on day 35. Analysis of the immune cell population in the blood showed no significant differences between the groups. However, the portion of CD21 lymphocytes in PBMCs was higher and a tendency for a reduced frequency of B-lymphocytes was noted in the probiotic group. Study 3: Challenge with *Salmonella* in the presence of *Enterococcus faecium* - Only provisional conclusions will be presented owing to the ongoing integrative evaluation of all available data and clinical parameters of our research network along with the statistical evaluation.

Presently, it can be stated that CD4 / CD8 double-positive lymphocytes from the blood as well as CD16 positive cells from the spleen are higher in probiotic treated animals. Funding by DFG through research network FOR 438 Schm 442/8-4 Contact: schmidt.mfg@vetmed.fu-berlin.de

6.13 FAT MOBILISATION SYNDROME AND BLOOD COAGULATION IN DAIRY COWS

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Problem: During the fat mobilization pro- and anti-inflammatory cytokines are released from the adipose tissue. These could cause a shock reaction and the lipolysis is increased by insulin inhibition. Dystocia with placental retention and endometritis following bacterial determined endotoxin release are caused by enhanced cytokine development. Cytokines could activate the coagulation cascade, that hemostasis disorders and a disseminated intravascular coagulopathy are found. Normal coagulation factors can not be formed by disorder of the liver function.

Aim: The aim of the study was to evaluate, if dairy cattle with fat mobilization syndrome or endometritis have coagulation disorders in dependence of disease at admittance into the clinic.

Material and methods: Blood was collected from 60 cows which were admitted into the clinic because of abomasal displacement and accompanied diseases. From these 60 cows, a basal therapy was given to group 1 (n=25) and additional a single dose dexamethasone was given to group 2 (n=25). 10 cows died in spite of intensive care. Comparative 36 healthy cows were tested. The numbers of thrombocytes and leucocytes were analyzed in EDTA blood and a number of hemostatic parameters like prothrombin time (PT), fibrinogen (FIB), reptilase time (RT), Antithrombin (ATIII), fibrin degradation products (D-Dimers), factor XIII (F XIII) and the activated protein C (APC) in citrated blood. Additional as acute phase proteins haptoglobin and Procalcitonin (PCT) were tested. **Results:** 50 cows could discharge from clinic after 4 days. 6 cows died within 24 hours and 4 cows died after 4 days. Fibrinogen was increased at first day and decreased while treatment. Antithrombin and factor XIII decreased in cows which died faster than in cows which were cured. The fibrin degradation products were slightly increased and were sunk while treatment. Cows which died had highly increased d-dimers. Procalcitonin and haptoglobin were increased at ill cows and were sunk like treatment.

Conclusions: Dairy cattle which suffer from fat mobilization syndrome or endometritis have a poor prognosis concerning the acute phase proteins fibrinogen, haptoglobin and procalcitonin and the hemostatic parameters especially antithrombin, factor XIII and d-dimers. A single dose of dexamethasone has no adverse effects to the coagulation, but shows a stabilized effect. An additional administration of heparin is indicated.

6.14 METABOLISM IN HEIFERS WITH DIFFERENT BODY CONDITION, THE ROLE OF ADIPOSE TISSUE

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Problem: Overconditioning of high yielding dairy cows before parturition often result in a combination of metabolic, digestive, infectious and reproductive conditions. These cows mobilise the most fat in comparison with healthy cows. The obesity is generalized throughout the body with extensive fatty metamorphosis in the liver. Recent studies in humans and other animals have shown that fat tissue is not a simple energy storage organ, but releases a lot of molecules which exerts important endocrine and immune functions. Adipocytes secrete numerous bioactive substances, including hormones, growth factors and cytokines. Abdominal fat accumulation has been shown to play crucial roles in the development of the human metabolic syndrome. **Aim:** This study describes the effect of body condition on metabolic profiles, hormones and cytokines in Holstein cows during the peripartal period. Maybe there are some similarities with the components of the human metabolic syndrome.

Material and methods: For this experiment serum was collected of al 45 heifers shared in three groups. 15 cattle were fed with a high energetic diet until they become pregnant, 15 heifers were chosen because of their high back fat thickness, 15 cattle were fed in optimum conditions. Blood samples were taken one week a.p., three days p.p. and about one month after parturition to determine total protein, urea, bilirubin, glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate, cholesterol, aspartate aminotransferase, haptoglobin, insulin, IGF-I and TNF-alpha. For the examination of TNF-alpha it was necessary to arrange an ELISA.

Results: In high back thick fatness cows insulin concentrations were significant higher at the third day p.p. than in the other groups; the high back thick fatness cows showed the highest glucose concentrations during the hole sample period. This fact argues for the existence of an insulin resistance. TNF-alpha levels were similar in all groups during the period, but we found a positive correlation between TNF-alpha and NEFA concentrations in high back thick fatness cattle. In high back thick fatness heifers and in cattle, which were fed with a high energetic diet, the serum concentrations of IGF-I were significant lower (< 100 ng/ml) at day three p.p. in contrast to the heifers in optimum conditions. Not only a positive correlation existed among the IGF-I and the NEFA concentrations, but also a negative correlation among IGF-I and haptoglobin in all groups. Haptoglobin levels were quite similar in all groups during the sampling period. There was a positive correlation among haptoglobin and NEFA concentrations.

Conculsions: In conculsion the study shows that adipose tissue may be directly involved in the pathogenesis of many postpartum diseases.

6.15 INTRAVENOUS INSULIN TOLERANCE TEST IN RABBITS: AN EXPERIMENTAL STUDY

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Insulin resistance is the main feature of metabolic syndrome in both humans and animals and an important cause for impaired glucose tolerance. Intravenous insulin tolerance test is a simple and specific test for determination of peripheral insulin sensitivity. This study aimed to develop an intravenous insulin tolerance test in rabbits and to evaluate some indices of insulin sensitivity. Twelve clinically healthy New Zealand white rabbits weighing between 2.5 and 3.4 kg were used. Blood samples were collected prior to (0 min), and 5, 10, 15, 20, 25, 30, 45 min after regular human insulin (0.1 U/kg) was administered via the auricular vein. Blood glucose concentration was measured immediately after collection by means of glucose meter using one drop whole blood. The following indices of insulin sensitivity were evaluated: index of insulin sensitivity (Kiis), calculated on the basis of initial and minimal blood glucose concentration after insulin administration; rate constant for glucose disappearance (K_{glucose}), estimated from the slope of the regression line of the logarithm of blood glucose concentration versus time; half-life time for blood glucose (t_{1/2}_{glucose}); area under the blood glucose concentration versus time curve (AUC_{glucose}); mean residence time for blood glucose (MRT_{glucose}); minimal glucose concentration (C_{min}) and time (T_{min}) to reach C_{min}. Glucose concentration decreased progressively after insulin administration. The average time to reach minimal blood glucose concentration (C_{min} = 2.89 ± 0.15 mmol/l) was T_{min} = 18.2 ± 1.22 min. Kiis, K_{glucose}, t_{1/2}_{glucose}, AUC_{glucose} and MRT_{glucose} were 0.53 ± 0.03, 5.1 ± 0.61 % min, 16.4 ± 2.22 min, 284.9 ± 16.48 mmol.min/L and 21.7 ± 0.37 min, respectively. The results of the present study indicate that the intravenous insulin tolerance test and deriving indices of insulin sensitivity could be used for evaluation of insulin sensitivity in rabbits when they are used as experimental animals in a research setting to study deterioration of glucose homeostasis.

6.16 THE LEVEL OF SERUM IONISED CALCIUM, ASPARTATE AMINOTRANSFERASE, INSULIN, GLUCOSE, BETAHYDROXYBUTYRATE CONCENTRATIONS AND BLOOD GAS PARAMETERS IN COWS WITH LEFT DISPLACEMENT OF ABOMASUM

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The purpose of this study was to determine concentrations of serum glucose, aspartate aminotransferase (AST), insulin, beta-hydroxybutyrate (BOH) and ionised calcium (Ica) in dairy cows with left displaced abomasum (LDA) and to compare these parameters before and after surgical correction of LDA. Eighteen Swiss-Holstein dairy cows with LDA were used in this study. Clinically healthy post parturient cows (n: 10) from a local dairy farm were used as control group. Blood samples were collected from the jugular vein from all the cows. Surgery was performed in cows with LDA. Blood samples from cows with LDA were collected 24 hours after surgery. The abomasum was repositioned followed by an omentopexy. Six of the 18 cows with LDA had clinical ketosis as detected with urine dipstick. The mean concentrations of insulin, BOH, glucose and AST in cows with LDA at admission time were increased compared with the healthy cows. But the mean concentration of Ica at admission time was slightly decreased compared with healthy cows. The mean BOH concentration was decreased 24 hours following surgery compared with values on admission time. However, the mean serum AST levels were increased both at admission time and 24 hours after surgery compared with healthy cows. The levels of blood gas parameters in cows with LDA were not significantly different in comparison with healthy cows, although hyperbasemia in six of 18 cows with LDA was determined.

In conclusion, the results of this study indicated that serum insulin, glucose, AST and BOH levels were increased in dairy cows with LDA. Serum BOH and Ica levels were decreased 24 hours after surgery compared with values on admission time. All cows with LDA used in this study had subclinical/clinical ketosis. We could say that ketosis might be a risk factor for the displacement of the abomasum.

6.17 INFLUENCE OF HUMIC SUBSTANCES ON THE IMMUNE RESPONSE OF RATS WITH SPECIAL REGARD TO THEIR POSSIBLE ADVERSE EFFECTS.

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Humic substances (HSs) are biologically very active and complex materials. Due to their chemical properties HSs can react with several compounds. It has been shown that HSs influence the immune system in a positive manner and due to their good chelating ability they alter the intestinal absorption and utilization of macro (i.e. Ca, P) and microelements (i.e. I, Mn) which may lead to hypothyroidism and alteration of bone composition. To understand the effect and mechanism of HSs, and their possible value in animal nutrition we have to take into consideration that HSs are not chemically homogenous, their main components are Fulvic acid (FA) and Humic acid (HA). FA is absorbable quickly in the intestines and is eliminated from the body within hours whereas HA is practically not absorbable. The aim of our experiment was to determine the effect of FA and HA on the immune response, macro and microelement composition of the femur and the thyroid function of the experimental animals with special regards to the possible adverse effects.⁷² Whistar CRL:(WI) BR, male, SPF rats (71±1 g) were used. Following the 4-day-long adaptation period animals were divided into 9 dietary groups. One group received the control diet, 4-4 treatment groups received FA and HA supplemented (0.1, 0.2, 0.4 and 0.8%) diets. Animals were housed individually. Feed and water were provided ad libitum. On the 2nd day of the experiment animals were immunized with ovalbumine (200 µl/100 g body weight) ip. On the 26th day of the experiment animals were euthanized and insanguinated. ELISA was used to analyze the immunological parameters collected from the serum samples.

Results: both the 0.4% HA and FA supplementation significantly ($p < 0.05$) increased the serum immune titer against ovalbumine (350 and 418% compared to the control respectively). FA increased the plasma TSH concentration in a dose related manner (16, 24, 35 and 55% respectively) and in 0.4 and 0.8% concentration significantly ($p < 0.05$) decreased the Mn content of femur ash. HA supplementation in 0.1, 0.2 and 0.4% decreased the Ca concentration of the femur ash (-4.2, -5.8, -6.9% respectively), while the P concentration increased (3.3, 6.2, 6.5% respectively). The Zn concentration of femur ash in the HA groups followed the pattern of Ca. The total femur ash contents were not influenced by the treatments.

Conclusion: both FA and HA were strong immunostimulants. The detected mild goitrogenity and the influence on the mineral content of bone ash - as possible negative effects - seem to be negligible in the applied concentration of FA and HA. Since these effects were dose related, in a higher dietary concentration of FA and HA their possible negative effects should be taken into consideration.

6.18 ORAL LIPOPOLYSACCHARIDE FAILS TO INITIATE AN ACUTE PHASE RESPONSE IN PERIPARTURIENT DAIRY COWS

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Feeding dairy cows diets containing high proportions of grain immediately after parturition is associated with enhanced release of endotoxin in the ruminal fluid. Translocation of endotoxin into the bloodstream has been reported and endotoxin has been implicated in several metabolic disorders such as laminitis, fatty liver, liver abscesses, infertility, and displaced abomasum. Making gastrointestinal mucosa tolerant to endotoxin by repeated administration of lipopolysaccharide (LPS) might prevent translocation of endotoxin and its harmful effects.

The objective of this study was to evaluate acute phase responses of transition dairy cows to repeated oral administration of LPS. Sixteen pregnant multiparous Holstein dairy cows were assigned to 2 groups 2 wk before the expected day of parturition. Cows were administered orally twice per week for 3 consecutive wk starting 2 wk before parturition the following treatments: 2 mL of saline (control), or 2 mL of saline containing LPS from *E. coli* 0111:B4. The amount of LPS administered each wk was 0.01, 0.05, or 0.1 µg/kg BW (~650 kg cow). Blood samples were obtained from the jugular vein twice per wk before administration of the treatment as well as once per week during wk 2, 3, and 4 after parturition and serum amyloid A, haptoglobin, lipopolysaccharide binding protein, and cortisol were measured.

Results indicated that plasma SAA and haptoglobin increased in both groups of cows during the first wk after parturition; however, no differences between the two groups were observed in the plasma concentrations of SAA or haptoglobin. Although plasma LBP was lower in LPS-treated cows during the first wk after calving no differences between the overall LBP means were evidenced between the two groups. In addition, no differences in the concentration of plasma cortisol were obtained between the control group and the group of cows treated repeatedly with oral LPS.

In conclusion, repeated oral administration of increasing amounts of LPS during 3 wk around parturition did not affect plasma concentrations of acute phase proteins measured and plasma cortisol. This suggests that endotoxin introduced into the oral cavity was not allowed to translocate into the blood circulation and that tolerance to LPS was induced in cows treated with oral LPS. Further research is needed to better understand the mechanism(s) by which repeated exposure to oral LPS failed to induce an acute phase response in dairy cows.

6.19 INTRAVENOUS ADMINISTRATION OF LIPOPOLYSACCHARIDE MOODULATES ACUTE PHASE RESPONSES IN DAIRY CATTLE

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Immediately after parturition dairy cows are fed diets containing rapidly fermentable carbohydrates. This is associated with a 20-fold increase in the amount of endotoxin in the ruminal fluid. Translocation of endotoxin into the blood circulation has been reported by several investigators. In response to endotoxin the host produces a variety of proteins known as acute phase proteins aimed at neutralizing and eliminating endotoxin from circulation. Recent work has shown that single intravenous administration of lipopolysaccharide (LPS) modulates immune responses in dairy cows. However, there is no information on immune effects of repeated intravenous administration of LPS in dairy cows.

The objective of this study was to evaluate acute phase responses of transition dairy cows to repeated intravenous administration of LPS. Sixteen pregnant multiparous Holstein dairy cows were assigned to 2 groups 2 wk before the expected day of parturition. Cows were infused into the jugular vein with either 100 mL of saline (control), or 100 mL of saline containing LPS from *E. coli* 0111:B4 at 0.01, 0.05, or 0.1 µg/kg BW (~650 kg cow) twice per week for 3 consecutive wk starting at 2 wk before the expected day of parturition. Blood samples were obtained from the jugular vein twice per wk before administration of the treatment as well as once per wk during wk 2, 3, and 4 after parturition and serum amyloid A (SAA), haptoglobin, lipopolysaccharide-binding protein (LBP), and cortisol in plasma were measured.

Results indicated that plasma SAA was not different between the two treatment groups. Serum amyloid A reached peak values 1 wk before parturition, remained high during wk 1 after calving and declined to the lowest levels 6 wk postpartum. On the contrary, plasma haptoglobin was higher in cows treated with LPS during the first 2 wk after calving. Furthermore, cows infused with LPS had lower plasma concentrations of LBP 1 wk before and 1 wk after calving. In addition, plasma cortisol was higher in cows infused with LPS during the whole experimental period.

In summary, results of this study demonstrate that repeated intravenous administration of LPS in periparturient dairy cows modulates production of acute phase proteins and plasma cortisol. Further research is warranted to elucidate the mechanism(s) by which repeated exposure to LPS modifies immune responses of transition dairy cows.

6.20 INTRAVENOUS LIPOPOLYSACCHARIDE MODULATES BLOOD METABOLITES IN PERIPARTURIENT DAIRY COWS

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Lipopolysaccharide (LPS) is a constituent of the outer membrane of all Gram-negative bacteria, including *Escherichia coli*, which is released in large amounts in the rumen fluid during feeding of diets with high content of readily digestible carbohydrates. Translocation of endotoxin into the bloodstream is associated with deleterious health effects to the host. Several investigators have reported short-term metabolic effects of single infusions of LPS to dairy cows; however, there are no reports regarding effects of repeated intravenous administration of LPS on blood metabolites related to carbohydrate and lipid metabolism.

The objective of this study was to evaluate metabolic responses of transition dairy cows to repeated intravenous administration of LPS. Sixteen pregnant multiparous Holstein dairy cows were assigned to 2 treatment groups 2 wk before the expected day of parturition. Cows were administered orally, twice per week, for 3 consecutive wk starting 2 wk before parturition the following treatments: 100 mL of saline (control), or 100 mL of saline containing LPS from *E. coli* 0111:B4. The amount of LPS administered each wk was 0.01, 0.05, or 0.1 $\mu\text{g}/\text{kg}$ BW (~650 kg cow). Blood samples were obtained from the jugular vein twice per wk before administration of the treatment as well as once per week during wk 2, 3, and 4 after parturition and plasma non-esterified fatty acids (NEFA), beta-hydroxy butyric acid (BHBA), glucose, lactate, cholesterol, and insulin were measured. Records of all clinical metabolic diseases were obtained during the 2 wk before and up to 8 wk after parturition. Our data showed that plasma NEFA and lactate increased in both groups of cows after parturition reaching peak values 2 wk postpartum. No differences between the two groups were evidenced regarding NEFA and lactate. Also, concentration of BHBA in plasma increased in both groups linearly and reached the highest values 5 wk postpartum; however, no changes between the two groups were obtained. Cows treated with LPS had higher plasma glucose during 1 wk before and 4 wk after parturition, whereas plasma cholesterol was lower in several time points before and after parturition in cows treated with intravenous LPS. Concentration of insulin in plasma was lower in LPS treated cows during the whole experimental period. The group of cows treated with intravenous LPS had higher incidence of clinical metabolic disorders after parturition compared to the control group.

In summary, our data showed that repeated intravenous infusion of LPS from *E. coli* 0111:B4 in transition dairy cows lowered plasma insulin and cholesterol, modified plasma glucose, had no effect on plasma NEFA, BHBA, and lactate and increased the incidence of metabolic disorders in periparturient dairy cows.

7 MACRO AND MICRO MINERAL METABOLISM

7.1 THE ETIOLOGY AND PREVENTION OF MILK FEVER AND SUBCLINICAL HYPOCALCEMIA

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Abstract

The periparturient cow undergoes a transition from non-lactating to lactating at calving. The animal is tremendously challenged to maintain calcium homeostasis. Those that fail can develop milk fever, a clinical disorder that is life threatening to the cow and pre-disposes the cow to a variety of other disorders. Guidelines for monitoring the incidence of hypocalcemia and methods for treating milk fever are reviewed. The physiologic factors that cause milk fever and strategies for prevention of milk fever will be discussed; focusing on the effects diet cation-anion difference can have on tissue sensitivity to parathyroid hormone. Another major risk factor for milk fever is hypomagnesemia, which is observed when animals are fed inadequate amounts of magnesium or some factor is present in the diet that prevents adequate absorption of magnesium. Moderate hypomagnesemia impairs the ability of the cow to maintain calcium homeostasis and hypocalcemia occurs.

Keywords: milk fever, DCAD, hypomagnesemia, hypocalcemia, anionic salts

Introduction

Inadequate blood calcium (Ca) concentrations can cause a cow to lose the ability to rise to her feet as Ca is necessary for nerve and muscle function. The result is the metabolic disease known as milk fever, though it is more properly termed periparturient hypocalcemia or periparturient paresis, as an elevated body temperature is not typically observed. It is a particular concern in the newly calved cow, where the sudden demand for calcium at the onset of lactation severely tests the calcium homeostatic capabilities of the cow. Less severe disturbances in blood Ca concentration cause reduced feed intake, poor rumen and intestine motility, poor productivity, and increases susceptibility to other metabolic and infectious disease. Mechanisms for maintaining normal blood Ca concentration perform efficiently most of the time but occasionally these homeostatic mechanisms fail and hypocalcemia ensues. Understanding how and why these mechanisms fail may allow the practitioner to develop strategies to avoid these disorders. Surveys in the USA suggest around 5 % of cows will develop milk fever each year and the incidence of subclinical hypocalcemia – blood Ca values

between 8 and 5.5 mg/dl during the perparturient period- is around 50% in older cows. Milk fever and subclinical milk fever should be considered gateway diseases that greatly reduce the chance for full productivity in the ensuing lactation. Hypocalcemia reduces rumen and abomasal motility increasing the risk of abomasal displacement. Hypocalcemia reduces feed intake so that greater body fat mobilization occurs in early lactation. Hypocalcemia reduces all muscle contraction including the teat sphincter muscle responsible for closure of the teat orifice after milking, thus increasing the risk of mastitis. More recently we have demonstrated hypocalcemia directly impairs immune cell response to an activating stimulus.

Ca Homeostasis and Monitoring for Hypocalcemia

Blood Ca in the adult cow is maintained between 8.5 – 10 mg/dl (2.1 – 2.5 mmol/L). Typically the nadir in blood Ca concentration occurs between 12 and 24 hours after calving and blood samples obtained around this time can reveal the extent of hypocalcemia experienced by a dairy herd. Nearly 25% of heifers will have blood Ca concentration below 8 mg/dl. About 50% of older cows will fall into this category. In well managed herds following a good anionic salt program or other effective milk fever control measures the above figures can be cut in half and the number of cows exhibiting clinical milk fever can be reduced to 1% or less. Acute hypocalcemia can also occur under many conditions involving infections, such as mastitis or metritis, especially if endotoxins are elaborated. As a rule the blood Ca concentration is below 8, but above 6 mg/dl. It is due to re-distribution of Ca within organs and will not be discussed further other than to be a reminder that not all hypocalcemic cows have the syndrome known as milk fever.

In order to prevent blood Ca from decreasing at the onset of lactation the cow must replace extracellular Ca lost to milk. She does this by withdrawing Ca from bone and by increasing the efficiency of absorption of dietary Ca. The dairy cow (as are most mammals) is programmed to go into a state of lactational osteoporosis, mobilizing bone Ca to help her achieve normocalcemia in early lactation. This will typically result in loss of 9-13% of her skeletal Ca in the first month of lactation (which is reversible in later lactation). Though it might stress her bones the main objective - to maintain normocalcemia - can be achieved. Bone Ca mobilization is regulated by parathyroid hormone (PTH) which is produced whenever there is a decline in blood Ca. Renal tubular reabsorption of Ca is also enhanced by PTH. However the total amount of Ca that can be recovered by reducing urinary Ca excretion is relatively small as only small amounts of calcium are typically lost to urine each day. A second hormone, 1,25-dihydroxyvitamin D, is required to stimulate the intestine to efficiently absorb dietary Ca. This hormone is made from vitamin D by the kidney –but only in response to an increase in blood PTH. Put simply, hypocalcemia and milk fever occur when cattle do not extract enough Ca from their bones and diet to replace the Ca lost to milk. Several nutritional factors are involved in the breakdown of Ca homeostasis that results in milk fever.

Factors Impairing Ca Homeostasis At The Cellular Level

Metabolic Alkalosis

Metabolic alkalosis predisposes cows to milk fever and subclinical hypocalcemia (Craig and Stoll, 1947). Metabolic alkalosis blunts the response of the cow to PTH (Gaynor, et al., 1989; Goff, et al., 1991; Phillippo, et al., 1994). We now believe the conformation of the PTH receptor is altered during metabolic alkalosis rendering the tissues less sensitive to PTH (Figure 1). Lack of PTH responsiveness by bone tissue prevents effective utilization of bone canalicular fluid Ca, sometimes referred to as osteocytic osteolysis, and prevents activation of osteoclastic bone resorption. Failure of the kidneys to respond to PTH also reduces renal reabsorption of Ca from the glomerular filtrate. More importantly, the kidneys fail to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. Therefore enhanced intestinal absorption of dietary Ca that normally would help restore blood Ca to normal, fails to be instituted. Metabolic alkalosis is largely the result of a diet that supplies more cations (K, sodium (Na), Ca, and Mg) than anions (chloride (Cl), sulfate (SO₄), and phosphate (PO₄)) to the blood. In simplest terms, a disparity in electrical charge in body fluids occurs in animals fed these diets because a greater number of positively charged cations enter the blood than negatively charged anions. To restore electroneutrality to this high cation, positively charged blood, a positive charge in the form of a hydrogen ion (H⁺) must be lost from the blood compartment. A reduction in H⁺ concentration is equivalent to an increase in the pH of the blood (Stewart, 1983). For a more detailed description of how dietary cation-anion balance influences blood pH the reader is referred to recent reviews on this subject (Constable, 1999); Goff, 2000). Adding readily absorbable anions to the diet increases the total negative charges in the blood allowing more H⁺ to exist and the blood pH decreases – it is more acidic.

Hypomagnesemia

Cow plasma Mg concentration is normally between 1.8 and 2.4 mg/dl (0.75 and 1.0 mmol/L). Hypomagnesemia affects Ca metabolism in two ways. 1. By reducing PTH secretion in response to hypocalcemia (Littledike, et al., 1983) and 2. by reducing tissue sensitivity to PTH (Rude, 1998).

The integrity of the interaction between PTH and its receptor is vital to Ca homeostasis. Hypomagnesemia, independent of metabolic alkalosis, can also interfere with the ability of PTH to act on its target tissues. When PTH binds its receptor on bone or kidney tissues, it normally initiates activation of adenylate cyclase, resulting in production of the second messenger, cyclic AMP. PTH-receptor interactions should also cause activation of phospholipase C in some tissues, resulting in production of the second messengers diacylglycerol and inositol 1,4,5-triphosphate. Both adenylate cyclase and phospholipase C have a Mg⁺⁺ binding site which must be occupied by a Mg ion for full activity (Rude, 1998). In man, it is well recognized that

hypomagnesemia can cause hypocalcemia and that Mg therapy alone restores the serum Ca concentration to normal; Ca and/or vitamin D therapy are ineffective (Rude, 1998). Field evidence suggests that blood Mg concentrations below 0.65 mmol/L in the periparturient cow will increase the susceptibility of cows to hypocalcemia and milk fever (van de Braak, et al., 1987).

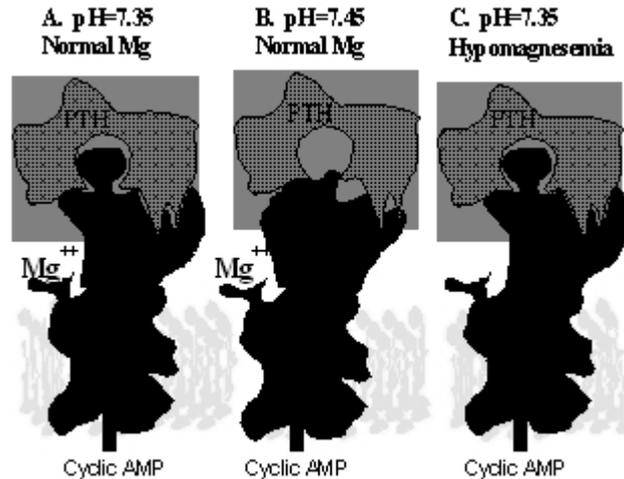


Figure 1. Parathyroid hormone (PTH) effects at the surface of target bone and kidney cells.

Panel A. Under normal conditions, PTH released in response to hypocalcemia interacts with its receptor, located on the surface of bone and kidney cells, in a lock and key fashion. This stimulates G-proteins and adenylate cyclase (adenylate cyclase complex) resulting in production of cyclic AMP, which acts as a second messenger within the cytosol of target cells. This initiates mechanisms such as bone Ca resorption and renal production of 1,25-dihydroxyvitamin D to restore blood Ca concentration to normal levels.

Panel B. Alkalotic conditions induced by high potassium diets induce a change in the shape of the PTH receptor protein so that it is less able to recognize and bind PTH, resulting in failure to activate the cell by producing cyclic AMP.

Panel C. Mg is required for function of the adenylate cyclase complex. Hypomagnesemia reduces ability of PTH stimulated cells to produce cyclic AMP, resulting in failure to activate the cell.

Maintenance of normal plasma Mg concentration is nearly totally dependent on a constant influx of Mg from the diet. Mg is well absorbed from the small intestine of young calves and lambs. As the rumen and reticulum develop these sites become the main, and perhaps the only, sites for net Mg absorption (Martens and Rayssiguier, 1980). Mg absorption from the rumen is dependent on the concentration of Mg in solution in the rumen fluid and the integrity of the Mg transport mechanism (Martens and Gabel, 1986).

The soluble concentration of Mg in rumen fluid is obviously dependent on the magnesium content of the diet. However, Mg solubility declines sharply as rumen pH rises above 6.5 and solubility can be a problem on higher forage diets. Forages also can

contain trans-aconitic acid. A metabolite of trans-aconitic acid, tricarballoylate can complex Mg and is resistant to rumen degradation and may play a role in hypomagnesemic tetany (Cook, et al., 1994).

Active transport of Mg across the rumen wall is necessary when diet Mg is not in great supply. Unfortunately, high K concentration in the rumen fluid depolarizes the apical membrane of the rumen epithelium reducing the electromotive potential needed to drive Mg across the rumen wall (Martens and Schweigel, 2000). Thus a ration that might otherwise be adequate in Mg results in a Mg deficient state when diet K is excessive.

A second pathway for absorption of Mg exists that is not affected by K. Unfortunately, this passive transport process only operates at high rumen fluid Mg concentrations, which allow Mg to flow down a concentration gradient into the extracellular fluids of the cow (Martens and Schweigel, 2000). The concentration of Mg in rumen fluid needed to utilize concentration gradient driven absorption of Mg is greater than 4 mmol/L (Care, et al., 1984; Ram, et al., 1998). The minimum level of Mg required in the diet to prevent negative Mg balance in the face of high K levels in ruminants is approximately 0.35% (Ram, et al., 1998). Thus, Mg content of the close-up dry cow ration and the early lactation ration should be between 0.35 and 0.4% as insurance against the possibility that the active transport processes for Mg absorption are impaired.

Assessing Mg status

Sampling the blood of several cows within 12 hrs after calving provides an effective index of Mg status of the periparturient cows. If serum Mg concentration is not at least 1.8 mg/dl (0.8 mmol/L) it suggests inadequate dietary Mg absorption and that hypomagnesemia may be limiting productivity as well as contributing to hypocalcemia in the herd. Cows with blood Mg between 1.15 and 1.8 mg/dl (0.5 and 0.8 mmol/L) have few obvious clinical symptoms, though they often are slow to eat and are not producing milk up to their potential. Clinical signs of hypomagnesemia, such as recumbency, convulsions, nystagmus; are only observed when blood Mg falls below 0.4-0.5 mmol/L. Tetany is generally accompanied by severe hypocalcemia. Hypomagnesemia is very amenable to prevention by increasing dietary magnesium content and form.

Reducing Diet Cation-Anion Difference to Prevent Hypocalcemia

In theory all the cations and anions in a diet are capable of exerting an influence on the electrical charge of the blood. The major cations present in feeds and the charge they carry are Na (+1), K (+1), Ca (+2), and Mg (+2). The major anions and their charges found in feeds are Cl (-1), SO₄ (-2), and phosphate (assumed to be -3). Cations or anions present in the diet will only alter the electrical charge of the blood if they are

absorbed into the blood. Trace elements present in diets are absorbed in such small amounts that they are of negligible consequence to acid-base status. Organic acids such as the volatile fatty acids are generally absorbed in the undissociated form so that they carry both a positive and negative charge into the blood. They also are rapidly metabolized within the liver so they have only a small effect on general acid-base balance under most circumstances.

The difference between the number of cation and anion particles absorbed from the diet determines the general acid-base balance of the body and therefore the pH of the blood. The cation-anion difference of a diet is commonly described in terms of mEq/kg DM (some authors prefer to use “mEq/100 g” diet DM) of just Na, K, Cl, and SO₄, although it must be kept in mind that Ca, Mg, and P absorbed from the diet will also influence blood pH. The relative merits of the various DCAD equations proposed is the subject of an accompanying report from this conference. However, experimental evidence from our laboratory (Goff et al., 2004) and the meta-analysis of Charbonneau et al., (2006) support the concept that dietary sulfate (S) is only about 60% as effective as chloride as an acidifying agent in the diet of the prepartum cow, suggesting an appropriate DCAD equation could be $(Na + K) - (Cl + 0.6 S)$. While DCAD equations provide a theoretical basis for dietary manipulation of acid-base status they are not necessary for formulation of mineral content of prepartum dairy cow rations in this author's opinion because, with the exception of K and Cl, the rate of inclusion of the other macrominerals can be set at fixed rates.

The USA National Research Council (NRC, 2000) requirement for Na in the diet of a late gestation cow is about 0.12%. A small amount of salt is added to the diet to prevent pica, which often is manifest as a desire to drink urine from the floor. Unlimited access to NaCl is to be avoided in late gestation because it will increase the risk of udder edema, not because it greatly affects acid-base status.

At least two studies have clearly demonstrated that inclusion of Ca in the diet at NRC required levels or several fold above NRC required levels does not influence the degree of hypocalcemia experienced by the cow at calving (Goff and Horst, 1997; Beede, et al., 2001). It appears from these studies that close-up diet Ca concentration should be maintained between 0.85 and 1.0% Ca.

To ensure adequate concentrations of Mg in the blood of the periparturient cow the dietary Mg concentration should be 0.35-0.4%. This higher dietary Mg concentration allows the cow to take advantage of passive absorption of Mg across the rumen wall.

Dietary P concentration should be fed at a level to meet the NRC requirement for P in the late gestation cow. This is generally about 0.4% P for most cows. A diet supplying more than 80 g P/day (Barton, 1978; Kichura, et al., 1982) will block renal production of 1,25-dihydroxyvitamin D and will actually cause milk fever.

Dietary S must be kept above 0.22% to ensure adequate substrate for rumen microbial amino acid synthesis. Corn (maize) silage diets are notoriously low in sulfur. Diet S

should be kept below 0.4% to avoid possible neurological problems associated with S toxicity (Gould, et al., 1991).

Now, with the exception of K and Cl, the “variables” in the various proposed DCAD equations have become more or less “fixed”. The key to milk fever prevention (at least with Holstein cows) is to now keep K as close to the NRC requirement of the dry cow as possible (about 1.0% diet K). The key to reduction of subclinical hypocalcemia, not just milk fever, is to add Cl to the ration to counteract the effects of even low diet K on blood alkalinity. For formulation purposes the concentration of Cl required in the diet to acidify the cow is approximately 0.5% less than the concentration of K in the diet. In other words, if diet K can be reduced to 1.3%, the Cl concentration of the diet should be increased to 0.8%. If dietary K can only be reduced to 2.0% the diet Cl would need to be roughly 1.5% to acidify the cow. This level of Cl in the diet is likely to cause a decrease in dry matter intake. Chloride sources differ in their palatability and since achieving low dietary K can be difficult it is prudent to use a palatable source of Cl when formulating the diet. Ammonium chloride (or ammonium sulfate) can be particularly unpalatable when included in rations with a high pH. At higher pH, a portion of the ammonium cation is converted to ammonia, which is highly irritating when smelled by the cow. Prilling the Cl (and SO₄) salts reduces the unpleasant taste of the salts. In our experience hydrochloric acid has proved the most palatable source of anions. Hydrochloric acid can be extremely dangerous to handle when it is procured as a liquid concentrate. Several North American companies now manufacture hydrochloric acid based anion supplements, which are safe to handle. Some also include magnesium in a highly soluble form to reduce the possibility that hypomagnesemia is present in the herd and contributing to hypocalcemia

These are simply guidelines for anion supplementation used by this author and are based on inclusion of Ca, Na, S, Mg, and P at the levels outlined above. Urine pH of the cows provides a cheap and fairly accurate assessment of blood pH and can be a good gauge of the appropriate level of anion supplementation (Jardon, 1995). Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to ~7.8). For optimal control of subclinical hypocalcemia the average pH of the urine of Holstein cows should be between 6.2 and 6.8, which essentially requires addition of anions to the ration. In Jersey cows the average urine pH of the close-up cows has to be reduced to between 5.8 and 6.3 for effective control of hypocalcemia. If the average urine pH is between 5.0 and 5.5, excessive anions have induced an uncompensated metabolic acidosis and the cows will suffer a decline in dry matter intake. Urine pH can be checked 48 or more hrs after a ration change. Urine samples should be free of feces and made on midstream collections to avoid alkalinity from vaginal secretions. Anion supplemented diets are generally fed for the last 3 wks before calving, though the length of time these diets need to be fed to induce a compensated metabolic acidosis is no more than 4-5 days.

Sulfate as a Milk Fever Preventing Agent Independent of an Acidifying Effect

Roche et al., (2002) have done a number of studies which provide evidence that sulfate may have milk fever preventing qualities that are independent of an acidifying activity. In these studies with pasture based animals daily drenching with magnesium sulfate was slightly more effective at maintaining plasma calcium levels at normal levels than was drenching the animals with magnesium chloride, though magnesium chloride proved to reduce urine pH to a greater degree. Lean et al., (2006) performed a meta-analysis on a data set similar to the one used by Charbonneau et al., (2006). They found that increasing dietary sulfate was among the better indicators of a diet that would prevent milk fever. Their analysis also indicated this effect was not dependent on an acidifying action of the sulfate salts. It remains to be seen what the mechanism of action of the sulfate salts might be. An experiment to test the effect of diet sulfate, with dietary Mg held constant and most other minerals and protein held constant, performed in at-risk older cows might provide further insight.

Feeding a Ca Deficient Diet To Stimulate PTH Secretion Pre-Calving To Prevent Hypocalcemia

When cows are fed a diet that supplies less Ca than they require, the cows are in negative Ca balance. This causes a minor decline in blood Ca concentration stimulating PTH secretion, which in turn stimulates osteoclastic bone resorption and renal production of 1,25-dihydroxyvitamin D. At parturition the cow's osteoclasts are already active and in high numbers and the lactational drain of Ca is more easily replaced from bone Ca. If provided with Ca in the lactation ration, the previous stimulation of enterocytes by 1,25-dihydroxyvitamin D will allow efficient utilization of dietary Ca and the cow avoids hypocalcemia (Green, et al., 1981). This works even in the face of metabolic alkalosis as metabolic alkalosis reduces but does not totally eliminate tissue PTH sensitivity. Prolonged exposure to high PTH levels induced by the low Ca diet overcomes the reduced tissue sensitivity to PTH.

The 2000 NRC lists the Ca requirement of the cow in terms of absorbable Ca, since the availability of Ca in diets varies. The absorbable Ca requirement (NRC, 2000) of the late gestation cow is approximately 14 g / day in Jerseys and up to 22 g in large Holsteins. A truly low Ca diet must supply considerably less absorbable Ca than required by the cow if it is to be capable of stimulating PTH secretion. For example, a 600 kg cow consuming 13 kg DM must be fed a diet that is less than 0.15% absorbable Ca if it is to provide less than 20 g available Ca/ day. Low Ca diets are more practical under grazing situations. In these cases the total dry matter intake of pasture may be just 6-7 kg DM/ day and the grasses being grazed can be less than 0.4% Ca, which would provide < 28 g total Ca and somewhere around 9-10 g absorbable Ca / day (Sanchez, 2003). It is important to note that after calving the animal is switched to a high Ca diet.

Recently two methods have been developed to reduce the amount of dietary Ca available for absorption. The first method involves incorporation of zeolite (a silicate particle) into the ration, which binds Ca and causes it to be passed out in the feces. At present the method is unwieldy because very large amounts of zeolite must be ingested each day (0.5-1 kg/day for 2 wks before calving) and the effects of zeolite on P and trace mineral absorption are not clear (Thilising-Hansen, et al., 2002). However, by chemically modifying the zeolite it is theoretically possible to increase the affinity and the specificity of the zeolite for Ca, which may allow its practical use. The second method involves oral administration of vegetable oils which bind Ca to form an insoluble soap preventing absorption of diet Ca (Wilson, 2003). It is unclear whether the oils might also tie up magnesium. These methods have been successfully used in cattle fed diets containing 30-50 g Ca / day. They irreversibly bind enough dietary Ca to cause the reaction typically seen when the diet provides <15 g absorbable Ca/day.

Vitamin D Supplementation

A reasonable practice is to supplement the dry cow with 20-30,000 IU vitamin D / day in the diet. Earlier literature often recommended feeding or injecting massive doses (up to 10 million units of vitamin D) 10 days -2 weeks prior to calving to prevent milk fever. These vitamin D doses pharmacologically increased intestinal Ca absorption, and sometimes prevented milk fever. Unfortunately the dose of vitamin D that effectively prevented milk fever was very close to the dose causing irreversible metastatic calcification of soft tissues. Lower doses (500,000-1 million units of vitamin D) actually induced milk fever in some cows because the high levels of 25-OH D and 1,25-dihydroxyvitamin D resulting from treatment suppressed PTH secretion and renal synthesis of endogenous 1,25-dihydroxyvitamin. These animals become hypocalcemic once the exogenous source of vitamin D that had maintained elevated intestinal Ca absorption rates is cleared from the body. In some cases the ability to begin endogenous production of 1,25-dihydroxyvitamin D was suppressed for a week after calving (Littledike and Horst, 1980).

Treatment with 1,25-dihydroxyvitamin D and its analogues can be more effective and much safer than using vitamin D but problems associated with timing of administration remain. The problem of suppression of renal 1,25-dihydroxyvitamin D production can be minimized by slow withdrawal of the exogenous hormone over a period of days after calving (Goff and Horst, 1990).

Oral Ca Treatments at Calving To Prevent Hypocalcemia

Ca administered to the fresh cow may arguably be called a treatment rather than a preventative measure for hypocalcemia. Briefly, the concept behind oral supplementation is that the cow's ability to utilize active transport of Ca across intestinal cells is inadequate to help her maintain normal blood Ca concentrations. By dosing the animal with large amounts of very soluble Ca orally it is possible to force

Ca across the intestinal tract by means of passive diffusion between, not across, intestinal epithelial cells. Best results are obtained with doses of Ca between 50 and 125 g Ca / dose. Ca chloride has been used but can be caustic. Large or repeated doses of calcium chloride can induce an uncompensated metabolic acidosis in the cow, especially if the cow is already being fed an acidogenic diet (Goff and Horst, 1993). Ca propionate is less injurious to tissues and is not acidogenic. It has the added benefit of supplying propionate, a gluconeogenic precursor (Pehrson, et al., 1998). For best control of hypocalcemia a dose is given at calving and again 24 hrs later. Larger or more frequent dosing can be toxic. Toxic doses of Ca can be delivered orally – about 250 g Ca in a soluble form will kill some cows. The benefit of adding oral Ca on top of a properly formulated low DCAD program does not seem to warrant the added expense (Melendez, et al., 2002).

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7.2 EFFECTS OF CA SOURCE ON CA TRANSPORT ACROSS THE ISOLATED BOVINE RUMEN EPITHELIUM

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Problem: The site of Ca absorption along the bovine gastrointestinal tract, the cellular mechanisms involved and its hormonal regulation is still under discussion. At least for small ruminants there is clear evidence that the well characterized intestinal mechanisms in monogastric species cannot be fully applied. Furthermore, the rumen wall has also to be considered as a site for Ca absorption.

Aims: As different Ca salts are used for prevention of milk fever, the experiments aimed at studying the Ca flux rates from the mucosal to the serosal compartment of bovine rumen epithelium as affected by the Ca source and the transepithelial Ca concentration gradient.

Material and methods: Rumen tissues from 9 animals were obtained from the local slaughter house within less than 30 min after stunning and bleeding, the epithelial tissues were stripped from the muscle and serosal layer and the tissues were then mounted in Ussing chambers with an exposed surface of 2 cm² and incubated in a modified Krebs-Henseleit-buffer with a physiological mixture of short chain fatty acids at the mucosal side of the tissues. Ca flux rates from the mucosal to the serosal side of the tissues (J_{ms}) were measured under short-circuit conditions with ⁴⁵CaCl₂ under basal Ca concentrations of 1.2 mmol/l and after increasing the mucosal concentrations up to 3.7 mmol/l. In five animals basal Ca concentrations were adjusted by CaCl₂ and the increases were adjusted by either CaCl₂, Ca-propionate or Ca-formiate and in four animals both, basal and increased Ca concentrations were adjusted by either CaCl₂, Ca-propionate or Ca-formiate. For each Ca source 4 chambers were incubated simultaneously.

Results: Under basal Ca concentrations the mean J_{ms} ranged between 13 and 19 nmol*cm⁻²*h⁻¹ irrespective of the Ca source. In response to higher Ca concentrations Ca flux rates increased significantly and ranged between 68 and 86 nmol*cm⁻²*h⁻¹ with no significant differences between the Ca sources. The short circuit currents and the tissue conductances ranged between 0.4 and 0.7 μEq*cm⁻²*h⁻¹ and 8 and 10 mS*cm⁻², respectively, and were not affected by different Ca treatments.

Conclusions: The data from these experiments confirm data from small ruminants that the rumen wall has to be considered as a site for active and/or passive Ca absorption which can be significantly stimulated by increased mucosal Ca concentrations. For this the Ca source appears to be of minor relevance since no differences could be detected when different Ca sources were applied. The molecular basis of ruminal Ca transport is not yet fully understood, however, from recent gene expression experiments it may be postulated that basolateral Ca extrusion may be mediated by both, Ca-ATPase and Na/Ca exchange.

7.3 RUMEN PROTECTED RICE BRAN TO STIMULATE CALCIUM HOMEOSTASIS IN THE CLOSE UP PERIOD OF DAIRY COWS

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Milk fever is a short episode of hypocalcaemia that dairy cows can suffer around calving. A low calcium diet before calving to activate Ca homeostasis, although practically unfeasible, seems to be the most effective preventive strategy (Thilsing-Hansen et al. 2002). Ca antagonists like zeolites have shown to stimulate homeostasis (Thilsing-Hansen et al. 2002b, Katsoulos et al. 2005). Cereal brans, and especially rice bran, can reduce Ca availability in humans (Jahnen et al. 1992). This experiment describes the effect of a rumen protected rice bran on Ca homeostasis in dry cows. Stabilised rice bran was rumen protected by coating with hydrogenated palm fat (FCRB: 30% additional fat). FCRB was tested in situ (Ørskov and Mc Donald, 1979) to evaluate bypass level of dry matter (DM) and phytic acid. Seven non lactating, pregnant, multiparous dairy cows were used in 3 feeding periods: P1: adaptation P2: feeding of FCRB and P3: withdrawal of FCRB. Feed intake was monitored daily for 20 days. Urine samples were taken daily. From day 7 to day 13 (P2), 2 kg/day of FCRB were fed. Urine was analysed for pH, Ca and creatinine. The daily repeated measures of urinary Ca creatinine ratio (CCR) and Ca creatinine ratio over calcium intake (CCRCI) were analysed after logarithm transformation with PROC mixed of SAS. Urinary parameters and nutrient intakes were also compared among the 3 periods. The in situ trial showed that, although phytic acid was degraded 3 times faster than DM, the bypass fraction (passage rate of 6%/h) was 34%. FCRB depressed dry matter intake (DMI) in P2 (P1;P2;P3: 14.4, 9.4 and 13.1 kg), resulting in a lower ($p<0.01$) Ca intake (P1;P2;P3: 40.16, 25.4 and 36.6 g). Urine pH was lower in P2 ($p<0.05$) (P1;P2;P3: 8.5, 8.4 and 8.5). CCR decreased from P1 to P2 ($p<0.01$) and increased from P2 to P3 ($p<0.01$), (P1;P2;P3 0.0089, 0.0048 and 0.0186). CCRCI only showed an increase ($p<0.01$) from P2 to P3 (P1;P2;P3 0.35, 0.11 and 0.64). CCR decreased at product introduction ($p<0.01$), and CCR and CCRCI peaked after withdrawal ($p<0.01$). Feeding FCRB affected urinary Ca excretion expressing influence in Ca homeostasis. Triggering the dormant Ca regulation in the close up period could prevent milk fever. Further research is needed for an alternative rumen protection technique.

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7.4 INFLUENCE OF HIGH CALCIUM SUPPLY ON MINERAL AND BONE METABOLISM OF DAIRY GOATS AND MILK SHEEP IN THE PERIPARTAL PERIOD

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An increased mobilization of Ca from bone and increased absorption from the gastrointestinal tract are required for Ca homeostasis in early lactation. High Ca supply during the last weeks of gestation may play a role in the development of hypocalcemia by disabling the Ca homeostatic mechanisms. Dairy goats were chosen as a model for cattle and compared to milk sheep. The aim of the study was to investigate the influence of high Ca content in diets ante partum (ap) on mineral metabolism of goats and sheep to get an idea about the changes possibly occurring in cattle. 12 goats and 10 sheep (housed individually, fifth lactation) were divided into two groups each: GH, SH (goat resp. sheep high; 2.5xCa requirement); GC, SC (goat resp. sheep control; Ca according to requirement). Blood samples were collected during the last 2 weeks (w) of gestation (ap) until 8 w post partum (pp). Serum Ca, carboxyterminal telopeptide of type I collagen (ICTP, bone resorption marker) and osteocalcin (OC, bone formation marker) were analyzed. Bone mineral content (BMC) and density (BMD) were quantified weekly until 8 w pp. Colon mucosa biopsies were obtained from goats to perform immunohistochemistry (IHC) for Vitamin D receptor (VDR) 3 w, 1 w ap, at parturition and 1 w pp. Mean ICTP concentrations of the 4 groups increased from parturition until 4 days pp, stayed at this level until 1 w pp and decreased thereafter. In contrast, mean OC concentrations in all groups were low during the last 2 w ap until 1 w pp, but increased until 8 w pp. BMC and BMD decreased from 1 w ap until parturition and increased until 8 w pp. Both parameters were always higher in sheep compared to goats. Total and ionized Ca concentrations were decreased at parturition. First results of IHC reveal that nuclei and cytoplasm of enterocytes stain positively for VDRs. Strongest immunoreactions were observed in intermediate and superficial glandular cells. During late gestation, immunoreaction for VDR was lower compared with samples taken 1 w pp. The increase of ICTP concentrations in all groups indicates that bone is resorbed at parturition disregarding the Ca content in the diet. This is consistent with the findings regarding BMC and BMD. At the same time, less Ca is embedded in bone, as indicated by the low OC concentrations.

In conclusion, an oversupply of Ca during late gestation in goats and sheep does not influence Ca homeostatic mechanisms, since neither bone parameters nor VDR revealed differences. Biopsy technology and IHC are useful tools to assess changes in VDR expression in relation to varying demands for Ca and show that in dairy goats and sheep, as in cows, an influence of gestation and lactation on VDR is obvious, but not necessarily dependent on the Ca supply during gestation.

7.5 MILK FEVER AND BONE METABOLIC ACTIVITY IN DAIRY COWS

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Milk fever with incidence of 5 - 10% in all multiparous dairy cows in intensive dairy production in Slovenia is one of the most economically important metabolic diseases. Calcium (Ca) demand after calving rises to multiple value of that during the dry period, which puts cows in risk of developing milk fever if all homeostatic mechanism for Ca balance are not working properly. Beside more effective Ca absorption from the gastrointestinal tract, Ca resorption from skeleton plays an important part of maintenance of normocalcaemia. In the present study bone metabolism in mature dairy cows in intensive dairy production, within 48 hours after calving, was evaluated by measuring blood serum bone resorption biomarker C-terminal telopeptide of type I collagen (CTX) and bone formation biomarker bone-specific alkaline phosphatase (bALP) beside the classical panel (minerals Ca, iP, Mg and total alkaline phosphatase (ALP)). 5 Holstein Friesian (HF) cows without signs of any disease and 12 HF cows with milk fever and no other signs of disease were included in the study. All the cows were in 4th or higher lactation. Samples of venous blood were drawn before classical therapy of milk fever was instituted or within 48 hours after calving in healthy cows. Mean total serum Ca value in cows with milk fever was $1,01 \text{ mmol/L} \pm 0,29 \text{ mmol/L}$ and in healthy cows it was statistically significantly ($p < 0,05$) higher, $1,94 \text{ mmol/L} \pm 0,04 \text{ mmol/L}$. All the cows were also hypophosphataemic with lower values in milk fever cows. Some cows with milk fever were also hypermagnesaemic, but the difference in blood serum Mg and aP concentrations between the two groups were not statistically significant. Mean total ALP and bALP activity were higher in cows with milk fever ($65 \pm 17,7 \text{ U/L}$ and $21,3 \pm 8,5 \text{ U/L}$) than in healthy cows ($57 \pm 7,6 \text{ U/L}$ and $18,9 \pm 1,2 \text{ U/L}$). Mean concentration of blood serum CTx was lower in cows with milk fever ($0,212 \pm 0,091 \text{ ng/L}$) than in healthy cows ($0,319 \pm 0,115 \text{ ng/L}$), but as for bALP not statistically significantly.

From the results we can conclude that response of bone metabolism in cows with milk fever is improper. Instead of more intensive resorption of the bone tissue in cows with milk fever, there is more intensive bone formation and less intensive resorption compared to healthy cows. Reasons for such response of bone metabolism still have to be elucidated. One of the suspects for this is sex hormone estrogen, which has anabolic effect on bone metabolism, and reaches highest blood values at the time of calving.

7.6 RENAL EXCRETION OF ELECTROLYTES AND UREA IN BEEF CATTLE

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Urolithiasis is one of the most important problems in intensive beef cattle breeding in Italy and it has typically a technological/alimentary pathogenesis. Urinary calculi formation usually results from a combination of physiological, nutritional and management factors. Diet containing excessive P and Mg and low levels of Ca and K, predisposes to a Ca–P imbalance and high urinary phosphate excretion possibly leading to urolith formation.

The objective of the present study was to analyse changes in blood parameters and urine composition in relation to nutritional variations and the risk of developing urolithiasis. 34 beef calves from a farm with high incidence of urolithiasis were monitored from 60Kg to 250Kg of b.w. in 3 different periods: I milk feeding, II weaning, III final diet. Calves were randomly assigned to 2 boxes: in period I they were fed milk replacer and had free access to hay and concentrate; in period II they were slowly adapted to hay, concentrate and cracked corn; in period III box1 didn't change diet (higher risk of urolithiasis) while in box2 concentrate was slowly substitute with corn silage, nucleus and pulps. Clinical conditions were regularly recorded and concentrate samples collected to determine mineral composition. Urine specific gravity has been measured with a refractometre, pH with a portable pHmeter and a chemical-physical examination of urine was made with Combur Test® 7. Blood and urine samples were simultaneously collected for urea (U), creatinine (Cr), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl) determination. The relative fractional excretion (%CrX) of electrolytes were calculated. Calves were always in good clinical condition. Significant variations on renal excretion were recorded in period III, especially for %CrU (Mean and SD respectively in box1 and box2: $66.61 \pm 9.2, 10 - 43.75 \pm 5.48$) %CrCa ($0.27 \pm 0.49 - 0.48 \pm 0.48$) %CrP ($8.42 \pm 13.94 - 1.28 \pm 2.83$) %CrMg ($2.62 \pm 4.46 - 10.19 \pm 6.96$) %CrK ($53.27 \pm 77.58 - 34.50 \pm 11.44$) %CrCl ($1.78 \pm 1.83 - 1.45 \pm 0.63$). These variations show important differences between box1 and 2: calves feeding risk diet had higher renal excretion of U, P, K and Cl associated to lower excretion of Ca and Mg and these variations are probably related to mineral composition of the diet (excess in P, lack of Ca).

All variations could be attributed to many factors (renal regulation of acid-base balance, water intake) but are primarily related to nutritional (especially mineral intake) and management factors. Further studies are needed on urinary concentration of Ca-P-Mg and ratios between themselves inasmuch as they seems to be more significant parameters to predict risk of urolithiasis in calves and to suggest diet variations.

7.7 NEW RESULTS OF TRACE ELEMENT RESEARCH IN CATTLE

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Introduction

Trace elements are essential inorganic components as well in feed as in food, which concentration is lower than 100 mg per kg dry matter. The trace elements iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), selenium (Se), iodine (I), molybdenum (Mb), chromium (Cr) and fluorine (F) are the „classical trace elements“, whereas aluminium (Al), arsenic (As), boron (B), barium (Ba), bromine (Br), cadmium (Cd), caesium (Cs), lithium (Li), nickel (Ni), lead (Pb), silicon (Si) are “new or ultra trace elements” (Spolders and Flachowsky 2006). The vital necessity and the physiological importance of these ultra trace elements are not verified in all cases. Some trace elements (e.g. Cu, Zn) release special effects in higher dosages, whereas other trace elements have toxic effects (e.g. As, Cd, Cu, F, Mo, Pb).

In the last ten years besides the requirements respectively recommendations of the animals, questions to the safety of trace elements use for human, animals and environment have obtained increasing importance. Consequences of this situation were questions of the European Commission to the SCAN (2003a, 2003b) and the EFSA (2004) on an evaluation to the safety and environmental relevance of copper, zinc and iodine as feed additives.

This discussion resulted in a reduction of some upper levels for trace elements in animal feeds (EU 2003, 2005). The range between the requirements for animals and the tolerable upper level is between 1:3 and 1:10 for the trace elements copper, zinc and iodine (Table 1). Another way which is discussed very contrary is the use of organic formulations of trace elements (complex compound of trace element with amino acids or proteins) in feeds. The bioavailability of these products should be higher than that of inorganic formulations, but this thesis are verified in practical studies only in individual cases (Jongbloed et al. 2002). In the following article the trace elements copper, zinc and iodine should be in the focus of interest, because these three trace elements are involved in the international discussion during the last years.

Table 1: Requirements (GfE 2001) and upper levels (EU 2003, 2005) for the trace elements copper, zinc and iodine for dairy cows

Trace element	Requirement (mg/kg DM)	Upper level (mg/kg DM)	Ratio
Cu	10	35	1:3.5
Zn	50	150	1:3
I	0.5	5	1:10

Copper

Copper has an important role in the antioxidant system and is an essential component of the enzymes copper-zinc superoxide dismutase and ceruloplasmin. A copper deficiency could result in increased susceptibility to bacterial infections and greater mortality. Dairy heifers supplemented with copper had lower *E. coli* numbers and somatic cell counts in milk, lower clinical scores and lower peak rectal temperatures than control heifers fed a low copper diet (Scaletti et al. 2003). An adequate copper supply is just as important for the health of the animals as the exactly diagnostic. In the last years there was an increasing reporting about copper deficiencies, diagnosed by copper serum concentrations below the reference values for copper given by the laboratories. But the most animals had no clinical signs of a typical copper deficiency, such as copper glasses. Feeding the double amount of copper (22.6 mg/kg dry matter) resulted in nearly the same copper concentrations in serum (12.0 $\mu\text{mol/l}$) compared to the controls (11.1 $\mu\text{mol/l}$), which received a ration with a copper content of 11.2 mg/kg dry matter (Öhlschläger et al. 2007). This control ration was in agreement with the recommendations of the German Society of Animal Nutrition and Physiology (GfE 2001) for copper (10 mg/kg dry matter). Other studies with higher copper contents in the ration (30 and 80 mg per kg dry matter) confirm, that the copper concentration in the serum was nearly constant (Table 2).

Table 2: Influence of different copper supplies (mg per kg DM) on copper concentration in blood serum ($\mu\text{mol/l}$)

Cu in the ration (mg/kg DM)	Number of animals	Duration of experimentation	Cu in serum ($\mu\text{mol/l}$)	Author
5 80	14 14	60	12.7 (day 60) 13.1 (day 60)	Du et al. (1996)
15 30	24 24	83	9.2 9.3	Chase et al. (2000)
15 30	25 25	70	13.5 (day 70) 14.6 (day 70)	Yost et al. (2002)
11.2 22.6	15 15	250	9.9 (day 250) 10.6 (day 250)	Öhlschläger et al. (2007)

The copper concentrations are located on the minimum level of the reference values ($< 12 \mu\text{mol/l}$), but without any clinical signs of a copper deficiency. Consequently, the serum concentration of copper is a not qualified indicator for the copper supply of dairy cows and the reference values for copper in serum must be adapted to the recommendations of the GfE (2001). It is provided over a great range, that the supply meets the requirements in dependence to their intake (Figure 1), because the recommendations of the GfE are adapted to the permanent changing requirements of the animals (Flachowsky and Martens 2006).

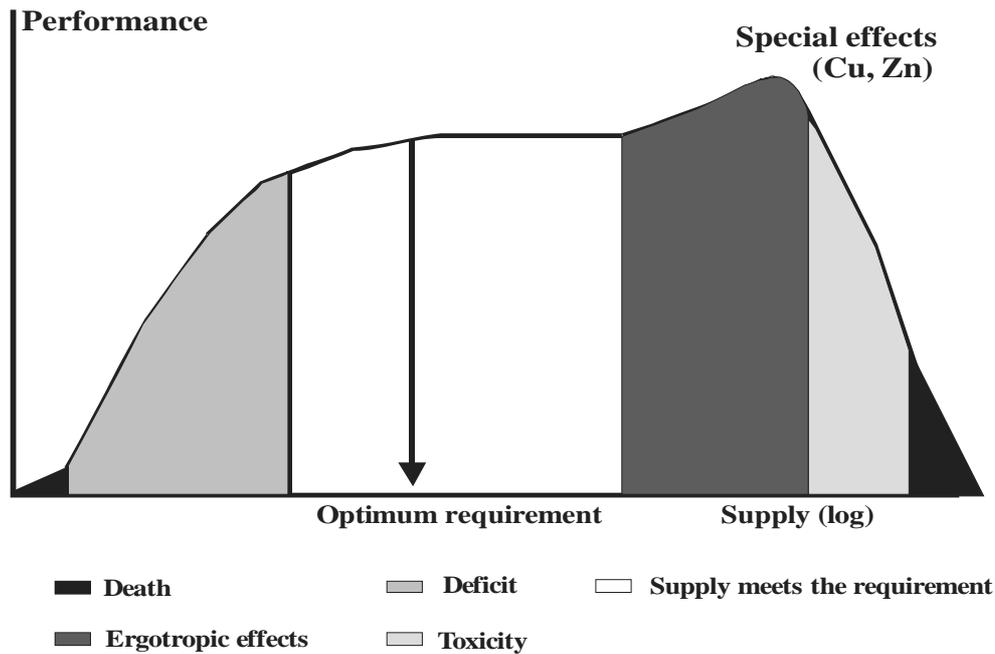


Figure 1: Dependence of the performance to the supply with trace elements (biological dose-response-curve)

Öhlschläger (2006) suggested an adapted reference value of 8-18 $\mu\text{mol/l}$ for copper in serum. A suitable indicator for the copper supply is the liver. The correlation between copper intake and copper content in the liver is higher ($r = 0.52$) than the correlation between copper intake and copper concentration in the serum ($r = -0.30$, Sun 2007). A copper supply according to the recommendations of the GfE (2001) resulted in a copper liver content of 376 mg/kg DM, whereas the copper liver content increased up to 518 mg/kg DM, when the copper supply was two times higher than the recommendations (Sun 2007). Both copper liver contents are on a high level; normal values for copper liver contents are 35-100 mg/kg DM. These results are in good agreement with other studies, in which copper liver contents were observed from 170 up to 826 mg/kg DM, when the copper content in the ration was 10 up to 40 mg/kg DM (Table 3).

Table 3: Influence of different copper supplies (mg/kg DM) on copper liver content (mg/kg DM)

Cu in the ration (mg/kg DM)	Number of animals	Duration of experimentation	Cu in liver (mg/kg DM)	Author
15 30	24 24	83	395 612	Chase et al. (2000)
10 40	12 12	61	431 826	Engle et al. (2001)
15 30	25 25	70	170 240	Yost et al. (2002)
11.2 22.6	10 10	28	376 518	Sun (2007)

Zinc

Zinc deficiency decreases resistance to a number of pathogens (Shankar and Prasad 1998); a number of zinc dependent enzymes are involved in protein synthesis and cell division. Although severe zinc deficiency clearly impairs animal health, controlled studies in cattle (Spears and Kegley 2002) suggest that marginal zinc deficiency does not affect animal health. The zinc recommendation of the GfE (2001) for dairy cows is 50 mg per kg dry matter, but the feeding in practice resulted in at least the double amount of zinc in the ration for dairy cows. Is this quantity of zinc necessary for the animals or only for the company of the feed additives? Feeding a ration with a zinc content of 62.5 mg/kg DM resulted in a serum concentration of 11.5 $\mu\text{mol/l}$, which is below the reference values. Feeding the double amount of zinc (113.2 mg/kg DM) increased the serum concentration of zinc into the reference value up to 13.5 $\mu\text{mol/l}$. In both groups there were no clinical symptoms of a zinc deficiency (Öhlschläger et al. 2007). The adapted reference value for zinc in the serum could be 8-17 $\mu\text{mol/l}$ (Öhlschläger 2006). In other studies (Wright and Spears 2004) a significant increasing of zinc concentration in the serum was only detected for rations with a zinc content of 500 mg/kg DM, which is not allowed in the EU. The upper level for zinc in feed additives is 150 mg/kg DM (EU 2003). In contrast to copper, the serum is a better indicator for the zinc supply ($r = 0.31$), whereas there is no correlation between zinc intake and liver zinc content ($r = 0.16$, Sun 2007). The zinc content in the liver is independent on the zinc supply. In studies with zinc contents between 17 and 300 mg/kg DM the zinc content in the liver differs between 100 and 360 mg/kg DM (Table 4). All these zinc liver contents are higher than the reference values (40-100 mg/kg DM).

Table 4: Influence of different zinc supplies on the zinc content in the liver

Zn in the ration (mg/kg DM)	Number of animals	Duration of experimentation	Zn in liver (mg/kg DM)	Author
17 40	20 20	21	100 106	Engle et al. (1997)
60 150 300	20 20 20	84	233 208 387	Kincaid et al. (1997)
62.5 113.2	15 15	250	300 360	Sun (2007)

Iodine

Iodine is a constituent of the thyroid hormones triiodothyronine (T₃) and thyroxine (T₄). A thyroid hormone deficiency due to dietary iodine deficit or inhibited iodine uptake of the thyroid causes goitre, either without signs of illness (compensatory goitre) or with more or less pronounced clinical symptoms of hypothyroidism (Laurberg et al. 2002). The German Society of Nutrition recommends a daily intake of 200 µg iodine for young people and adults (D-A-CH 2000). According to newer monitorings (Gärtner et al. 2001), the recommended iodine intake is met in a magnitude of two third and big parts of population renally excrete less than 100 µg per person and day representing a first-degree iodine deficiency according to the World Health Organisation (WHO) classification.

The still insufficient dietary iodine supply of the population is counteracted by iodine concentration of food – on the one hand in a direct manner by the use of iodised salt in baked goods and sausage production (Großklaus and Jahreis 2004), on the other hand indirectly by the concentration of the trace element in animal products mainly milk (Kaufmann et al. 1998) administering a feed with increased iodine contents.

In the EU, an upper limit of 5 mg iodine per kg feed of the dairy cow (EU 2005) represents the manifold feed iodine addition of the animal nutrition societies in a magnitude of 0.5 – 0.6 mg/kg DM (GfE 2001).

Nevertheless the level of the practised feed iodine supplementation there was an milk iodine concentration increase from 20 µg/l detected in Eastern Germany before 1985 to more than 100 µg/kg in the 1990s up to now (Flachowsky et al. 2006). Monitorings in the German states show concentrations in a range of 100 to 200 µg iodine/kg milk (Schöne et al. 2003, Bader et. al. 2005, Launer und Richter 2005). In a dose-response experiment (Schöne et al. 2006), five Holstein cows were fed four iodine doses as calcium iodate-hexahydrate in 4 periods of 14 days each. In addition to the testing of the diet without iodine supplement (iodine content 0.2 mg/kg DM) the supplementation levels were 1.3, 5.1 and 10.1 mg/kg DM. Rising iodine supplements increased

significantly the iodine concentration of serum and milk (48, 66, 131 and 290 μg iodine/l serum; 101, 343, 1215 and 2762 μg iodine/kg milk in the sequence of the groups, Table 5). The iodine supplements to rations for dairy cows resulted in increased iodine concentrations in the milk up to a level that the upper limit of 500 $\mu\text{g}/\text{d}$ for human iodine supply is exceeded by consuming 250 ml milk.

Table 5: Iodine concentration of serum and milk and excreted iodine with milk (Schöne et al. 2006)

	Iodine content (mg/kg DM)			
	0.2	1.3	5.1	10.1
Serum-I ($\mu\text{mol}/\text{l}$)	48 ^a \pm 12	66 ^b \pm 16	131 ^c \pm 37	290 ^d \pm 75
Milk-I ($\mu\text{g}/\text{kg}$)	101 ^a \pm 32	343 ^b \pm 109	1215 ^c \pm 222	2762 ^d \pm 852
I-excretion with milk (mg/d)	2.22 ^a \pm 0.66	7.21 ^b \pm 2.30	24.0 ^c \pm 4.2	51.9 ^d \pm 15.8

a, b, c, d ($p < 0.05$)

There are also indications that the use of iodized udder disinfection solutions contribute to the iodine concentration in the milk. For example, the application of an iodized dip solution providing a free available iodine concentration of 0.3 % through nonoxinol(9)-iodine increases the iodine concentration in the milk by 54 $\mu\text{g}/\text{l}$, indicating that this transfer is only marginal as compared to the effect of dietary iodine supplements. However, one litre milk after dipping the udder nearly meets the daily iodine requirements of adults, which is widely accepted to be 200 $\mu\text{g}/\text{d}$ (Table 6).

Table 6: Iodine concentration in the milk with or without the use of iodized dip solution (Spolders et al. 2006)

Dipping	Iodine concentration ($\mu\text{g}/\text{kg}$)
No dipping	100 ^a \pm 23
Iodized dip solution	154 ^b \pm 42

a < b; $p < 0.05$

Conclusions

Trace elements such as copper, zinc and iodine are in major interest of food security and safety in the last ten years. On the one hand, feeding trace elements to the animals may contribute to avoiding deficiencies in animals and humans, but on the other hand, questions of consumer protection and environmental aspects are of major concern, which resulted in the reduced upper levels for some trace elements. To combine these two different aspects is a main field of researches in animal nutrition for the future.

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7.8 DIFFERENT ANIONIC SALTS HAVE A DIFFERENT IMPACT ON THE ACID-BASE STATUS AND THE CALCIUM METABOLISM IN DAIRY COWS

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To evaluate the impact of different anionic salts (AS) on the metabolism of dairy cows, a study-design including a controlled feed intake has been carried out. Eleven mature, non-lactating, non-pregnant, Holstein-Friesian-Crossbred cows received 2000 meq of either one of the three chloride salts (CaCl₂, MgCl₂, NH₄Cl), four sulfate salts (CaSO₄, CaSO₄ D10, MgSO₄, (NH₄)₂SO₄), two combinations of AS (CaCl₂+MgSO₄, CaSO₄+NH₄Cl), sodium chloride (NaCl) or water as control via a rumen cannula. Salts and controls were assigned in an 11 x 11 Latin square and cows were randomly distributed. Each treatment period lasted fourteen days and was discontinued to the following TP by a washout period (WOP) of the same duration. Whole blood, serum and urine samples were taken during treatment (TP) and WOP. Samples of whole blood were tested for pH, base-excess (BE) and bicarbonate concentrations [HCO₃⁻]. In urine samples, pH and NABE were analysed. Calcium, sodium, magnesium and chloride concentrations were measured in serum and urine samples. All AS tested induced a metabolic acidosis visible in small reduction of blood pH, BE and [HCO₃⁻] ($p < 0.001$) and remarkable changes in urinary pH, NABE ($p < 0.001$) and urinary calcium excretion ($p < 0.001$). However, sulfate salts induced alterations of ABS which did not differ from those induced by NaCl. MgSO₄ had the lowest potential to alter acid-base status (ABS), differing significantly from CaCl₂, MgCl₂ and CaSO₄ D10, a natural occurring by-product of gypsum industry. NaCl decreases NABE significantly ($p < 0.05$), this reduction did not differ from that induced by MgSO₄ ($p > 0.05$). CaCl₂ had a significant greater impact on ABS than most of the sulfate salts. Beside these different reactions no significant differences were detectable between the potential to alter ABS and urinary calcium excretion between the other chloride (MgCl₂, NH₄Cl) and sulfate salts (CaSO₄, CaSO₄ D10, (NH₄)₂SO₄).

To conclude, CaCl₂ has the greatest impact of changing ABS and MgSO₄ has the weakest one. Due to the present results, there is no indication that chloride salts in general have a greater impact on ABS than sulfate salts. All AS tested induced an increase of urinary calcium excretion clearly exceeding the threshold value of 5mmol/l, which is an indicator of an activated calcium mobilisation.

7.9 ADVANTAGES AND PROBLEMS OF FEEDING ZEOLITE A IN PREVENTION OF HYPOCALCEMIA FOR DAIRY COWS

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Problem: subclinical hypocalcemia is an important disease for high yielding cows. There exist some strategies in the prevention of hypocalcemia, one of these strategies is the addition of zeolite A as a calcium-binder to the ration of dry cows. However such additives could influence the feed intake negatively and this was an important problem. **aim:** the aim of the presented study was to prove the influences of different doses of zeolite A on feed intake and mineral metabolism around calving.

Material and methods: A total of 80 pregnant dry cows between the 1st and 6th lactation were assigned to 4 groups (I-IV). They were fed a TMR (48 % maize, 32 % grass silage, 20 % concentrate on dry matter basis) ad libitum, starting 4 weeks before calving. Additionally, at the last two weeks ante partum the experimental groups (II, III and IV) received an average daily dose of 12, 24 and 46 g zeolite per kg DM of TMR, which was mixed into the TMR. Individually DM intake was recorded daily. Blood samples from the V. jug. ext. were taken 28, 14 and 7 days before the expected day of calving, on the day of calving and on day 1, 2 and 7 after calving. The serum was analysed for Ca, Mg, and Pi.

Results: The average daily feed intake amounted to 11.4 kg DM in the first 2 weeks in all 4 groups. During the last 2 weeks the feed intake of the groups I to IV was reduced to 10.0; 10.8; 9.4 and 7.2 kg DM per day. The reduction was only significant in group IV. The zeolite supplementation in higher doses (III and IV) showed a stabilizing effect on serum calcium around calving for cows with a lactation rank higher than 3 (2.27 and 2.31 mmol Ca/L), whereas cows in groups I and II showed a subclinical hypocalcemia (Ca < 2 mmol/L). For cows in the 1st and 2nd lactation hypocalcemia had no relevance, because the mean serum Ca around calving was higher than 2 mmol/L. The mean serum Mg decreased at calving in group IV (0.99 and 0.94 mmol/L), but only for older cows significantly. In the other groups the mean serum Mg was nearly constant (1.1 mmol/L). In all 4 groups a reduction of mean serum Pi was observed (from 1.75 down to 1.17 mmol/L, group I and from 1.58 down to 0.60 mmol/L, group IV). However, the concentration decreased into ranges of a hypophosphatemia only in group IV (< 0.80 mmol/L).

Conclusions: A daily amount of 24 g zeolite A/kg DM TMR feeding for 2 weeks before calving seems to be the maximum zeolite dosage for reducing subclinical hypocalcemia frequency without a significantly decrease of feed intake.

7.10 PHARMACOKINETIC EVALUATION OF A SINGLE ORAL AND AN INTRAVENOUS CALCIUM TREATMENT IN CALVES AND COWS

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There are several ways to prevent milk fever in dairy cows. We posed the question: would orally administered CaCl₂ in a paste formulation effectively elevate blood calcium concentration? Therefore, the evaluation of the expected efficiency of a single oral treatment with Ca paste was aimed in a pharmacokinetic study.

A two-phase experiment was performed to study the absorption and elimination of Ca in normocalcaemic, milk-fed calves (n₁=8) that were modelling the same species, but lacking a functional rumen, and in non-pregnant normocalcaemic cows (n₂=8). After an initial blood sampling from the tail vessels of cows, and the jugular vein of calves, we administered one tube (295 g) Recovin Ca Paste (180.5 g CaCl₂) per os to each of 4 non-pregnant cows, and 0.59 g paste/kg bwt to each of 4 calves (361 mg CaCl₂/kg bwt). Each of 4 other cows intravenously received a 500 ml Ca-gluconate infusion (50 g Ca-gluconate), while each of further 4 calves, 50 mg Ca-gluconate/kg bwt, iv. Additional blood samples were collected at 5, 15, 30, 45 min. and 1, 2, 4, 6 and 12 h after each treatment. Treatments and samplings were repeated not earlier than 7 days later, in a crossover design. After anaerobic blood collection into 5 ml heparinized syringes, the cooled samples (+4°C) were measured within 30 min., using a portable blood gas and electrolyte analyzer (ABL-77). Blood actual ionized Ca concentrations (Ca²⁺) were measured.

Calcium absorption was fast from the Ca paste both in cows and in calves (absorption half life /t_{1/2a}/: 7.2 min. for calves, 3.0 min. for cows). The highest Ca²⁺ concentration /T_{max}/ occurred after 62.9 min. in calves and 27.3 min. in cows and this elevation was more pronounced in calves. Changes in Ca²⁺ concentrations after iv. treatment were more pronounced and longer in cows than in calves (elimination half life /t_{1/2el}/: 74.4 min. for cows, 9.3 min. for calves). While blood Ca²⁺ concentration returned to the initial value by 45 min. in calves, it remained elevated even in the samples taken at 2 to 4 hours in cows.

In conclusion, our results helped to establish the beneficial effect of an oral calcium paste on blood Ca²⁺ concentrations even in normocalcaemic calves and cows. However, this treatment type provided a slower absorption and a more prolonged effect than the iv. treatment. Additionally, it was more pronounced in calves than in cows. Supports: OTKA T-043505, Jorgen Kruuse A/S, Marslev, Denmark, Ghislandi and Co. Ltd., Budapest, Hungary.

7.11 RENAL PHOSPHORUS EXCRETION RELATED TO CRUDE FIBRE SUPPLY AND THE USE OF CALCIUM CHLORIDE - RELEVANT TO UNDERSTAND UROLITHIASIS IN FATTENING CATTLE?

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Problem: Struvite urolithiasis, one of the common disorders in intensive cattle fattening, is thought to be associated with a high renal P excretion and an alkaline urine pH. The aim of this study in fattening bulls was to investigate possible influences of feeding intensity (crude fibre content) on the P metabolism, especially renal excretion. Furthermore, the effect of CaCl₂ - an established struvite prophylactic in other species - was studied. The results are supposed to contribute to establishing dietary recommendations for prophylactic purposes.

Material and methods: 5 consecutive balance trials (10 d adaptation, 10 d collection of faeces and urine, blood samples on the 21st d) were conducted with 3 fattening bulls (beef cattle cross-bred, 250 - 400 kg body weight). Diets based on maize silage and soybean meal supplemented with minerals and vitamins according to animals' requirements (P intake: 23 g/d). Crude fibre content varied (14.8 - 24.0 % of DM) due to varying proportions of barley and hay in the diet. In two balance trials coated CaCl₂ was added to the ration (75 g/d). Effects on P metabolism (apparent digestibility, excretion, concentration in serum) as well as on acid-base-balance (pH in urine and blood) were studied. Urine pH was estimated immediately after the micturition. P was analyzed using a colorimeter.

Results: Crude fibre content in the diet had no significant effect either on P concentration in urine or on absolute renal P excretion. The most interesting finding was the individually strong different reactions of the animals. One bull, which always showed the highest apparent digestibility rates of P (47.5 - 57.1 %) and had higher P values in serum (2.43 - 2.98 mmol/l), excreted 7 - 20 % of the ingested P via urine, whereas the renal P losses of the other bulls (apparent digestibility of P: 30.6 - 45.5 %, P concentration in serum: 2.18 - 2.60 mmol/l) never exceeded 4 % of ingested P. The addition of CaCl₂ lowered blood pH slightly, but urine pH was reduced significantly from > 8.0 to < 6.5.

Conclusions: Like in sheep where genetically based differences in P metabolism are well known results of these investigations indicate comparable conditions in cattle. High efficient P absorber have higher P concentrations in the blood and excrete the P surplus via urine. Presumably only those animals are disposed for struvite formation in the urine, explaining the fact that in large fattening units only a minor percentage of animals is affected despite of identical feeding and housing conditions. The use of CaCl₂ might be a useful dietetic measure. The chosen dosage of 75 g coated CaCl₂/animal and day (equals 20 g/100 kg body weight) was adequate for the acidification of urine and poses no threat to the animals' health.

7.12 MAGNESIUM BALANCE IN LACTATING DAIRY COWS SUPPLIED WITH DIFFERENT POTASSIUM AND MAGNESIUM LEVELS

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Magnesium balance in lactating dairy cows supplied with different potassium and magnesium levels. Low amounts of Mg in the diet or decreased bioavailability may cause hypomagnesemia in dairy cows. The K content of the ration is considered an important risk factor in the development of hypomagnesemia in ruminants since it reduces the digestibility of Mg. In many areas with intensive dairy production K content in forage often is higher than 30 g/kg DM. Most studies of the interactions between potassium and magnesium have been performed in small ruminants or in dry dairy cows.

Thus an aim of this study was to investigate the interactions of dietary K intake typical for forage-based diets on Mg balance in lactating dairy cows. Six lactating multiparous cows of the Swedish Red and White breed in mid-lactation were used. Two concentrations of Mg (1.9 and 4.3 g/dry matter (DM) and three concentrations of K (19, 28 and 37 g K/kg DM) were obtained by adding appropriate amounts of MgO and KHCO₃ to the diet. The experimental set up was a 6x6 Latin square design with a 2 x 3 factorial arrangement of treatments. Each experimental period lasted 14 d (9 d treatment adaptation period and 5 d data collection).

The results of the present study show that supplementation with K to the ration did not affect the Mg apparent absorption, urinary Mg excretion or plasma Mg concentration. The Mg balance, estimated as the Mg losses in milk and urine, was positively related to Mg intake but not affected by K intake. The apparent Mg digestibility ranged from 0.12 to 0.24 with no effect of mineral supplementation on the digestibility. The urine excretion of Mg and plasma Mg were positively associated in a curvilinear action. A similar association has been shown in dry cows. However, in the present study with lactating cows, the urine excretion was markedly higher. It is possible that there is productivity-related increment of inescapable urinary Mg losses in lactating cows. Cows supplemented with Mg in the diet had wetter feces in line with previous observations showing that high levels of may cause diarrhea.

In conclusion: In this trial with lactating dairy cows an increased potassium intake did not negatively affect apparent magnesium absorption. It is possible that the requirement of Mg supplementation to lactating dairy cows fed potassium-rich diets has been blown up. An intake of 1.9 g Mg/kg DM was too low for lactating cows fed grass silage-based diet.

7.13 TRACE ELEMENTS IN CATTLE: COPPER AND IT'S "FRIENDS"

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Prophylactic procedures to prevent diseases become more and more important in cattle rearing. Adequate supply in trace elements is required. Copper (Cu) deficiency is regarded as the second most common mineral deficiency of cattle in the World (Telfer et al, 1996; Black und French, 2004). Two different copper deficiencies are known: a primary copper deficiency induced by a simple deficiency of copper in the diet, and a secondary, resulting from the reduction in copper absorption or utilization by the antagonistic effects of molybdenum (Mo) and iron (Fe). 340 cattle samples were analysed as serum/plasma pairs regarding the concentration of Cu, Mo and Fe with ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) during July 2006 until March 2007. The Cu-plasma analyses showed that only 24% of these samples were below the reference value for Cu (80 µg/dL). Compared to these results, 84% of the corresponding serum samples showed a Cu deficiency (below 80µg/dL). Serum clotting reduces Cu by up to 80% compared to the plasma values (in confirmation to Laven & Livesey, 2006). In contrast to copper the analyses of Mo in bovine plasma and serum resulted in comparable values. 4500 bovine serum and plasma samples were analysed for Mo. 80% of Mo-concentrations were below 26 µg/L. Only a few samples (< 1%) showed higher values (max. 3300 µg/L). A correlation between low Cu and high Mo values in plasma, or low Cu values with high Fe concentrations could not be found. Additionally, hair analyses of Cu (N = 173) and Mo (N = 99) were performed. 85 % of the hair samples (3-times analyses of each hair sample) showed Cu values between 5 and 20 mg/kg (49%: 6,6-10,4 mg/kg; 4 %: below 6,6 mg/kg), 15 % of the samples showed higher values up to 100 mg/kg). The Mo concentrations in the hair samples varied between 0 up to 800 µg/kg or even higher).

The comparison between Cu and Mo in hair (N = 99), or Cu hair/plasma (N = 107) and Mo hair/plasma (N = 70) showed no correlation. The analysed cattle samples with low copper plasma concentrations showed mainly a primary copper deficiency. A secondary copper deficiency caused by a molybdenum intoxication could not be found in this set of samples.

7.14 THE ROLE OF SALIVARY GLANDS IN THE MINERAL HOMEOSTASIS IN RUMINANTS

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To study the role of salivary glands in mineral homeostasis, the present study was undertaken in sheep fitted with a large rumen cannula. On the day of experiment, a salivary collector was slipped into position at the base of the oesophagus so that a continuous collection from all the salivary glands could be achieved. The Na concentration in the mixed saliva and plasma were nearly same, whereas the salivary K concentration was approximately double as compared to plasma K concentration in Na replete sheep. The concentration of PO₄ in ruminant saliva was about 8-10 times higher than that of plasma PO₄ concentration. The effects of varying the concentrations of plasma Na, K and PO₄ were undertaken to study the role of salivary glands in handling these ions. It has been observed that there were immediate and positive correlations between Na, K and PO₄ concentrations in plasma and saliva. Infusing 72 mM KCl for 90 minutes increased the plasma K level by 2 fold with a corresponding increase of K in the saliva. Intravenous infusion of sodium phosphate at 45 mmol/hr in sheep increased the plasma PO₄ 5-7 fold and plasma Na to 10% with significant increases in the PO₄ and Na in the saliva. It was observed that intravenous infusion of 1 mg of angiotensin-II (a stimulant of aldosterone secretion) over a period of 2 hrs was responsible for causing a sudden drop in the salivary flow rate from 9.5 ml/min to 4.2 ml/min and a decrease of about 25% in the ratio of Na and K in saliva. Intravenous infusion of 0.5 mg aldosterone in Na replete sheep resulted in a decreased ratio of Na and K in saliva. The sum total of Na and K remained the same in the final saliva. The daily rates of secretion of Mg and Ca in saliva can be 25-30% of their contents in the extracellular fluid. Infusion of hormones PTH and PTHrP (100 microgram in 2 hrs) quickly increased the plasma Ca concentration without having much effect on the salivary Ca concentration. It was only a long term increase in plasma Ca concentration induced by the administration of Vit D₃ or an analogue that an increase in the salivary Ca concentration could be observed. Thus, impairment of the absorption of Mg and Ca from the reticulo-rumen, especially of Mg could contribute to the development of the clinical signs of acute hypomagnesaemia. This impairment is exacerbated by both increased dietary intake of K and dietary Na depletion (leading to increased salivary K concentration) and consequent increase in ruminal K concentration. The net absorption rates of both Mg and Ca ions from the reticulo-rumen are decreased by this increased ruminal concentration of K ions.

It is concluded that in ruminants the salivary glands are actively involved in mineral homeostasis and the aetiology of certain production diseases.

7.15 TRACE ELEMENTS IN EQUINE SERUM: A CURRENT SURVEY - SELENIUM, COPPER AND ZINC

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A sufficient supply of trace elements is very important for performance, especially in the competing horse. Insufficient supply can lead to a decrease in performance, and deficiency symptoms as sore muscles and changes in skin- and coat metabolism are more likely to be seen. Between July and December 2005, a total of over 5800 equine serum samples were analysed regarding copper, zinc and selenium concentration. Analyses were performed on ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy). All three trace elements were analysed simultaneously from the same diluted sample. Copper: 95% of the horses showed copper concentrations within the normal range (50-150µg/L) and were considered as adequately supplied. Zinc: 66% of the zinc concentrations were within the normal range (500-1300 µg/L). 33% of the horses showed zinc concentrations lower than the reference values. Selenium: Only 51% of the horses showed selenium concentrations within the normal range (100-200 µg/L). 42 % showed selenium levels lower than the reference values and 7% showed elevated concentrations. Regarding all three trace elements, only 1/3 of the tested horses showed a sufficient supply. 40% showed an undersupply of at least one of the three trace elements. An excess concentration in all three trace elements is very rare (ca. 1%). For this study a large number of equine serum samples was analysed.

Results showed a high incidence of undersupply of one or more trace elements. Qualifying these concentrations in sport horses or horses with poor performance could provide helpful information.

7.16 TRANSITION COW TREATMENT WITH CALCIUM GEL

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57 Holstein cows were paired according to parity and previous incidence of milk fever. Cows in the different treatment group received doses of calcium chloride in a gel formulation 48, 24 and 6-0 hours prior to expected calving and at 0, 6, and 24 hours after calving. Cows in the control group received no calcium chloride gel treatment. A blood sample was taken from the tail vein before treatment with gel and was subsequently analyzed in the laboratory. Calcium, inorganic phosphorus, magnesium, glucose and parathyroid hormone were analysed in cow blood serum of different treatment and control groups. The lowest calcium range in control group of cows without symptoms of milk fever were detected 24 hours after calving - $1,74 \pm 0,19$ mmol/l. Inorganic phosphorus at the same time was normal. The lowest calcium level of sick cows was 24 hours after calving ($1,34 \pm 0,45$ mmol/l). The lowest inorganic phosphorus level of sick cows was observed at 48 hours postpartum ($0,75 \pm 0,17$ mmol/l). Treated cows with calcium gel did not avoid milk fever.

7.17 TOXIC AND TRACE ELEMENTS IN INTENSIVE SWINE IN GALICIA (NORTHWEST SPAIN)

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Problem: The toxic metals cadmium, lead, arsenic and mercury are very important because they do not have any function in animals and when they reach certain concentrations they can cause toxic effects. The trace metals such as copper, zinc, iron and selenium have many functions in animals, but below or above certain concentrations animals can show deficiency or toxicity symptoms. Diet is the main source of these metals for man and animals, and that is why European Union, as well as other countries, have a legislation to regulate the maximum metal content in animal products for human consumption.

Aim: The aims of this study were to evaluate toxic and essential metal concentrations in intensive swine meat and offal in NW Spain, compare these to metal contaminants in cattle from the same region, and relate them to maximum acceptable concentrations.

Material and methods: Samples of liver, kidney and muscle from 63 pigs aged 6 months were randomly collected at slaughter, acid-digested and levels of metals determined by ICP-OES and ICP-MS. Results and discussion Average concentrations in liver, kidney and muscle respectively were 0.073, 0.308 and 0.009 mg/kg fresh weight for cadmium; 0.004, 0.008 and 0.003 for lead; 0.013, 0.011 and 0.003 for arsenic; 0.001, 0.002 and 0.001 for mercury. These concentrations were lower (lead and arsenic) or higher (cadmium and mercury) than those found in young cattle from the same region and maximum admissible concentrations established by the European Union were not exceeded in any sample. For the essential metals these concentrations were 14.9, 5.63 and 6.85 mg/kg for copper; 81.3, 28.9 and 42.5 for zinc; 195, 51.6 and 26.5 for iron; 1.17, 2.51 and 0.656 for selenium.

Conclusions: The concentrations of toxic metals cadmium, lead, arsenic and mercury in liver, kidney and muscle of pigs in NW Spain are low and admissible maximum concentrations established by the European Union were not exceeded in any sample. In the case of the essential metals analysed, their concentrations in pigs in our study are within the adequate ranges for this animal species.

7.18 INFLUENCE OF BREED ON COPPER STATUS IN BULL CALVES

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Problem: The copper deficiency and toxicity are common in beef cattle. There is a wide variety of researches about variation in susceptibility and tolerance among breeds of sheep, whereas, little information is available concerning differences among bovine breeds. The identification of tolerant and sensitive breeds could help us to optimise the beef cattle production. **AIM:** The aim of this research is to evaluate the influence of breed on different blood parameters in relation to copper status in feedlot bull calves receiving copper supplementation.

Material and methods: Blood samples were collected, monthly, from bull calves (n=30) Galician blonde, Holstein fresian and crosses between the two breeds. Since the age of three months until the tenth month approximately (slaughtered date). Samples were acid digested and copper concentration determined by ICP-OES.

Results: During all the research, the average serum copper concentration, blood copper concentration and ceruloplasmin activity were within physiological ranges for this animal species. Holstein fresian calves showed, generally, the lowest mean copper concentration in blood, serum and ceruloplasmin activity. Galician blonde showed the highest mean copper concentration in serum and ceruloplasmin activity. The crosses between the two breeds had intermediate levels of copper concentration in serum.

Conclusions: Under the circumstances of this research, the breed did not show a significant influence on blood parameters of bull calves copper status (plasma copper, serum copper and ceruloplasmin activity) along all the study.

7.19 FACTORS INFLUENCING BOVINE MATERNAL AND FETAL HEPATIC MINERAL CONCENTRATIONS

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Studies have shown significant transference of mineral elements from the pregnant cow to the developing fetus. Maternal mineral status during pregnancy influences fetal and postnatal mineral status. The aim of this study was to characterize parameters that influence bovine maternal and fetal hepatic mineral concentrations. Liver samples were collected from 181 pairs of pregnant cows and their fetuses at an abattoir over a period of 13 months. Breed, sex, fetal numbers and measured crown-rump length were recorded at time of collection. Inductively coupled plasma atomic emission spectroscopy (ICP/MS) was used to assay 9 minerals (calcium, copper, cobalt, iron, magnesium, manganese, molybdenum, selenium, zinc) in all samples. Mineral concentrations were determined on a wet weight (WW) and converted to a dry weight (DW) basis. Liver dry matter (DM) content was determined by drying an aliquot sample in a convection oven. Fetal gestational age was estimated from measured crown-rump length. Paired T-test was used to determine differences between fetal and maternal mineral values. Regression and ANOVA were used to determine significant parameters influencing fetal and maternal hepatic mineral concentrations. Of the 181 total paired samples 78.5% and 21.5% were from dairy and beef cows, respectively. Dairy cows were predominately Holstein breed, while beef cows were Hereford, Angus or crossbreds. Twin fetuses were found in 11 cows (10 dairy; 1 beef). Mean (range) fetal age was 6.4 (3.8-9.4 months). Fetal sex and twin pregnancy did not influence hepatic mineral concentrations. Mean fetal (0.22) and adult (0.31) liver DM ratios were different ($P < .0001$). Mean fetal-maternal pair differences for all mineral concentrations were different ($< .0001$) from zero, except selenium (WW) concentration across all data and within dairy breed samples. Breed (dairy or beef) influenced a number of mineral relationships. Within the beef cow samples, no differences between maternal and fetal concentrations were found for WW cobalt, copper or selenium and cobalt DW. Fetal mineral concentrations were lower compared to maternal values for cobalt, copper, manganese and molybdenum (WW and DW) and WW magnesium. In beef cow samples, fetal DW copper concentration was greater ($P < .01$) compared to maternal. Fetal, but not maternal, liver DM ratio increased ($P < .0001$) with gestational age. Gestational month influenced fetal mineral concentrations more than maternal values and these effects were further influenced by breed.

These data show maternal and fetal hepatic mineral concentrations cannot be interpreted with one set criteria. Differences in DM content suggest using DW values and must be adjusted for gestational age and breed depending upon the mineral in question.

7.20 BREED EFFECTS ON BOVINE FETAL AND MATERNAL HEPATIC MINERAL CONCENTRATIONS

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A number of factors have been identified as influencing trace mineral status of the dam and its fetus. Previously, breed (dairy vs. beef) was identified as a significant factor affecting hepatic mineral status. Aim of this study was to better characterize breed effects on maternal and fetal hepatic mineral concentrations. Liver samples were collected from 181 pairs of pregnant cows and their fetuses at an abattoir over a period of 13 months. Breed, sex, fetal numbers and measured crown-rump length were recorded at time of collection. Inductively coupled plasma atomic emission spectroscopy (ICP/MS) was used to assay 9 minerals (calcium, copper, cobalt, iron, magnesium, manganese, molybdenum, selenium, zinc) in all samples. Mineral concentrations were determined on a wet weight (WW) and converted to a dry weight (DW) basis. Liver dry matter (DM) content was determined by drying an aliquot sample in a convection oven. Fetal gestational age was estimated from measured crown-rump length. Regression and ANOVA were used to evaluate interrelationships and breed effects between fetal and maternal hepatic mineral concentrations. A total of 181 fetal-maternal paired samples were collected from 142 dairy and 39 beef cows, including 11 sets of twins (10 dairy; 1 beef). Dairy cows were predominately Holstein breed, while beef cows were Hereford, Angus or crossbreds. Mean (range) fetal age was 6.4 (3.8-9.4 months). Breed (dairy vs. beef) was found to influence fetal and maternal values and their interrelationships. Within all fetuses, both WW and DW concentrations of magnesium decreased ($P < .0001$) and manganese and molybdenum increased ($P < .0001$) with gestational age. Dairy fetuses had increasing calcium WW and decreasing iron DW concentrations, while beef fetuses showed increasing ($P < .001$) zinc (WW, DW) and decreasing ($P < .03$) cobalt and selenium DW concentrations. Dairy fetuses had greater WW and DW iron ($P < .02$) and selenium ($P < .001$) and lower ($P < .02$) WW magnesium, calcium and manganese content compared to beef fetuses. In general, maternal hepatic mineral concentrations were not influenced by gestational stage; however, some differences between breeds were identified. In dairy cows, manganese ($P < .0005$) and molybdenum ($P < .04$) content (WW, DW) declined with gestational age. Magnesium (DW) content declined ($P < .05$) over gestation in beef cows. Beef cows had higher hepatic manganese ($P < .03$) and lower copper ($P < .0001$), zinc ($P < .05$) and selenium ($P < .0004$) content compared to dairy cows.

In the current study, beef cattle showed lower hepatic copper, zinc and selenium content, possibly reflecting lower rates of mineral supplementation. Fetuses were capable of assimilating mineral similarly across gestation, but hepatic content reflected maternal status.

7.21 CAPRINE ARTHRITIS ENCEPHALITIS VIRUS (CAEV), A PATHOLOGICAL STUDY

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Caprine arthritis encephalitis virus (CAEV) is an RNA virus that belongs to the genus lentivirus within the family Retroviridae.

The objectives of the study were to evaluate the incidence of CAEV infection in Oman, and to assess the pathological changes and the pattern of the disease in the lungs of infected indigenous goats. Histopathological study was carried out on lung samples. They were collected from slaughtered indigenous goats (6-24 months). Specimens were fixed in 10 % buffered-formalin, processed, sectioned and stained with H&E and special Stains. The histopathological lesions were characterized by interstitial pneumonia, peribronchial, perivascular and or/parenchymal lymphocytic infiltration or lymphoid nodules with or without germinal centers. The lymphoid follicles often compressed bronchiolar lumens. Dense fibrosis in the interstitial, interlobular, peribronchial and perivascular areas had been observed. These lesions were detected in the lungs of clinically healthy animals and no macrothological alterations were observed in the organs.

The results suggest that these animals had been exposed to CAEV. It is highly likely that these animals represent a subpopulation of goats in early non-clinical phase of CAEV.

7.22 COMPARISONS BETWEEN BOVINE MATERNAL AND FETAL HEPATIC MINERAL CONTENT

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Understanding nutrition's role in animal health has prompted a need for accurate assessment and interpretation of mineral status relative to disease potential. Adult animal tissue mineral concentrations and their interpretation have been well defined. Diagnostic criteria for fetal hepatic mineral concentrations are not well established. The aim of this study was to generate preliminary data for assessing bovine fetal hepatic mineral concentrations. Liver samples were collected from 181 pairs of pregnant cows and their fetuses at an abattoir over a period of 13 months. Breed, sex, fetal numbers and measured crown-rump length were recorded at time of collection. Inductively coupled plasma atomic emission spectroscopy (ICP/MS) was used to assay 9 minerals (calcium [Ca], copper [Cu], cobalt [Co], iron [Fe], magnesium [Mg], manganese [Mn], molybdenum [Mo], selenium [Se], zinc [Zn]) in all samples. Mineral concentrations were determined on a wet weight (WW) and converted to a dry weight (DW) basis. Liver dry matter (DM) content was determined by drying an aliquot sample in a convection oven. Fetal gestational age was estimated from measured crown-rump length. Population statistics were determined for fetal and maternal mineral values. Regression and ANOVA were used to evaluate interrelationships and breed effects between fetal and maternal hepatic mineral concentrations. Paired fetal-maternal samples were collected from 142 dairy and 39 beef cows, including 11 sets of twins (10 dairy; 1 beef). Mean (range) fetal age was 6.4 (3.8-9.4 months). Raw hepatic mineral concentrations (WW and DW basis) across maternal and fetal samples spanned from very low to very high values relative to current reference information. Mineral supplementation rate and subclinical disease information were unavailable. No evidence of clinical disease was observed. Though fetal and maternal hepatic mineral content on a WW basis were statistically different, range of the raw values were very similar. Only fetal Fe (258.1 ppm) and Zn (169.4 ppm) showed greater ($P < .002$, $.007$) values across gestational age compared to maternal concentrations (87.0 ppm; 65.5 ppm), respectively. Wet weight value comparisons are confounded by differences in hepatic DM content and gestational age effects on fetal mineral concentrations. Hepatic DW mineral concentrations showed more distinct statistical differences between maternal and fetal values across gestation, though this was somewhat breed dependent. Fetal and maternal hepatic mineral concentrations are distinct populations requiring specific diagnostic criteria for interpretation. Interpretation of mineral WW concentrations is confounded by DM differences and changes over time and criteria based on DW values are recommended.

7.23 SERUM CALCIUM, PHOSPHOROUS AND MAGNESIUM CONCENTRATIONS OF DAIRY CATTLE IN DIFFERENT STAGES OF LACTATION IN GARMSAR DAIRY FARMS

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Calcium, phosphorous and magnesium are the most crucial minerals which play important roles in the body. Any deficiency in metabolism of mentioned elements may lead to outbreak of over 15 metabolic disorders in dairy cattle. During four different seasons, jugular blood samples were collected from 400 dairy cattle in different stages of lactation and gestation of dairy farms of Garmsar. In laboratory sera were separated from blood samples and kept at - 20 centigrade degree till analysis of samples. Measurement of calcium, phosphorous and magnesium concentration in collected samples were carried out by colorimetric method. Statistical analysis of results was done by t- student test followed by post- hoc test. The results of the present study showed that serum calcium concentration of dairy cattle in Garmsar dairy farm (8.76 ± 0.068 mg/dl) is less than normal range for cattle (9- 12 mg/dl). Lowest serum calcium and phosphorous concentrations of Garmsar dairy cattle were found in the winter season and for magnesium was in spring. Highest serum calcium and phosphorous concentrations were found in the spring season and for magnesium was in autumn. Serum calcium and phosphorous concentrations of pregnant dairy cows were higher than no pregnant cows but the difference was not statistically significant. Serum concentrations of three defined elements in heifers in the present study were significantly higher than multiparous cows.

7.24 INVESTIGATION ON TRACE ELEMENT BALANCE IN SAXON FEED PRODUCTION FARMS

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The trace element circulation was balanced and judged in select Saxon food building businesses. Examined the elements became manganese, zinc, copper and selenium. The being of use animal edging of the businesses was 0.8 for 1.6 GV per hectare. The area equipment lay at 863 - 4.216 hectares. Estimate and laboratory analytical became the input and output examined. In addition, indicators were examined. These should clarify the supply situation of the ground, plant and animal. For the examined trace elements a positive balance was determined in all businesses. Main reason is the low usage of the elements about the animal. Animal raw materials are not considerable trace element straps. A clear luxury equipment was traceable in the food rations. Lasting has 2 possible reasons: 1. Remained unconsideredly the salary results of the singles food means. 2, The antagonistic effects between the trace elements cause defect despite over supply. This leads to a further increase of the trace element. 2 possible facts give up the mineral input is secure: 1. Around balanced trace element balance sheets lowered by 80%. 2. The being of use animal edging is lowered by approx. 0.4 GV per hectare at same feeding practice.

8 RUMEN DIGESTION AND ACID BASE BALANCE

8.1 SCFA, PROTONS AND RUMINAL EPITHELIUM: THE GOOD, THE BAD, THE BARRIER

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Energy Requirements and intraruminal SCFA Production

Data obtained by indirect calorimetry and in feeding trials led to the calculation that 293 kJ/kg LW^{0.75} and 3.3 MJ/kg milk (4% fat) are daily needed on a NEL basis in lactating dairy cows for maintenance and milk production, respectively (Kamphues et al., 2004). Consequently, the daily energy requirement of a dairy cow weighing 650 kg and producing 35 kg milk summarizes up to 160 MJ NEL per day. 160 MJ equals the energy content of about 140 hamburgers.

Ruminants convert the biggest part of this energy to short-chain fatty acids (SCFA; mainly acetate, propionate, butyrate) by the aid of microbes contained in their forestomach. The majority of microbially produced SCFA is subsequently absorbed directly from the rumen. According to studies by Allen (1997) in cannulated dairy cows, the ruminally degraded organic matter (RDOM) ranges from 5.7 to 15.4 kg/d. Taken into account that, on average, ~7.4 Mol of SCFA are produced per kg RDOM (Allen, 1997), the microbial release of SCFA ranges from 42 to 114 Mol/d, which is equal to about 3 to 8.4 kg of pure acids. Effective buffering and absorption of SCFA are essential requirements to prevent sustained acidification of the ruminal content with all its detrimental consequences for the forestomach microflora and the ruminant host. Moreover, the high acid production also implies an increased risk for the ruminal epithelium.

Removal of Protons from the ruminal Content

The removal of H⁺ by either buffering or removal of acids from the rumen is classically believed to occur mainly via three main routes (Gäbel et al., 2002):

1. Buffering by HPO₄²⁻ inflow with the saliva and subsequent outflow of H₂PO₄⁻ to the psalter and distal parts of the gastrointestinal tract (GIT).
2. Buffering by HCO₃⁻ inflow with the saliva, conversion to H₂CO₃ and subsequently to CO₂ and H₂O. CO₂ is removed either by absorption across the ruminal wall or by eructation.

3. Absorption of undissociated SCFA across the ruminal wall via lipophilic diffusion.

Allen (1997) calculated the quantities of H^+ removed by the three pathways. Results of his calculation are summarized in Table 1.

Table 1: Estimated amount of H^+ elimination from the rumen on various routes. Daily SCFA production was calculated as 74 Mol. Data from Allen (1997)

Route	Amount (Mol/d)	Percentage of total produced (%)
SCFA absorption	39	53
$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$; CO ₂ removal by eructation and absorption	21	28
Outflow of H^+ to distal GIT after buffering (mainly by saliva HPO_4^{2-})	11	15
Total	71	96

The calculation in Table 1 underlines SCFA absorption as the primary route of hydrogen ion removal from the rumen. With some diets, up to 85 % of the SCFA can be taken up by the ruminal epithelium (Peters et al., 1990; 1992; Dijkstra et al., 1993; Lopez et al., 2003). By this, the capacity of the ruminal wall for SCFA absorption is not only important for the energy supply of the animal but is also vital for the maintenance of intraruminal pH (Müller et al., 2002; Gäbel and Aschenbach, 2006).

Pathways of SCFA Absorption and intraepithelial Catabolism

H^+ removal by ruminal SCFA absorption includes both a direct and an indirect pathway. The direct pathway is the lipophilic diffusion of undissociated SCFA (HSCFA); the indirect pathway is an exchange mechanism which couples SCFA outflow to HCO_3^- inflow (Ash and Dobson, 1963; Gäbel et al., 1991). Although many textbooks still postulate a predominance of the lipophilic diffusion pathway, there are several hints that lipophilic diffusion of HSCFA has to be regarded of minor importance. Those hints can be derived from studies on the influence of pH on SCFA absorption. Theoretically, a decreasing pH would increase [HSCFA] and should lead to an increased elimination of HSCFA by lipophilic diffusion.

In fact, however, the stimulation of SCFA absorption after lowering the ruminal pH did never reflect the relative increase in [HSCFA]. Additionally, the effect seemed only pronounced if the pH was lowered to subphysiological levels (Table 2). Another question mark on the predominance of lipophilic diffusion is set by a study of Voelker and Allen (2003), who reported that the rate of SCFA absorption was slower, not higher, at lower ruminal pH.

Various studies have offered convincing evidence that the small effect of pH on SCFA removal is due to permeation of SCFA in their dissociated form (SCFA⁻). The permeation of dissociated SCFA⁻ was hypothesized to occur via a transport protein that couples SCFA⁻ absorption to HCO₃⁻ secretion. The existence of such a protein had first been derived from studies in the washed reticulorumen. These studies had shown that the ruminal wall secretes about 1 mole of HCO₃⁻ for 2 mole SCFA absorbed (Ash and Dobson, 1963; Gäbel et al., 1991).

Subsequent experiments in isolated ruminal epithelia (Kramer et al., 1996; Sehested et al., 1999; Bilk et al., 2005; Bilk et al., 2007) were in accordance with the existence of an apical transport protein functioning as an SCFA⁻/HCO₃⁻ exchanger. The molecular identity of this transporter is still under investigation. Transcripts for anion exchangers like the 'down regulated in adenoma' (DRA) and the 'putative anion transporter' (PAT) were demonstrated in the ruminal epithelium, making these proteins likely candidates for SCFA⁻/HCO₃⁻ exchange (Bilk et al., 2005). At variance, Kirat et al. (2006) presented a model with monocarboxylate transporters (MCT) working as apical transport proteins for SCFA⁻ uptake in exchange for HCO₃⁻ excretion.

Following absorption, SCFA are partly metabolized within the ruminal epithelium (Bergman, 1990). Among the three SCFA, butyrate is degraded to the highest extent (Kristensen and Harmon, 2004). Butyrate is mainly degraded to β-hydroxybutyrate, but also to acetoacetate and lactate (Weigand et al., 1975). The high degree of n-butyrate degradation has to be regarded as beneficial since n-butyrate may induce detrimental morphological changes due to its effects on cell proliferation and gene expression (Gálfi et al., 1991, 1993).

Table 2: Influence of luminal (mucosal) pH on the absorption of acetate (ac.), propionate (pr.), and butyrate (bu.).

pH	HSCFA concentration ratio ^a	Ac.	Pr.	Bu.	Measured parameter	Reference; species; type of study
6.0	1:	-	196	-	Dis. ^b (mmol/h)	Weigand et al., 1972; calves; washed reticulorumen
4.8	8.6	-	315	-		
6.6	1:	3.2	3.6	4.4	Cl. ^b (l/h)	Thorlacius and Lodge, 1973; cows; washed reticulorumen
5.5	11	3.6	6.5	10.9		
6.8	1:	0.75	0.90	1.15	Cl. ^b (l/h)	Sündermann, 1986, and unpublished
4.6	63	1.41	2.42	2.65		

						data; sheep; washed reticulorumen
7.2	1:	0.21	0.35	0.28	Fr.dis. ^b (1/h)	Dijkstra et al., 1993; cows; washed reticulorumen
6.3	8:	0.33	0.51	0.46		
5.4	51:	0.35	0.54	0.53		
4.5	168	0.35	0.67	0.85		
6.8	1:	0.31	0.35	0.54	Fr.dis. ^b (1/h)	Lopez et al., 2003; sheep; intraruminal infusion
5.7	12:	0.34	0.43	0.64		
5.3	25	0.35	0.51	0.72		

^a Concentration ratio of undissociated short-chain fatty acids (HSCFA) was calculated by applying the Henderson-Hasselbalch equation assuming a pK value of 4.79.

^b Dis.: Disappearance; Cl.: Clearance; Fr. Dis.: Fractional disappearance

Regulation of intracellular pH in the presence of SCFA and their protons

While the lipophilic diffusion of HSCFA helps to keep intraruminal pH stable, it directly acidifies the cytosol due to the dissociation of HSCFA into H^+ and $SCFA^-$. $SCFA^-/HCO_3^-$ exchange also displaces acid load from the rumen into the cytosol of ruminal epithelial cells since buffer capacity is shifted from cytosol to lumen. The consequence is an increased concentration of H^+ inside the cell. Metabolism of SCFA does not really give relief since other acids with low pK values are released (β -hydroxybutyrate, acetoacetate and lactate; Weigand et al., 1975; Rapoport and Radebrecht, 1977). Thus the epithelial cell is in need of effective mechanisms for acid extrusion. Functional, immunohistochemical, and structural studies have indicated that various mechanisms are available to maintain intracellular pH. A sodium/proton exchange (NHE) leads to a direct extrusion of protons (Müller et al., 2000; Graham et al., 2007). NHE is very effective since it is driven by the sodium gradient from the extracellular fluid to the cytosol. The exchange of H^+ extruded for Na^+ taken up leads to a rapid counter regulation of an intracellular acidification (Müller et al., 2000). NHE is supported by a proton transfer via the monocarboxylate transporter 1 (MCT1) on the basolateral side of the ruminal epithelial cell (Müller et al., 2002; Kirat et al., 2006; Graham et al., 2007). MCT1 couples proton extrusion to outflow of ketonic acids and lactate. By this, the potentially detrimental catabolites derived from the intraepithelial SCFA degradation are also effectively extruded (Müller et al., 2002).

Intraepithelial buffer capacity is enhanced by an inflow of HCO_3^- from the basolateral side. HCO_3^- uptake is due to a coupling of HCO_3^- and Na^+ inflow via a sodium bicarbonate cotransporter (NBC1) the transmembranal Na^+ gradient being the driving force (Huhn et al., 2003).

Barrier Failure during Acidosis

The ruminal epithelium has to be regarded as a system which has evolutionary adapted to effective transfer of SCFA at slightly acidic luminal pH. The capacity for SCFA transfer is combined and coupled with pathways to defend intracellular pH in the face of huge H^+ fluxes. However - at extreme feeding conditions - the defence mechanisms are overwhelmed. The inherent loss of buffer capacity by the $SCFA^-/HCO_3^-$ exchange and the enhanced inflow of HSCFA at low luminal pH entail a severe disturbance of pH_i in the ruminal epithelial cells.

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8.2 ABSORPTION OF GLUCOSE FROM THE FORESTOMACH OF DAIRY CATTLE

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Problem: Carbohydrates are mainly metabolized to short chain fatty acids by the forestomach microflora. However, feeding of large amounts of easily fermentable carbohydrates to dairy cattle also carries a potential that glucose accumulates in the rumen, facilitating the development of ruminal acidosis. **Aim:** The aim of the present study was to investigate if glucose can be absorbed directly from the reticulo-rumen by the sodium/glucose cotransporter, SGLT-1. Another aim was to check whether glucose may be partly metabolized by the epithelium during absorption. **Methods:** In four lactating Holstein Friesian cattle, the cannulated reticulo-rumen was completely emptied, washed and isolated. Antibiotics- and SCFA-containing buffer solution (15 L, pH 6.7) was filled into the reticulo-rumen and spiked with 14 mM ¹³C-labeled glucose. Buffer samples were obtained at 0 and 60 min. In a second experiment, a solution containing 0.5 mM glucose was applied. After obtaining samples at 0 and 60 min, the inhibitor of SGLT-1, phlorizin (0.1 mM), was added to the intraruminal buffer and glucose disappearance monitored from 90-150 min. Blood was sampled from mesenteric artery, hepatic vein and portal vein to evaluate the ¹³C enrichment of blood glucose. **Results:** At 14 mM initial glucose concentration, the intraruminal glucose pool decreased from 208 ± 31 to 82 ± 24 mmol in 1 h (P < 0.05). The ¹³C fraction of blood glucose did not change in the portal vein between the addition (1.090 ± 0.000%) and the removal of the ruminal glucose (1.094 ± 0.007%). However, there was an increase in ¹³C enrichment 1 h after buffer removal (to 1.113 ± 0.005; P < 0.05). No differences were observed between the ¹³C fraction of blood glucose from portal, hepatic and arterial samples. At 0.5 mM initial glucose concentration, the intraruminal glucose pool decreased from 7.35 ± 0.26 to 4.37 ± 0.50 mmol within 1 h (P < 0.01). In the presence of phlorizin, intraruminal glucose still tended to decrease further from 3.94 ± 0.38 to 2.96 ± 0.65 mmol within 1 h (P = 0.064). **Conclusions:** Dairy cattle can absorb glucose very efficiently from the forestomach. In this study, the fractional disappearance rates of glucose from the reticulo-rumen were more than three times higher than those in sheep (1) and comparable to the fractional disappearance rates of short chain fatty acids from the bovine reticulo-rumen (2). SGLT-1 seems to play a minor role, pointing to a dominance of facilitated glucose absorption. Glucose appears to be almost completely converted to gluconeogenic precursors during absorption. 1) Aschenbach JR, Borau T, Gäbel G (2002). J Nutr 132:1254. 2) Dijkstra J, Boer H, van Bruchem J, et al. (1993). Br J Nutr 69:385. The work was supported by Pfizer Global Research.

8.3 INFLUENCE OF CHOP LENGTH OF CORN SILAGE ON PERFORMANCE, FREQUENCY OF DISPLACED ABOMASUM AND PARAMETERS OF METABOLISM OF LACTATING COWS

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The influence of chop length of corn silage on feed intake, milk yield, fertility and the incidence of certain diseases is discussed controversially. In this study the hypothesis was reviewed whether the chop length of corn silage in rations heavy on corn silage influences the ruminal fermentation processes creating a ruminal acidosis and therefore having a negative effect on the health of cows. Over a period of 20 weeks 30 cows were assigned to group A and 29 cows to group B. Both groups were fed a TMR (dry matter %: 52,4 % corn silage, 9,3 % grass silage, 17,1% protein supplement, 19% concentrate, 1,1% propylene glycol, 0,41% minerals) which only differed in the chop length of the corn silage. The corn silage in group A had a length of 5mm in group B it was 21mm. During this time the milk yield, the body weight and the feed intake were measured daily. The chewing activity was determined twice a week. The data on fertility and the overall health of the cows was taken from the herd management program. Every two weeks the milk composition was measured. During the first four weeks blood, urine and rumen fluid samples were taken once a week. After the four weeks they were only taken every four weeks until the 20th week. The blood was tested for AST, CK, GLDH, β -hydroxybutyrat, bilirubin, urea, cholesterol, free fatty acids, calcium, phosphor, magnesium). The urine was tested for pH, NABE, concentration of ammonium, BAQ, calcium, magnesium, sodium, potassium, chlorine, phosphor and the rumen fluid was tested for pH, concentration of fatty acids and pattern of the fatty acids. On these dates the back fat thickness was also measured. There were no differences found concerning feed intake, milk yield and fertility. The frequency of a displaced abomasum lay in group A at 33,3% while group B only had 6%. The examination of the urine samples provided a set of significant differences concerning the group and the week. The results showed a more acidotic metabolism for the group fed the short corn silage compared with the group fed the long corn silage. The short corn silage versus the long corn silage had a significant negative influence on the health of cows. The group fed the short corn silage showed not only a higher incidence of displaced abomasum but also these animals had a significant higher activity of ASAT. In all significant differences between the groups group A always ranges in the more acidotic area but most of the data is still in the limit. This shows the difficulty in diagnosing a subacute ruminal acidosis in dairy herds. Reliable diagnostics are even more important because the subacute ruminal acidosis is a common problem in dairy herds and has huge negative effects on the animal health, but often stays undetected.

8.4 ACID-BASE STATUS IN RUMEN FLUID AND URINE OF DAIRY HOLSTEIN COWS - A COMPARATIVE STUDY

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Subclinical rumen acidosis is an important metabolic disturbance in high yielding dairy cows during the first third of lactation. Different methods have been described to assess the acid-base status of rumen fluid. The direct measurement of rumen pH requires the time consuming sampling of rumen fluid by tube or the not riskless transcutaneous rumen puncture. Therefore other substrates (milk, urine) have been described to be useful assessing rumen acid-base status. The aim of the study was to investigate the rumen acid-base status in cows during transit period and first third of lactation and to assess diagnostic value the urine NABE, ratio between urine bases and acids and urine pH for prediction of rumen acidosis. The study comprised 348 sample pairs of urine and rumen fluid. Samples were simultaneously taken from healthy dairy cows between 14 days before and 90 days after parturition. Cows were kept in 10 different farms and had milk yields higher 8000 kg/year. Cows were fed a corn/grass silage based ration and concentrates according to their milk yield. None of the cows did receive anionic salts. Rumen and urine pH were measured with a pH meter as cow-side test. NABE and base/acid ratio were calculated after titration according the Jørgensen-Kutas method. Physiological range for rumen pH was taken between 6.2 and 7.2; pH below 6.0 was considered subclinical rumen acidosis and above 7.5 subclinical rumen alkalosis. Physiological ranges for urine parameters were between 7.0 - 8.4 for urine pH, 2.5 - 4.8 for urine base-acid ratio and 80 - 220 mmol/L for NABE(Kraft and Dürr 2005). Subclinical rumen acidosis was found in 43 (12.3%), subclinical rumen alkalosis in 30 (8.6%) of the 348 samples; the rumen pH of the remaining 275 (79.1%) samples were within the physiological range. Rumen pH showed significant but week correlation coefficients to NABE ($r = 0.13$), urine base/acid ratio ($r_s = 0.13$), and to urine pH ($r = 0.19$). Overall, the sensitivities of the urine parameters to predict subclinical rumen acidosis (NABE 44.8%, b/a-ratio 55.8%, urine pH 2.3%) were moderate or low. Specificities for subclinical rumen acidosis of the parameters were 64.5% for NABE 47.9% for base/acid ratio, and 97.0 % for urine pH. We concluded that the measured parameters of urine acid base status showed a moderate or even week association to rumen pH. This is consistent with studies describing an association between NABE and rumen acid load but found NABE not predictive for rumen pH. Recent studies have found that urine acid-base status is mainly influenced by strong ions among which potassium plays the main role. The diagnostic value of these parameters seems to be limited to assess rumen acid-base status in healthy dairy cows during transit period and early lactation.

8.5 RUMENOCENTESIS IN PARALUMBAR FOSSA AND RUMEN PH VALUES IN SAMPLES OBTAINED BY DIFFERENT TECHNIQUES IN DAIRY COWS

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Subacute ruminal acidosis is a frequent health problem in dairy cows and feedlots. The simple and reliable technique of collection of rumen sample and determination of pH are important for the diagnosis of this ruminal disorder in the field conditions.

The objective of this study was to describe rumenocentesis in the ventral part of paralumbar fossa (RPF) and compare the ruminal pH values in samples obtained by rumenocentesis in two different sites of left flank and via oro-ruminal probe in dairy cows. Samples of rumen fluid were collected from thirty five Holstein cows between 10 and 50 d in lactation (35 to 52 kg milk/d) by: 1) RPF (needle inserted in the ventral direction), 2) rumenocentesis in the area located 15 to 20 cm caudoventral to the costochondral junction (RCCJ) of the last rib on a line parallel with the top of the stifle, and 3) oro-ruminal probe connected with pump, between 4 and 6 h after morning feeding of a total mixed ration. Cows were sedated with xylazine (20-25 mg/cow) administered intravenously. A 1.6 mm x 125 mm needle and 10 ml syringe were used for collection of rumen fluid. Rumen fluid samples were obtained by RPF, RCCJ and by oro-ruminal probe at the same time and the pH values were determined by a portable pH meter immediately after a collection. Rumenocentesis samples from paralumbar fossa had the lowest pH value, the difference between pH values from different sites of puncture in the left flank were not significant (0.04 unit of pH). The positive significant correlation ($r = 0.78$, $p < 0.05$) was found between rumen pH values obtained by rumenocentesis and oro-ruminal probe. Rumen pH value in samples obtained by oro-ruminal probe was 0.40 higher than in rumenocentesis sample. Rumenocentesis in paralumbar fossa is a suitable and fast technique for the collection of ruminal sample enabling adequate determination of rumen pH and a diagnosis of subacute ruminal acidosis in the field conditions, with the low risk of health complications for the cow and it is also more safe and comfortable for a veterinary surgeon than RCCJ.

8.6 BODY CONDITION AND DISLOCATION ABOMASI: COMPARATIVE INVESTIGATIONS INTO BACK FAT THICKNESS AND ADDITIONAL CRITERIA IN CATTLE

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Along with the increased average milk production the incidence of abomasal displacement has increased. The median incidence of abomasal displacement amounts 1 – 3 %. Risk factors for abomasal displacement are identified specially feeding management in the transition period, metabolic disorders around parturition combined with twins, dystocia, milk fever, retained placenta, metritis, and ketosis. Hypothetically the facts of fat mobilisation syndrome are the background of leftside abomasal displacement (LDA). The goal of this investigation was to check the development of body condition (back fat thickness) around parturition furthermore the kind of parturition and the sex of the calves in cows with abomasal displacement. The body condition, the kind of parturition, the milk yield and diseases during early lactation were investigated 1-2 weeks before and 2 weeks after parturition in 228 cows including heifers.

Results: Overconditioning (back fat thickness [BFTh] > 30 mm) before parturition resulted in cows falling ill with mastitis and abomasal displacement. These cows mobilised the most fat (Δ BFTh 12.7 mm) in comparison with healthy cows (Δ BFTh 8.9 mm) and cows suffering from different diseases after parturition (Δ BFTh 8.4 mm). Especially heifers (83%) which gave birth to heavier male calves fell ill with abomasal displacement, therefore needed assistance during labour because of dystocia. Besides abomasal displacement additional disturbances occurred in these heifers, e.g. 50% mastitis, and 30% retentio secundinarum in this investigation. The daily milk yield amounted to 13.1 kg in cows with DA and to 23 kg in healthy cows.

Conclusions: The presented results support the classification of abomasal displacement into the pathophysiology of fat mobilisation syndrome. Consequently overconditioning and stress during parturition need to be avoided to prevent abomasal displacement.

8.7 NEW TOOLS! FIRST CHARACTERIZATION OF TWO PRIMARY EPITHELIAL CELL CULTURES FROM PORCINE JEJUNUM AND COLON

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Background & Aims: At present, there are neither jejunal nor colonic porcine epithelial cell lines available for studying the epithelial barrier function. Therefore, we developed a procedure to isolate and culture jejunal and colonic porcine epithelial cells (pJECs and pCECs) grown on porous filters. **Methods:** Tissue obtained from slaughtered pigs was incubated in a dispase solution to separate cells, which were cultured on collagen-coated membrane-supports. By immunohistochemical detection of tight junction-associated protein zonula occludens-1 (ZO-1), cytokeratin and fibronectin, the epithelial origin of the cells and the expression pattern of tight junctions were verified. For functional characterization, the transepithelial resistance (TEER) was measured as an indicator of confluence. For the colonic cells, we also determined the transepithelial potential difference (Pd) to evaluate polarity and functional differentiation of the artificial epithelia. The potential difference was modulated by application of forskolin and NPPB. Ussing-chamber experiments using colonic epithelia of pigs were performed to compare the forskolin and NPPB effects in cultured cells and intact epithelia. Additionally, we detected CFTR expression in cultured colonic cells on mRNA-level using RT PCR.

Results: Isolated jejunal and colonic cells formed tight monolayers on membrane-supports and were maintained up to 3 weeks. Nearly all the cultured cells stained positively for cytokeratin and ZO-1, but not for fibronectin, indicating that most of them were cells of epithelial origin. TEER and Pd across pCEC monolayers increased with time, reaching maximum values after about 12 days in culture. Application of forskolin caused an increase of Pd. NPPB reduced both baseline Pd and forskolin-induced Pd increase. Similar forskolin and NPPB effects were detected in intact colonic epithelia as well. CFTR mRNA expression was detectable in pCECs.

Conclusions: In summary, jejunal and colonic cells cultured on permeable membranes, maintain their epithelial cell characteristics in long term culture. Colonic cells are normal polarized and the Pd generated across the artificial epithelia is at least partly mediated by CFTR channels. Supported by H.-W.-Schaumann Stiftung and DFG (PF 403/5-1).

8.8 CHANGES IN URINE PH AND ACID-BASE- AND ELECTROLYT-BALANCE IN COWS WITH LEFT OR RIGHT DISPLACED ABOMASUM AND SEVERAL SYSTEMIC DISEASES

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Dysfunctions of the acid-base balance (ABB) are known to be important in cows with displaced abomasum (DA). Hypochloremic alkalosis of the blood is considered as the common finding. Furthermore a paradoxical aciduria in urine is postulated. However a paradoxical reaction of urine is pathophysiologically questionable, because of the tendency of aciduria in all forms of decreased or absent feed intake. The aim of the study was to describe the electrolytes and ABB in blood, urine and rumen fluid in cows with DA and to investigate possible relations of the parameters. Blood, urine and rumen fluid were collected from 25 cows with DA before abomasal reposition. The following parameters were determined: pH amongst others in rumen fluid; pH, HCO₃, pCO₂, pO₂, base excess, K, Na, Cl in blood; pH, acids, bases, NH₄, net acid-base excretion (NBE) and minerals in urine. While rumen fluid pH (6,65; 6,3-6,9) was physiological in all samples, the tendency to metabolic acidosis and aciduria was conspicuous. Rumen fluid pH correlated neither with blood nor with urine parameters. The results confirm the relation between low NBE (-9; -38,6-7,2) and urine pH (7,3; 6,5-7,6) and the concentrations of the electrolytes in urine, especially of the potassium (72; 41,5-89,5 mmol/l). Decreased K-contents cause a reduce of NBE and the bases. This reflects the general compensation of the organism in periods with low dry matter intake and decreased K-excretion, while the elimination of H⁺ions increase (NH₄: 17; 7,8-36,8 mmol/l). The development of an aciduria is typical for cows with reduced dry matter intake in consequence of DA and decreased K-excretion as a compensatory reaction of the kidney towards the low potassium blood level. The rumen fluid tends to alkalinity. Blood, urine and rumen fluid parameters develop contrary.

8.9 INFLUENCE OF DIFFERENT FEEDING SYSTEMS ON THE DEVELOPMENT OF IMMUNOGLOBULIN G AND M SERUM CONCENTRATIONS IN NEWBORN DAIRY CALVES

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Investigations on the concentration of immunoglobulins in bovine colostrum and blood serum of neonatal calves for examination of the immunological status are still actual and of high clinical and scientific interest. One aim of this study was to compare the most common feeding systems of newborn calves with special regard to their efficiency in the absorption of immunoglobulin G and M.

Methods: Twenty-eight Holstein Friesian calves were randomly assigned to 4 treatment groups (7 calves per group). One liter colostrum from first milking was fed to treatment groups 1 to 3 at 2, 6 and 12 hours post natum. In the following the calves of group 1 received milk replacer exclusively. The probationers in group 2 were fed with whole milk up to day 10. In treatment group 3 the calves received milk replacer and 500 ml colostrum of first and second milkings at each feeding for 10 days. The calves in group 4 were fed 3 L of first milking colostrum by stomach tube 2 hours after birth and in the following time received milk replacer only. Blood samples were taken immediately after birth and at 2, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h post natum. Immunoglobulins G and M in serum and colostrum/milk were determined by a competitive ELISA whose necessary antibodies and antigens were commercially available.

Results: As it was expected, the IgG and IgM sera levels increased in all treatment groups after the first colostrum feed and continued. Mean serum IgG concentrations at 24 h of age were 15.74, 19.58, 19.96 and 12.82 mg/ml in groups 1 to 4 respectively. For IgM the mean serum concentrations were measured in groups 1 to 4 24 h post natum as follows: 3.18, 5.47, 7.39 and 1.55. These serum levels showed significant ($p=0,023$) differences between the four treatment groups at 24 h. Over the whole period of investigation the mean IgG and IgM concentrations of treatment group 2 and 3 took a higher course than those of the other two groups.

Conclusion: Feeding of whole milk respectively the additional administration of colostrum leads to tendentious higher immunoglobulin sera concentrations in newborn dairy calves. Whether this phenomenon is a consequence of immunoglobulin absorption through the intestine beyond 24 hours of life could not be explained by the result of this investigation. Further investigations on immunoglobulin resorption must take histological and molecularbiological aspects into consideration.

8.10 PROCESSING PARAMETERS FOR THE PRODUCTION OF HIGH-QUALITY SILAGES

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Problems: The silage quality is an important prerequisite for the performance and the health for the animals. Economic consequences have dry mass losses which appear at an improper silage preservation. The quality of the silages is influenced by the harvest crop and also influenced by processing parameters. Aims Aim of the examinations is the determination of the necessary processing parameters for limiting mould growth during the silage storage. The limit conditions of the influence factors storage density and outer air tightness have primarily to be determined and characterized. From the results for the mould growth into dependence of the silage density and the outer air tightness have to be derived for the required compaction effort when filling the bunker silos.

Material and methods: The practical silage making were carried out as bale silage making. Different silage densities were produced with the help of different reducing in round bale press. An extreme density was produced with the help of a compact roles press. Altogether 5 series of experiments were carried out, three from 1st grew up and two from 3rd grew up. The dry matter content varied between 32.2% and 63.7%, the raw fibre content between 24.2% and 29.7%. The crop was from areas managed extensively. For the determination of the influence of the outer air tightness the number of foil wrappings from 2 to 8 was varied. For the determination of the required compaction effort the top surface density was measured for different crops into dependence of the number of the tractor drives. The density measuring was carried out radio metrically.

Results: The rise of the storage density into the silages is the primary basis for the production of sufficient air tightness in all silage examinations. The outer air tightness quality had on the other hand secondary character. For sufficient air tightness a storage density of about 200-210 kg DM/m³ and a maximum gas permeability of the foil of about 1.7 l/m² can be derived from the results within 24 hours. Under these conditions the fermenting quality is safeguarded and the mould attack limited for silages from grass produced extensively. After that > 1.5 Tr. min. / t OS for compaction time are approximately required for all crops and dry matter. For these corresponding silo breadths must be available. For dry matter over 50% the required top surface densities are not reached any more.

Conclusions: The density of the silage has the biggest influence on the silage quality. Covering up the food with a foil does not suffice, if the silage density is inadequate. The silage density for grass shall be about 200 - 210 kg DM /m³. The compaction effort of the tractors shall be over 1.5 Tr.-min./t OS.

8.11 THE EFFECT OF LIVE YEAST ON RUMEN FERMENTATION PARAMETER CHANGES OVER A 4 WEEKS PERIOD IN DAIRY CATTLE

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Live yeast supplementation is getting more and more popular in dairy cows diets because of their potential to positively influence rumen fermentation. This experiment examines how the rumen fermentation changes in fistulated dairy cattle over a period of 4 weeks after live yeast (*Saccharomyces cerevisiae* CBS 493.94 - YeaSacc®1026) inclusion in the diet. Two fistulated Holstein cows were individually fed and adapted to a standard TMR for 16 days prior to the trial. During the next 4 weeks, the TMR remained unchanged but live yeast (10g/d, minimum 1×10^9 CFU) was added in the concentrate. TMR was composed from corn silage, grass silage, hay, beet pulp, concentrate, minerals and vitamins (CP 15.4%, CF 16.6 %, 6.9 NEL MJ/kg DM). Rumen fluid samples were taken 1 hour before feeding and 1, 3, 5 and 7 hours post-feeding during 3 consecutive days at the end of the adaptation period (control period). In the live yeast period, samples were collected at similar times on 2 consecutive days each week for 4 weeks. VFA, NH₃ and lactic acid were measured in rumen fluid samples. All significant differences among treatments were declared at $p < 0.05$. Total VFA quantities tended to increase after feeding with a maximum value at 3 to 5 hours after feeding time for both control and live yeast periods. There was no effect of live yeast supplementation length on total VFA concentration after feeding. Live yeast effect on acetate concentration showed a trend to a slight reduction without being significantly different. Average propionate concentration over the live yeast feeding period was numerically higher than control (1 hour post-feeding: 23.8 vs. 25.1 – 3h post-feeding: 23.8 vs. 29.9 - 5h post-feeding: 22.3 vs. 30.8 mmol/L respectively for control and live yeast). Lactate levels were lower than detectable 1 hour before and 7 hours after feeding in all treatments. Live yeast addition resulted in a reduced lactate concentration with the greatest reductions reached in weeks 2 to 4 of the experimental period. Average lactate levels were 8.3 vs. 3.2 1h post-feeding and 2.2 vs. 0.4 mmol/L at 5h post-feeding respectively for control and treatment thus showing a significant decrease when live yeast was fed ($p < 0.05$). Ammonia levels were numerically decreased with live yeast addition at 1 and 3h post-feeding (13.2 vs. 12.5 at 1h post-feeding and 13.2 vs. 11.7 at 3h post-feeding for control and treatment respectively). This difference reached significance at 1 and 3 hours post-feeding sampling time in week 4 on the experimental period ($p < 0.05$). This experiment demonstrated that *Saccharomyces cerevisiae* CBS 493.94 has a positive impact on rumen fermentation parameters by lowering lactic acid and ammonia concentration as well as increasing propionic acid production.

8.12 DEGRADATION OF CRUDE PROTEIN FROM DIFFERENT FEEDSTUFFS IN THE RUMEN OF DAIRY COWS MEASURED IN SACCO

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Increasing utilization of plant material for fuel production opens the need for a reliable nutritive valuation of by-products designated to feed farm animals. This study addresses the ruminal degradation of crude protein (CP) from wet and dried distillers grain and rape cake as by-products of the bio-fuel industry in comparison to other concentrates. Material: Two Holstein Friesian cows fitted with a rumen fistula were used to study the apparent ruminal degradation of CP in sacco according to MADSEN and HVELPLUND (1994). The following feedstuffs were investigated: a mix of 85% wheat and 15% barley (WB), rye (RY), pressed but wet distillers grain (wDG, from RY), dried distillers grain with solubles (dDG, from WB), rapeseed cake and meal (RSC and RSM) and soybean meal (SBM). The rape products originated from the same batch of seed. The feedstuffs contained CP (% of dry matter) as follows: WB 14.2, RY 11.0, wDG 22.8, dDG 38.7, RSC 30.8, RSM 39.5, and SBM 55.1. Disappearance of nitrogen was measured after 2,4,8,12,24 and 48h of incubation and fitted to equations (Ørskov & McDONALD 1979) to estimate the time-course of CP degradation, the effective degradation (ED) and the proportion of CP that apparently resisted to ruminal degradation (RCP). The rate of passage through the rumen was pre-set at 8%/hr.

Results: RY vs WB contained more rapidly soluble (sCP) and less insoluble but potentially degradable CP (dCP) and the degradation rate of dCP (DR) was higher. ED was higher for RY than WB (83 vs 77%), too. The distillers grains had similar DR, but sCP and especially dCP were higher in the dried than the wet feed. E was clearly elevated in dDG compared to wDG (75 vs 57%). The oil seed meals contained similar amounts of sCP, but dCP was higher in SBM. In RSM, DR was somewhat higher. ED was nearly the same for RSM and SBM (59 & 61%). RSC contained more sCP but less dCP than RSM. The DR of RSC was 2.9-fold greater than that of RSM. The ED of RSC (81%) reached 1.4-times the value found for RSM. The following RCP (% of DM) were calculated: WB 23, RY 17, wDG 43, dDG 25, RSC 19, RSM 41 and SBM 39.

Conclusions: Compared to the untreated grain, pressing the distillers product resulted in a washout of soluble fractions such that the remaining CP had an elevated RCP fraction, whereas drying did not result in any relevant alteration. Because different kinds of grain have been used to produce the distillers grains, this result should be treated cautiously. Rapeseed and soybean meals are obviously characterized by a similar effective ruminal degradation of CP. Producing rape cake instead of meal largely elevates the ruminal CP degradability. The result can not easily be transferred into practice as rape cakes with different nutrient compositions are found there.

9 GASTROINTESTINAL DISORDERS

9.1 I LIKE TO MOVE IT – MOVE IT: ENTERIC NERVOUS SYSTEM AND GUT MOTILITY

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Function and composition of the ENS

The gastrointestinal tract has to fulfil various tasks like absorption of nutrients, water and ions transport and mixing of luminal contents and defence against pathogens and noxious substances (Wood et al., 1999). All of these functions are controlled or at least modulated by the enteric nervous system (ENS) (Brookes and Costa, 2006). The ENS represents the largest accumulation of neurones outside the brain (Schemann, 2005). The ENS of sheep, for example, has been estimated to have 30,000,000 myenteric and more than 50,000,000 submucous neurones (Brookes and Costa, 2006).

Enteric neurones are organized in two major plexūs, the submucous plexus and the myenteric plexus. Both plexūs consist of ganglia and interganglionic fibre tracts (Fig. 1) (Brookes and Costa, 2006).

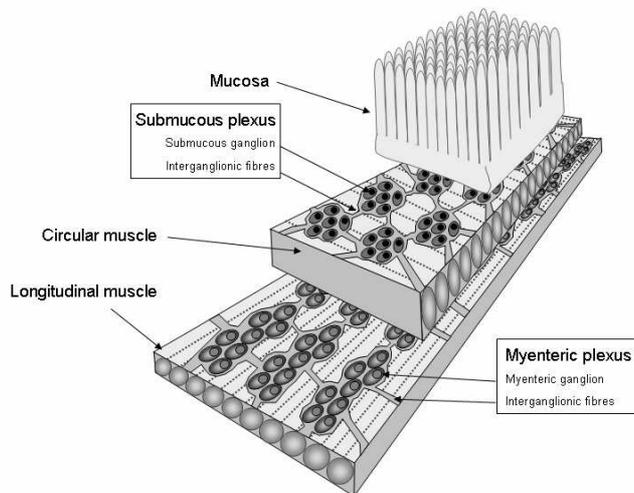


Figure 1: Anatomy of the intestinal wall. Both, submucous and the myenteric plexus consist of ganglia and interganglionic fibre tracts. Somata of the intrinsic neurones are localized within the ganglia.

The submucous plexus is located between mucosa and circular muscle layer. It mainly controls mucosal functions. The myenteric plexus is predominantly involved in regulation of motility. It is located between circular and longitudinal muscle layer (Fig. 1). Within the ganglia of both plexūs, sensory neurones, interneurones and motor neurones can be distinguished. These functionally different populations enable the ENS to control gastrointestinal functions independent from the central nervous system (Brookes and Costa, 2006).

Enteric neurones can be classified in various ways including electrophysiological properties, projection preferences and neurotransmitter expression.

The expression and the release of neurotransmitters play a key role in the crosstalk between enteric neurones and their target tissues. By now, a large number of neurotransmitter substances, known from the central nervous system, have been also found in the ENS (Brookes and Costa, 2006). Regarding the myenteric plexus, acetylcholine and nitric oxide seem to be the primary transmitters. All myenteric neurones express either a cholinergic or a nitrergic phenotype in combination with various neuropeptides and neuronal markers (Brookes, 2001b). The cocktail of neurotransmitters and neuronal marker synthesized by one neurone is named the neurochemical code of this neurone. The neurochemical code is strongly correlated with the function of the neurone. For the ileum of small rodents like guinea pigs the neurochemical code of submucous and myenteric neurones has been described in detail (Table 1) (Brookes, 2001b). However, it has to be kept in mind that region and species specific differences in the neurochemical code exist. Although expression and colocalisation of neurotransmitters have been analyzed only in selected regions, in large species like sheep and cattle, a multitude of neurotransmitters and neuronal markers have been identified in the neurones and fibres of the myenteric plexus; including cholineacetyl-transferase (ChAT), nitric oxide synthase (NOS), vasoactive intestinal peptide (VIP), substance P (SP), neuron specific enolase (NSE), calbindin (CALB), enkephalin (ENK), Neurotensin, neuropeptide Y (NPY), somatostatin (SOM), calcitonin gene related peptide (CGRP), galanin (GAL), gastrin releasing peptide (GRP), tyrosin hydroxylase (TH), dopamin- β -hydroxylase (DBH) (Barahona et al., 1998; Chiocchetti et al., 2004; Chiocchetti et al., 2005; Groenewald, 1994; Kitamura et al., 1986; Kitamura et al., 1987b; Kitamura et al., 1987a; Lalatta-Costerbosa et al., 2007; Pfannkuche et al., 2002a; Pfannkuche et al., 2002b; Pfannkuche et al., 2003a; Pfannkuche et al., 2003b; Pfannkuche et al., 2004a; Pfannkuche et al., 2004b; Vittoria et al., 2000; Wathuta, 1986; Wathuta and Harrison, 1987)

The enteric reflex repertoire

The enteric nervous system contains a library of programs for the necessary patterns of intestinal motility. Mixing and propulsion in the digestive state, the migrating motor complex in the interdigestive state and haustral formations in the large intestine are only some examples of outputs from the neural programs in the ENS (Wood, 2007). The generation of these motility patterns requires an intimate crosstalk of the functional different neuronal populations and the smooth muscle layers.

In this regard, the best known circuits are those of the peristaltic reflex, initiating propulsion of gut contents. The peristaltic reflex circuit can be divided in sensation of stimuli by primary afferent neurones, integration by enteric interneurones and initiation of smooth muscle activity by motor neurones (Fig. 2).

In this regard, the initial step for peristalsis originates from luminal contents stimulating intrinsic primary afferent neurones of the ENS. Intrinsic primary afferent neurones are able to sense distension of the gut wall and chemical stimuli (Furness et al., 1998). Stimuli can be directly receipt from intrinsic primary afferent neurones or by other cells in the intestinal wall, subsequently activating intrinsic neurones. Chemical stimuli, for example, are perceived by enterochromaffine cells in the intestinal epithelium subsequently releasing serotonin (5-HT). Serotonin triggers the excitation of primary afferent neurones and therefore plays a significant role in the enteric reflex pathways (Furness et al., 1998).

Intrinsic primary afferent neurones activate interneurones and motorneurones by the release of acetylcholine and substance P acting on nicotinic and neurokinin-3 receptors, respectively (Bornstein et al., 2004).

The final components of the peristaltic reflex are motor neurones that innervate the smooth muscle layers (Fig. 2). These neurones have polarized projection preferences to the circular muscle layer to ensure a contraction orally from the stimulus and a relaxation in anal direction (Brookes, 2001a). The oral contraction is driven by excitatory cholinergic motor neurones projecting in oral direction. The aboral relaxation is consequently mediated by inhibitory nitrergic neurones with a projection preference in anal direction (Fig. 2).

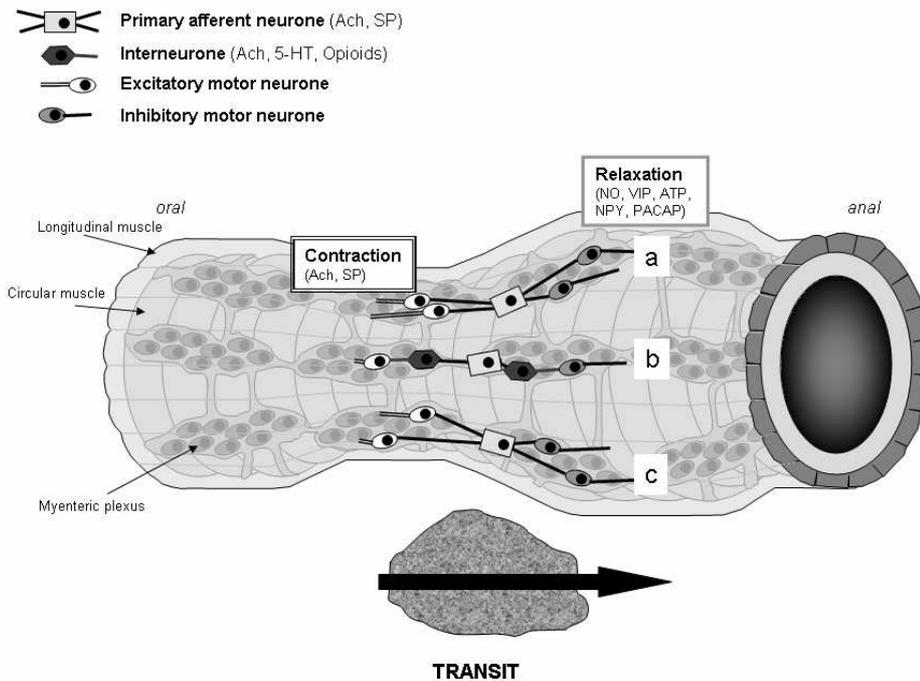


Figure 2 Neuronal circuits controlling the peristaltic reflex. Intraluminal stimuli are receipt by primary afferent intrinsic neurones which induce muscle responses via motor neurones (a, c) or via a chain of interneurones and motor neurones (b).

Besides the populations of intrinsic primary afferent neurones and motor neurones, also intrinsic interneurones may be involved in the initiation and propagation of peristalsis (Bornstein et al., 2004). Activation of interneurones might allow the reflex circuit to extend further up or down the gut than would otherwise occur. This feature is not selectively related to peristalsis, but also to other complex motor patterns. In this regard activation of distinct classes of interneurones might be involved in switching between different motility patterns (Brookes and Costa, 2006).

Besides the propagation of gut contents, the gastrointestinal tract also has the ability to store ingesta by reflexory accommodation. For the monogastric stomach, it is well known that passage of food through the pharynx and filling of the stomach induce receptive relaxation and adaptive relaxation, respectively (Desai et al., 1991a; Desai et al., 1991b). In the small and large intestine, accommodation occurs when the lumen is filled slowly (Ciccocioppo et al., 1994; Waterman et al., 1994). Fast increase in intraluminal pressure, in contrast, evokes peristalsis as described above (Waterman et al., 1994). The reflex accommodation of the intestine is mainly mediated by the activation of intrinsic inhibitor nitergic neurones, causing relaxation of the circular muscle layer (Ciccocioppo et al., 1994; Waterman et al., 1994). Under physiological conditions, reflexory accommodation allows the intestine to store chymus for subsequent degradation and resorption processes. However, during the pathogenesis of

impaction a slow filling of the intestine also occurs. This might lead to a further decrease in propulsive motility by reflexory relaxation.

Special Impact of neurotransmitters increasing or decreasing intestinal motility

As mentioned above, acetylcholine (ACh) is considered to be the primary and most important excitatory transmitter in the ENS. It is released from primary afferent neurones, interneurones and motor neurones. ACh binds to muscarinic (M_3) receptors on smooth muscle cells and also to muscarinic (M_1) and nicotinic receptors on enteric neurones (Maklouf and Murthy, 2006). The different localization of cholinergic receptors is not only present in monogastric animals, but was also detected in the gastrointestinal tract of ruminants (Stoffel et al., 2006). Binding of ACh to M_3 receptors induces a second messenger cascade leading to an increase of cytosolic calcium concentration (Maklouf and Murthy, 2006). A similar effect can be evoked by application of the cholinergic agonist betanecol (Pfeiffer et al., 2007). Butylscopolamine, in contrast, functions as spasmolytic agent by blocking the M_3 receptors.

Cholinergic neurotransmission can also be modulated by neostigmine, an inhibitor of the acetylcholine esterase. Neostigmine was found to increase the motility of the cecum and colon in cows (Steiner et al., 1995), but did not alter abomasal motility (Wittek and Constable, 2005). However, cholinergic neurotransmission is also involved in the control of abomasal contractions. In cows suffering from displaced abomasum, the abomasal longitudinal muscle layer was found to exhibit a decreased sensitivity to cholinergic neurotransmission (Geishauser et al., 1998). Together with an enhanced nitrergic component (Geishauser et al., 1998), this might be a significant factor in the pathogenesis of abomasal dislocation.

Regarding gastrointestinal motility of ruminants, some research has also focussed on serotonin (5-HT). Serotonin is not only released from enterochromaffin cells. It also functions as a transmitter of interneurones in the ENS (Bornstein et al., 2004). Various receptor subtypes for serotonin have been described. In the intestinal tract of cattle the receptor subtypes 1B, 2B and 4 seem to be the most abundant (Meylan et al., 2004; Ontsouka et al., 2006). Agonists for receptor types 2 and 4 induced a contractile response in isolated muscle strips from the abomasal antrum (Spring et al., 2003). The action of 5-HT was mediated through 5-HT₂ receptors on cholinergic motor neurones and 5-HT₄ receptors on smooth muscle cells (Spring et al., 2003). In contrast to the responses of the antroduodenal area a decrease of reticuloruminal myoelectric activity can be induced by intravenous application of 5-HT in conscious sheep (Plaza et al., 1996).

Serotonergic mechanisms might be also involved in the control of the distal parts of the gastrointestinal tract under pathophysiological conditions. In this regard, the expression of mRNA for all probably important receptor subtypes (1B, 2B and 4) was significantly lower in cows with cecal dilatation-dislocation (Engel et al., 2006).

For an undisturbed intestinal motility, both excitatory cholinergic and inhibitory nitrergic neuronal populations are essential. However, under physiological conditions, the intestinal muscle is always under an inhibitory tone from myenteric neurones. This assures that the lumen is not occluded, avoiding mechanical obstruction. The importance of this “neuronal brake” becomes obvious when myenteric ganglia are partly absent. Both, Morbus Hirschsprung in humans and the “lethal white foal syndrome” in horses are characterized by a congenital aganglionosis of intestinal segments (Hultgren, 1982; McCabe et al., 1990; Vonderfecht et al., 1983). This has been also described in a Holstein calf (Forzan and McClure, 2005) and in piglets (Stockhofe-Zurwieden et al., 2001). The aganglionic segments strongly contract and no passage of chymus can occur, leading to severe, life threatening constipations.

The intrinsic “neuronal brake” is mainly accomplished by the release of inhibitory neurotransmitters from the ENS. One of the most important inhibitory neurotransmitters is nitric oxide (NO), synthesized by enteric motor neurones and interneurones. NO induces a prominent relaxation in gastrointestinal smooth muscles (Hata et al., 2000; Onaga et al., 2000). Cotransmitters for NO are VIP, NPY and the pituitary adenylate cyclase activating peptide (PACAP) (Brookes, 2001b; Hata et al., 2000; Venugopalan, 1989). Although NO seems to be the primary transmitter, relaxation of the intestinal muscle in various region is driven by a cocktail of this substances (Zagorodnyuk et al., 1996).

Expression and release of inhibitory neurotransmitters have a significant impact on the pathogenesis of hypomotility. Nitric oxide seems to play a prominent role in this regard. It is not only released by enteric neurones, but also from vascular endothelium and immune cells. Local traumata as well as local and systemic inflammations seem to induce the release of NO (De Winter et al., 1997; Hellstrom et al., 1997). Elevated endotoxin levels in the body are known to slow down gastrointestinal motility (Tanabe et al., 2004). This action is at least partly mediated by NO. In this regard, direct stimuli for NO release are different inflammatory mediators, particularly prostaglandin E₂ (Rebollar et al., 2002). The interplay between endotoxines, inflammatory mediators and NO is discussed to be an important factor for the development of abomasal displacement. This is supported by the fact, that in cows with displaced abomasum an increase in nitrergic control of abomasal motility was found (Geishauser et al., 1998). However, it is still not clear if an upregulation of NO is the initial step for the decrease in abomasal motility or if a slowly occurring increase in abomasal pressure leads to enhances NO release.

When discussing intestinal hypomotility, also the group of endogenous opioids like enkephalines, endorphins and dynorphines has to be taken into account. All of this substances decrease the excitability of enteric neurones (Wood and Galligan, 2004). The depression of neuronal excitability does not singularly occur in the ENS, but represents the basal mechanism for endogenous pain release.

The release of opioids from enteric neurones is stimulated during abdominal surgery (Patierno et al., 2005). The opioids decrease excitability of enteric neurones involved in the peristaltic reflex. Consequently, intestinal transit is diminished.

Opioid induced constipation might also occur after pharmacological application of opioid agonists for anaesthesia or general pain management. Since the constipatory effect of opioids is mainly mediated by μ - and δ -receptors (Gray et al., 2005; Roger et al., 1994), the application of μ -Agonist (like Levomethadon) might be problematically in this regard. However, also κ -agonists like butorphanol have been found to have weak affinity to μ -receptors (Wongchanapai et al., 1998). In horses the application of butorphanol resulted in a decrease in colonic propulsion (Rutkowski et al., 1989).

Conclusion

The ENS is probably the most important level for the regulation of intestinal motility. The release of excitatory and inhibitory transmitters from afferent neurones, interneurones and motor neurones enables the ENS to precisely adapt motility patterns to the local requirements along the gut. The large number of neurotransmitters and receptors might also represent a point for pharmacological modulation of motility.

Table 1 Neuronal populations in the guinea-pig ileum (Modified from Brookes, 2001b)

Plexus	Chemical Coding	Function
SMP	ChAT/+Calb/+SP/+NMU	Primary afferent neurone
MP	ChAT/+Calb/+SP/+NMU	Primary afferent neurone
MP	ChAT/+Calb/+SP/+NMU	Primary afferent neurone, long aboral axon
MP	ChAT/+Calret/+SP	excitatory motor neurone, LM
MP	VIP/+NOS/+NPY/+GABA	inhibitory motor neurone, LM
MP	ChAT/+SP/+ENK/+NFP/+GABA/AP	excitatory motor neurone, CM
MP	VIP/NOS/ENK/+GABA/AP	short inhibitory motor neurone, CM
MP	VIP/NOS/NFP/+GRP/AP	long inhibitory motor neurone, CM
MP	ChAT/Calret/ENK/NFP/SP	ascending interneurone
MP	ChAT/5-HT/NFP	descending interneurone
MP	ChAT/SOM	descending interneurone
MP	ChAT/VIP/+NPY/+GRP	descending interneurone
MP	VIP/NOS/NFP/+NPY/+GRP/AP	descending interneurone
MP	VIP/ChAT/GRP	viscerofugal neurone
SMP	ChAT/NPY/NMU/CCK/CGRP/SOM/GAL/DYN	secretomotor
MP	ChAT/NPY/NMU/CCK/CGRP/SOM/GAL/DYN	secretomotor
SMP	ChAT/Calret	vasodilator
SMP	VIP/GAL/DYN/+NMU	secretomotor
MP	VIP/GAL/DYN/+NMU	secretomotor
SMP	VIP/GAL/DYN/+NMU	interplexus interneurone

Abbreviations: 5-HT: 5 hydroxytryptamine, AP: endogenous alkaline phosphatase, Calb: calbindin, Calret: calretinin, CCK: cholecystokinin, CGRP: calcitonin gene-related peptide, ChAT: choline acetyltransferase, CM: circular muscle, DYN: dynorphin, ENK: enkephalin, GABA: gamma amino butyric acid, GAL: galanin, GRP: gastrin-releasing peptide, LM: longitudinal muscle, MP: myenteric plexus, NFP: neurofilament protein triplet, NMU: neuromedin U, NOS: nitric oxide synthase, NPY: neuropeptide Y, SOM: somatostatin, SMP: submucous plexus, SP: substance P (or related tachykinins), VIP: vasoactive intestinal polypeptide.

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9.2 IN VITRO EFFECTS OF BETHANECHOL ON SMOOTH MUSCLE PREPARATIONS FROM THE ABOMASUM AND DUODENUM OF DAIRY COWS WITH LEFT DISPLACEMENT OF THE ABOMASUM IN COMPARISON WITH HEALTHY DAIRY COWS

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Displacement of the abomasum is a common disease in dairy cattle which has developed to a major economic problem. Left displacement of the abomasum (LDA) leads to decrease in milk production, high treatment costs, and increased culling rates, as well as to considerable discomfort for the animal. A disturbance of normal gastrointestinal motility followed by gas accumulation in the abomasal fundus is considered to be a prerequisite for subsequent displacement.

The objective of the present study was to investigate the *in vitro* effects of bethanechol (BeCh) on motility traits of smooth muscle specimens from the abomasum and duodenum of dairy cows with LDA in comparison with healthy dairy cows, and to determine the role of the muscarinic receptor subtypes M2 and M3 in the mediation of contraction. Smooth muscle specimens with longitudinal and circular orientation were isolated from the fundus, corpus and antrum pyloricum of the abomasum and from the duodenum of 12 cows with LDA and 30 healthy dairy cows immediately after slaughter. Concentration-response curves for the muscarinic agonist BeCh with or without prior incubation with a M2-receptor antagonist (AF-DX 116) and a M3-receptor antagonist (4-DAMP) were recorded in an organ bath setting. Analyzed motility variables included area under the curve (AUC), maximal amplitude (Amax) and basal tone (BT). The maximal attainable response (Vmax) and the effective concentration 50% (EC50) of BeCh were calculated for each variable. Statistical analyses were performed by use of the Wilcoxon signed-rank test and of ANOVA with a level of significance set at $\alpha=0.05$. Bethanechol induced a significant, concentration-dependant increase of 2 or all 3 investigated contractility variables in all locations and in both groups of cows. An inhibiting effect was present both for the M2- and for the M3-antagonist, with a stronger effect of 4-DAMP than of AF-DX 116. In comparison with healthy controls, Vmax was reduced and EC50 was increased in muscle preparations from cows with LDA. This effect was significant for Vmax of AUC and BT, and for EC50 of Amax. With regard to locations, the greatest reduction of contractility was seen in the corpus, whereas no significant differences were observed in the duodenum.

In conclusion, BeCh acting at M2 and M3 receptors caused contraction of muscle specimens from the abomasum and duodenum of cows with LDA, and thus may be of clinical use as a prokinetic drug for such cases. The contractile effect of BeCh is mediated by both muscarinic receptor subtypes M2 and M3, however M3-receptors appear to play a predominant role. Abomasal but not duodenal smooth muscle contractility, especially BT, was reduced in cows with LDA, indicating hypotonia of the displaced abomasum.

9.3 MUSCARINIC RECEPTORS IN THE BOVINE GASTROINTESTINAL TRACT: MRNA EXPRESSION AND RECEPTOR BINDING IN COWS WITH CECAL DILATATION/DISLOCATION IN COMPARISON WITH HEALTHY COWS

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Cecal dilatation/dislocation (CDD) is an economically important motility disorder in dairy cows. The motor functions of the gastrointestinal (GI) tract are tightly controlled by the enteric nervous system modulated among others by the parasympathetic nervous system. Acetylcholine interacts with muscarinic receptors (5 subtypes, M1-M5) on smooth muscle cells and nerve terminals to mediate gastrointestinal (GI) smooth muscle contraction. To investigate the role of muscarinic receptors in the bovine intestine, the mRNA levels and binding sites of M1 to M5 were measured in muscle tissues from the fundus abomasi, pylorus, ileum, cecum, proximal loop of the ascending colon (PLAC), and external loop of the spiral colon (ELSC) of 7 healthy slaughter cows. Furthermore, the mRNA levels of M2 and M3 in full-thickness biopsies from the ileum, cecum, PLAC and ELSC of cows with CDD (n=7) were compared with results from healthy control cows (n=7). The mRNA levels were measured by qRT-PCR. The inhibition of [³H]-QNB binding by M antagonists [atropine (M1-5), pirenzepine (M1), methoctramine (M2), 4-DAMP (M3), and tropicamide (M4)] served to identify receptor subtypes at the protein level. Maximal binding (B_{max}) was determined during saturation binding with atropine as a competitor.

Results for B_{max} and mRNA levels were tested for significant differences among locations and between groups by ANOVA using the general linear model of SAS, with Bonferroni corrections for multiple comparisons. The mRNA levels of M1, M2, M3 and M5 represented 0.2, 48, 50, and 1.8%, respectively, of total M, whereas mRNA of M4 was not detected. The mRNA levels of M2 and M3 in the ileum were lower (P < 0.05) than in other GI-locations. Atropine and antagonists for M1, M2 and M3 inhibited [³H]-QNB binding according to a one- or a two-site receptor model. The M4 antagonist had no effect on binding. Values of B_{max} were lower (P < 0.05) in the fundus, pylorus, and PLAC than in the ELSC, and lower in the pylorus than in the ileum. In cows with CDD, mRNA of M2 (cecum, PLAC and ELSC) and M3 (all locations) were reduced by 47 to 67% and 73 to 85%, respectively, as compared to healthy cows.

In conclusion, M2 and M3 appeared to be the most important muscarinic receptors in the GI tract of dairy cows. The decreased mRNA levels observed in cows with CDD may indicate involvement of these two receptor subtypes in the motility disorder leading to CDD.

9.4 INTESTINAL E. COLI POPULATIONS IN HEALTHY DOMESTIC PIGS

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Non-pathogenic, intestinal *E. coli* lacking virulence genes (commensal *E. coli*) support the physiological intestinal balance of the host, whereas *E. coli* with typical virulence factor gene profiles (pathogenic *E. coli*) can cause severe outbreaks of infectious diseases with high economical losses in pig production. Although there are many studies on pathogenic *E. coli*, little data is available about commensal *E. coli* in pigs. Furthermore, the division between commensal and pathogenic *E. coli* is not clear. Intestinal *E. coli* populations in pigs are individual and dynamic, however, the basis of transmission, colonization and distribution are still not known. In several studies we determined the intestinal *E. coli* populations in healthy domestic pigs and focused on correlations between colonization and occurrence of common porcine virulence genes and genes for virulence/colonization factors associated with extraintestinal *E. coli* (ExPEC), haemolysis and phylogenetic affiliation. We show that *E. coli* is not always the dominant intestinal Enterobacteriaceae species. Intestinal sections were often found free of Enterobacteriaceae and mucosa-adherent Enterobacteriaceae were scarce. The intestinal *E. coli* flora in healthy domestic pigs can be dominated by clones with a broad variety of typical porcine virulence factor gene profiles. In contrast, the majority of isolated haemolytic *E. coli* often harbours none of the typical porcine-associated virulence genes. Colonization by *E. coli* showing haemolysis, virulence/colonization factors and virulence/colonization factor gene profiles common to ExPEC can also dominate the intestinal *E. coli* flora. There does not seem to be a clear division between *E. coli* exclusively colonizing the small intestine and the large bowel or a distribution based on phylogenetic affiliation.

In conclusion, our data reveal that the occurrence and diversity of Enterobacteriaceae within one clinically healthy domestic pig population is clearly individual. Enterobacteriaceae do not appear to be an essential part of the microbiota of the jejunum, and high numbers of adherent bacteria do not seem to be essential for successful intestinal colonization. Hemolytic activity, iron acquisition systems and other ExPEC-common virulence/colonization factors support successful intestinal establishment. The multiple virulence gene patterns further suggest a dynamic transmission of virulence genes in *E. coli* populations. Finally, high numbers of *E. coli* carrying virulence genes and/or with haemolytic activity do not necessarily correlate with disease.

9.5 LAPAROSCOPIC ABOMASOPEXY IN DAIRY COWS WITH LEFT SIDED ABOMASAL DISPLACEMENT: LONG TERM POST SURGICAL PERFORMANCE

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Objective: To study complication rates, post surgical convalescence, and long term productivity of cows after surgical treatment of left sided abomasal displacement (LDA) by means of laparoscopic abomasopexy according to Janowitz.

Material and methods: Prospective case control study with 200 German-HF dairy cows with LDA from 70 herds. Additionally, each LDA cow was matched with two to three control cows from same herds according to lactation number and days in lactation. Complications during and after surgery were recorded. Blood samples were taken on the day of surgery and tested for liver enzymes, bilirubin, sodium, potassium, chloride and minerals in order to test for information about the outcome of the surgery. Survival time and culling rates were calculated, and causes for culling were recorded basing on DHI data and owner information. Milk yield and constituents (fat-, protein-, urea-content, fat/protein ratio) were analysed and compared between LDA cows and control cows, considering the lactation of surgery, the previous and the following lactation. Statistics were performed with SAS. Data were tested by t-test and non-parametric tests according to the results of testing for normal distribution. Chi-squared test was used to test for differences in frequencies. Discriminant analysis and logistic regression were used to test for the diagnostic accuracy of blood tests in predicting survival time. Performance data were analysed by means of the mixed model procedure considering the factors season of the year, lactation number, DA, days in milk, farm, and individual cow.

Results: One LDA cow died four days post surgery due to hepatic failure, three cows revealed relapses and underwent a second abomasopexy. No other significant complications were observed. Culling rate (three years of observation) and mean survival time in the LDA and control group was 88% and 81%, and 716 days and 783 days, resp.. Causes of culling were typical and almost same in both groups. The outcome of the LDA cows was unpredictable by results of blood parameters. 305d milk yield of LDA cows in the lactation of surgery was reduced in average by 392kg/cow and 153kg/cow compared to their previous lactation and to the corresponding lactation of controls, resp.. Comparing the lactation of LDA cows and controls the fat/protein ratio was temporarily increased but no differences were found in the previous and the following lactation.

Conclusion and practical relevance: Laparoscopic abomasopexy (Janowitz method) is regarded as sufficiently safe for correction of LDA in the field. Surgical intervention in order to correct LDA is justified in dairy cows, since survival time is only slightly and performance insubstantially and temporarily affected by LDA.

9.6 HEMATOLOGICAL AND BIOCHEMICAL VARIABLES IN COWS BEFORE AND AFTER SURGICAL TREATMENT OF ABOMASAL DISPLACEMENT

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Incidence of displaced abomasum (DA) in Slovenia increased to about 4% in last decade. Diagnosis of DA is usually not problematic for experienced practitioner. The investigation of blood variables is helpful for assessment of health status and prognosis in these cows. The aim of the research was to establish how the surgery of DA and later therapy influence haematological and biochemical parameters in blood. 54 black&white cows from surroundings of Ljubljana were included in the research, 41 cows had left and 13 right DA. Diagnosis was made with clinical examination. Blood samples were taken from each cow before, and 3 days after surgery. Haematological variables and concentrations of total bilirubin, glucose, BHB, Ca, aP, Mg, K, Na, Cl, total serum protein (TSP), Alb, urea and activity of CK, AST, GGT and GLDH were measured. Average values of haematological parameters before surgery were; RBC $6.70 \pm 0.96 \times 10^{12}/L$, WBC $8.05 \pm 2.98 \times 10^9/L$, Hb 113.9 ± 14.4 g/L and PCV 0.34 ± 0.05 L/L. After surgery they decreased statistically significantly. Before surgery higher percent of seg. neutrophils ($P = 0.001$) was established than after surgery. Before surgery statistically significantly higher average concentration of bilirubin (19.34 ± 13.79 $\mu\text{mol}/L$) was established than after surgery when it decreased to 13.08 ± 8.03 $\mu\text{mol}/L$ ($P < 0.001$). Average concentration of glucose was 5.63 ± 2.33 mmol/L and BHB 1.81 ± 2.00 mmol/L before surgery and after surgery the concentration of glucose was 3.15 ± 0.71 mmol/L ($P < 0.001$) and BHB 1.52 ± 2.04 mmol/L ($P = 0.349$). Before surgery the average concentrations of minerals were; Ca 2.13 ± 0.25 mmol/L, aP 1.68 ± 0.48 mmol/L and Mg 0.87 ± 0.15 mmol/L, after surgery they were Ca 2.24 ± 0.22 mmol/l ($P = 0.091$), aP 1.81 ± 0.41 mmol/L ($P = 0.101$) and Mg 0.82 ± 0.13 mmol/L ($P = 0.183$). The average concentrations of K before treatment were 3.99 ± 0.68 mmol/L, Na 141.88 ± 4.76 mmol/L and Cl 95.14 ± 8.18 mmol/L. After surgery they increased to K 4.95 ± 0.59 mmol/L ($P < 0.001$), Na 144.07 ± 3.42 mmol/L ($P = 0.029$) and Cl 103.88 ± 4.96 ($P < 0.001$). Values of urea ($P < 0.001$), TSP ($P < 0.001$), Alb ($P < 0.001$) and activity of CK ($P = 0.010$), AST ($P = 0.029$), GGT ($P = 0.026$) and GLDH ($P = 0.960$) decreased after surgery. The results indicate that values of most of investigated variables change because of abomasal reposition, rehydration, electrolyte and antimicrobial therapy and application of NSAID-s. The values of K and glucose became normal in 3 days after surgery. The values of bilirubin, AST, CK and GGT got near to the physiological values. Measuring of haematological and biochemical parameters is helpful for assessing the efficiency of therapy and prognosis.

9.7 COMPARATIVE ACETAMINOPHEN ABSORPTION TEST AS A DIAGNOSTIC TOOL FOR THE EVALUATION OF THE RETICULAR GROOVE REFLEX

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Problem and aim: This study was conducted in order to evaluate the comparative acetaminophen absorption test value in the assessment of the function of reticular groove reflex. Potentially, this test can be used in the diagnosis of calves with ruminal drinkers and also, in the evaluation of drugs that can induce reticular groove reflex in older ages in ruminants.

Materials and methods: Twelve 15-days-old ewe-lambs of Baluchi breed were selected. They sucked at least 4% of their body weight of reconstituted milk replacer from the bottle and nipple once daily, till 165 days of life, in addition to the usual milk and solid feeds they had access to. The comparative acetaminophen absorption test has been performed three times before weaning, at the time of weaning and 1.5 months after weaning. Each stage includes two substages on the same lambs: 1) sucking of a mixture of reconstituted milk replacer containing pure acetaminophen powder (50 mg/kg body weight) and 30% Barium sulfate suspension (10 ml/kg Body weight). 2) Feeding the same compound by stomach tube. The second substages were performed 1 week following the first ones. Radiography was performed immediately, following the sucking/tube-feeding of the mixture and evaluated by an expert radiologist. Heparinated catheters were inserted into the jugular vein and Jugular venous blood samples were taken according to the following schedule: Every 30 minutes up to 180 minutes following sucking and/or tube feeding of the mixture of the acetaminophen powder and barium sulfate, with a sample before starting the procedure (collectively, 7 blood samples).

The results of serum acetaminophen levels were interpreted according to the results of radiography (entrance of the mixture into the abomasum and/or the rumen). Serum acetaminophen levels were estimated by spectrophotometry. In statistical procedure, Normality of data was evaluated by Q-Q scatter plots and Kolmogorov-Smirnov test. Serum acetaminophen levels were compared with Paired t-test. Results: Serum acetaminophen levels in suckling procedure were significantly higher than tube feeding, at 60 minutes (highest difference occurred at first and second stages), and 90 and 120 minutes (only in first stage) following ingestion of the mixture of the acetaminophen powder and barium sulfate. In these stages, serum acetaminophen levels were above 30ug/ml in sucking procedure.

Conclusion: we propose that the comparative acetaminophen absorption test can be regarded as a test for the evaluation of the function of the reticular groove, with the aim of detecting a significant difference between two methods (sucking and tube-feeding). It may potentially be useful in evaluating calves with ruminal drinkers and drugs that can induce reticular groove reflex.

9.8 CLOSTRIDIUM PERFRINGENS TYPE C -INFECTION IN PIGLETS IN THE SWISS SWINE POPULATION

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Problem: Necrotizing enteritis in suckling piglets is caused by the spore-forming and toxin-producing *C. perfringens* type C. A typical clinical symptom is bloody diarrhoea of several piglets of a litter during the first day after birth. In certain cases the infection leads to death immediately without clinical manifestations. A treatment is futile when symptoms appear. *C. perfringens* type C produces alpha- and beta-toxin. The beta2-toxin can be found in *C. perfringens* in association with the alpha-toxin (type A) or with alpha- and beta-toxin (type C). Both are sensible for trypsin what explains the early occurrence of the disease.

Aim: The objective of this study is to investigate the presence of beta- and/or beta2-toxigenic *C. perfringens* in diseased and healthy animals and to find out predisposing factors for an outbreak of necrotizing enteritis in a swine herd. **Material and Methodes:** One hundred swiss farms with at least ten sows were selected randomly and classified in two groups. In the negative group farms are classified which had never necrotizing enteritis due to *C. perfringens* type C. The positive group includes the farms which had a case of necrotizing enteritis during this study or had a case before the beginning of the study and now they are using a vaccination containing beta-toxoid of *C. perfringens*. The data of the farms were collected by a questionnaire. In 20 herds faecal samples from sows and piglets were taken twice in an interval of 14 days and the material was analyzed for presence of *C. perfringens*. The detection of alpha-, beta- and beta2-toxin genes was performed by PCR. In contrast to other studies we also had data from healthy animals.

Results: We analyzed 1478 faecal samples. In 798 swab samples we detected *C. perfringens*. In 577 samples we could find beta2-toxigenic *C. perfringens* type A and in 86 samples *C. perfringens* with beta- and beta2-toxin genes. The beta-toxin gene was detected always in combination with the beta2-toxin gene. Furthermore the beta-toxin gene was detected only in the positive classified farms and exceptionally in a sample of one piglet of a farm classified in the negative group. Bad sanitation and localisation of breeding farms in regions with lot of other swine herds are predisposing for an outbreak of clostridiosis in piglets.

Conclulsion: Our results show that the prevalence of *C. perfringens* in swiss farms is 100 percent and in piglets nearly 95 percent. All farms with the beta-toxin gene were associated with an acute outbreak of necrotizing enteritis or an incorrect management of vaccination. This fact points out the important role of the beta-toxin gene for an outbreak of necrotizing enteritis in piglets. Beta2-toxigenic *C. perfringens* type A seem to be part of the normal flora.

9.9 EFFECTS OF FEED PARTICLE SIZE (COARSE/FINE) AND ADDITION OF ORGANIC ACIDS OR POTASSIUM DIFORMATE ON COUNTS OF E. COLI AND SALMONELLA IN CHYME AFTER AN EXPERIMENTAL INFECTION OF WEANED PIGS

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E. coli is responsible for high economic losses in pig production by causing fatal diseases whereas *Salmonella* represent as a zoonotic pathogen a high danger for human health. The aim of the experiment was to examine if and to what extent structure of feed and addition of free acids (75 % formic acid, 25 % propionic acid) or potassium diformate affect the passage of *E. coli* and *Salmonella* and the efficacy of the stomach barrier function. The experiments have been carried out with 80 pigs allotted into 4 groups in 4 stages of 20. Pigs were fed ad libitum all time. Cereals (74%) in the control diet were finely ground (2 mm screen), in test diets 0,9% organic acids (formic and propionic acid) or potassium diformate (1,2% in the diet) were used; in group 4 (without organic acid) the diet was coarsely ground (6 mm screen). Pigs were sacrificed 4-5 hours after oral application of a bouillon containing a *Salmonella* amount of $1.5E+08$ to $3.3E+09$ CFU/ml or an *E. coli* amount of $7.8E+08$ to $7.1E+10$ CFU/ml and chyme from stomach, small intestine and caecum was taken. Pigs were fed with 10 ml of the solution each. Counts were determined with classical culture methods. The results of the experiment show that the addition of free acids or potassium diformate leads to the most reduced counts of *E. coli* in stomach ($1,0E+04$ to $1,0E+06$ CFU/g). The coarse ground diet led to results of germ count from $1,0E+05$ to $1,0E+07$ CFU/g (with exception of one pig which had an uncommon high count of $1,0E+10$ CFU/g). Control diet showed results from $1,0E+07$ to $1,0E+08$ CFU/g. The average count of *Salmonella* in stomach was lowest in pigs fed diet with potassium diformate ($6.2E+04$ CFU/g). Pigs fed diet with free acid showed an average result of $1.4E+05$ CFU/g. There was no significant difference between coarsely ground diet ($8.6E+05$ CFU/g) and control diet ($1.1E+06$ CFU/g). The conclusions of these investigations are that either free acid or potassium diformate increase the efficiency of the stomach barrier. In more distal parts of the digestive tract their effect is less obvious presumably due to absorption of organic acids in the small intestine. A coarse structure of diets tends to result in positive effects concerning the stomach barrier and its functions. Combining a low grinding intensity and the addition of organic acids may reduce the risk of *E. coli* and *Salmonella* infections in pigs due to different effects within the gastrointestinal tract. Organic acids and/or potassium diformate act primarily in the cranial part whereas the coarse diet alters the starch content in the hindgut and the fermentation pattern (butyrate increases) as observed by Visscher 2006.

9.10 MRNA EXPRESSION OF MOTILITY-MEDIATING RECEPTORS IN INTESTINAL MUSCLE TISSUE FROM THE ABOMASUM TO THE SPIRAL COLON OF COWS SUFFERING FROM LEFT-SIDED ABOMASAL DISPLACEMENT

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Dairy cows frequently suffer from left-sided displacement of the abomasum (LDA), which causes important economical losses. Although the symptoms of LDA are well known, the pathogenesis of the disease remains unclear. Motor functions of the gastrointestinal (GI) tract are tightly controlled by the enteric nervous system modulated by the sympathetic and parasympathetic nervous systems, involving alpha- and beta-adrenergic receptors (AR) as well as muscarinic receptors (M) on nerve terminals and smooth muscle cells in the wall of GI organs. In addition, a non-adrenergic and non-cholinergic pathway also influences GI motor functions via motilin receptors (MTL-R). To determine whether levels of motility-mediating receptors vary in the GI tract of dairy cows depending on their health status, the mRNA levels of alpha2AD-AR, beta2-AR, M2, M3, and MTL-R in full-thickness samples and in smooth muscle specimens from the abomasum (fundus abomasi, pylorus), duodenum, cecum and external loop of the spiral colon (ELSC) of cows with LDA (n=8) were compared with those in healthy control cows (n=8). The mRNA levels were measured by qRT-PCR and normalized relative to GAPDH. Receptor mRNA levels were evaluated using the repeated procedure of mixed model (SAS). Differences between groups within locations were investigated using the student's t-test. The mRNA levels of alpha2AD-AR, beta2-AR, M2, M3, and MTL-R varied among GI locations ($P < 0.05$) in full-thickness samples and in smooth muscle tissues. In full-thickness specimens, the mRNA levels of all five receptors were lower ($P < 0.05$) in the duodenum of cows with LDA than in controls. In contrast, the mRNA levels of beta2-AR were higher ($P < 0.05$) in the ELSC of cows with LDA than in healthy cows. In smooth muscle tissues, the mRNA levels were lower ($P < 0.05$) in cows with LDA than in controls for alpha2AD-AR and beta2-AR in the fundus and pylorus, and for MTL-R in the pylorus. In duodenal samples, the mRNA levels of MTL-R, alpha2AD-AR and beta2-AR were lower ($P < 0.05$) in cows with LDA than in controls. In contrast, mRNA levels of M2 in muscle tissues from the duodenum and the ELSC were higher ($P < 0.05$) in cows with LDA than in controls.

In conclusion, unequal distribution of differences in mRNA levels between cows with LDA and healthy in full-thickness or in smooth muscle tissues indicates differences in receptor mRNA synthesis and/or turnover rates among various cell types within the GI wall. Furthermore, significant differences between tissue samples from cows with LDA and healthy dairy cows in mRNA levels for motility-modulating receptors were most frequently observed in the duodenum. This suggests that LDA might be caused by a primary motility disturbance at this location rather than in the abomasum itself.

9.11 ABOMASAL EMPTYING RATE IN HEALTHY LACTATING DAIRY COWS AT DIFFERENT STAGES OF LACTATION

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Abomasal hypomotility currently is believed to be a prerequisite for development of LDA or AV, and abomasal hypomotility results in a decrease in abomasal emptying rate. We determined the abomasal emptying rates of 21 healthy Holstein-Friesian cows at different stages of lactation and different milk yield. Our hypothesis was that dairy cows have a slower rate of abomasal emptying in early lactation, when the risk of LDA is greatest. D-xylose (0.5 g/kg body weight [BW], 50% solution) was injected into the abomasum in cows (group 1, 4 to 7 days in milk [DIM], n = 7), group 2 (90 to 120 DIM, milk yield of > 30 kg/day, n = 7) and in group 3 (> 300 DIM, < 15 kg/day, n = 7) by ultrasound guided transcutaneous abomasocentesis. Blood samples were periodically obtained from jugular vein before D-xylose administration (0 min) and at 30, 60, 90, 120, 150, 180, 240, and 360 minutes after administration. Serum D-xylose concentrations were measured using a commercially available enzymatic test. The serum D-xylose-time curve was modeled using the first derivative of Siegel's modified power exponential formula as described recently. The time to maximal serum D-xylose concentration (T_{max}) was used as an index of abomasal emptying rate. The abomasal emptying rates for cows in groups 1, 2, and 3 did not differ (T_{max} group 1, 108 ± 14 min; T_{max} group 2, 109 ± 18 min, T_{max} group 3, 105 ± 14 min). Maximal concentration (C_{max}) tended to be lower in early lactation, relative to mid and late lactation (C_{max} group 1, 0.83 ± 0.18 mmol/l; C_{max} group 2, 1.17 ± 0.35 mmol/l, C_{max} group 3, 1.09 ± 0.32 mmol/l). The results indicate that the increased incidence of LDA in the first month of lactation is not associated with an intrinsic decrease in abomasal emptying rate in healthy cows. This finding suggests that differences in abomasal emptying rate as a direct result of stage of lactation do not play an important role in the high incidence of LDA during the first month of lactation. However, the exclusion of diseased postparturient cattle in the present study means that our results may not be representative of the abomasal emptying rates of all postparturient cows some of which may suffer from a variety of postparturient diseases such as endotoxemia, hypocalcemia, and hyperglycemia that decrease abomasal motility and therefore emptying rate.

9.12 HISTOLOGICAL ASPECTS REGARDING REACTIVITY OF INTESTINAL MUCOSA IN NEWBORN LAMBS AND THE RISK OF APARITION OF PARASITIC ENTERITIS

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The research has been conducted on Karakul and Turcana crossbred lambs of 1-3 days of age; the purpose of this investigation was to reveal the development of lymphoid tissue associated of intestinal mucosa, local reactivity and the risk of apparition of parasitic enteritis. For the histological examination, samples of tissue were drawn from small intestine (duodenum, jejunum, and ileum) and from mesenteric lymphoid nodes, which were fixed in formaldehyde 10%. Subsequent, the samples were specific processed, included to paraffin, sectioned at 5µm and coloured through the M.G.G. and P.A.S.

Methods: The examination and microphotography were accomplished at M.C.5 with 10 oc., 10, 20, 40, ob. and 63, 100 immersion ob. Histological examination revealed the integrity of the epithelium with prominence of the brush border like and the taking over of the immunoglobulins as vacuole, through clathrin-coated vesicles, in a passage from the apical pole to the basal pole of the intestinal epithelial cells, the vacuole were surprised in lamina propria, with distension of central lacteal; diffuse lymphoid infiltration in lamina propria of villosity and Peyer`s patches with immature polimorphic cellular population with outlining of the germinative centers and a dome zone, in submucosa; anizocaria, reflecting a polymorphic mezenchymal population. In lymphoid nodes, there were observed primary nodules in forming, without germinative center, and in subcapsulary sinus a low cellular population. The lambs are extremely vulnerable to the parasitic intestinal aggression due to the fact that the local defensive system is in forming and the reactive capacity is minimum, at this age. Key words : lambs, newborn, small intestin, lymphoid tissue, reactivity.

9.13 NEW ASPECTS IN THE PATHOGENESIS OF ABOMASAL DISPLACEMENT IN DAIRY COWS

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Problem: It is supposed that disorders of abomasal motility are the main cause for the occurrence of abomasal displacement, but the predisposing factors for these disorders are still not completely understood. **Aim:** The aim of several studies was to identify different factors, which cause an abomasal displacement.

Materials and methods: - Electromyographic long-time measurements of abomasal motility in 5 healthy Holstein cows - Feeding studies with concentrate-rich diets- Measurement of the electromyographic activity of the abomasum and duodenum in 25 cows with left abomasal displacement during the first week after surgical treatment - Examinations to quantify the content of substance P (SP), vasoactive intestinal polypeptide (VIP) and neurofilament 200 (NF 200) in biopsies of the abomasal wall from 20 slaughtered Holstein cows and 20 Fleckvieh cows, as well as from 20 Holstein cows suffering from left abomasal displacement **Results:** Long-time measurements showed that the stage of lactation influences abomasal motility. No variation of the duodenal myoelectric activity during the dry period could be detected. In contrast abomasal activity increased significantly during this period and decreased significantly in the first weeks after calving. The long-time measurements also showed a circadian rhythm of abomasal and duodenal motility. During daytime a higher myoelectrical activity was recorded in comparison to the nighttime. The abomasal motility was significantly reduced, when the animals received a concentrate-rich diet, but the endotoxin- and cytokine-levels were still physiological. The 25 cows suffering from abomasal displacement showed decreased abomasal motility also during the first week after surgical treatment. In the immunohistochemical examinations of abomasal biopsies the content of stimulating SP was higher and the content of inhibiting VIP was lower in Fleckvieh cows than in Holstein cows. In cows with abomasal displacement, both, the content of SP and VIP were significantly decreased in comparison to healthy Holstein cows.

Conclusions: The measured disorders of abomasal motility can explain not only the occurrence of displacement, but also the high rate of relapses after a conservative therapy. Concentrate-rich diets induce an abomasal atony, but this was not the effect of endotoxemia. The differences detected concerning the content of SP, VIP and NF 200 in the abomasal wall suggest, that in Holstein cows the stimulating effects on the motility are smaller and the inhibiting influence seems to be more distinct than in Fleckvieh cows. In addition to further influencing factors, this could explain why Holstein cows do suffer from abomasal displacement more frequently than Fleckvieh cows.

9.14 EFFECT OF MILK, MILK REPLACER AND ORAL REHYDRATION SOLUTIONS ON ABOMASAL LUMINAL CONDITIONS AND ON THE ACID-BASE STATUS IN DAIRY CALVES

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Introduction: Oral rehydration solutions (ORS) cause an increase in the pH in the abomasal fluid, which could influence abomasal clotting of milk.

Material and methods: 11 healthy dairy calves (age: 23 (21-27) days, body weight: 54 (50-64) kg) were surgically instrumented with an abomasal plastic cannula. Calves were fed with cows milk, milk-protein milk replacer (MR) and different ORS at 8, 14 and 22:30 hours (12 % of body weight/day). Overall 168 feeding times were analyzed. Before and after feeding abomasal fluid was collected. pH, area under pH-curve (AUC) and [strong ion difference = SID3] were determined. The acid-base status of the calves was estimated by the parameters of Henderson-Hasselbalch and Stewart in blood. In-vitro milk clotting was determined after addition of chymosin and/or hydrochlorid acid by measuring viscosity. **Results:** After feeding of milk the abomasal pH increased from 2.0 ± 0.4 to 4.7 ± 0.4 within 30 min. When calves were fed with MR the abomasal pH reached higher values (5.2 ± 0.7). After 4-6 hours the fasting values were reestablished. With increasing [SID3]-data in the test meal significant higher abomasal pH-values and AUC-data were obtained. If the ORS were prepared in water the increase in pH in abomasal fluid took only 2-3 hours. The abomasal pH correlated with the measured [SID3] in abomasal fluid. In the measurement of in-vitro milk clotting cows milk reached higher values of viscosity than MR. Some mixtures of MR and ORS failed milk clotting at the original pH-level. After acidification with hydrochlorid acid at pH ~5.5 enzymatic milk clotting was detectable in every case. Also, acidification without chymosin at pH 4.7 caused an increase in viscosity. After every test meal there was a decrease in [acid total=Atot]. If we fed ORS with [SID3] >77mmol/l, higher levels of [SID3] were detected.

Conclusion: Effective ORS for calves with diarrhoea should have [SID3]-values >80 mmol/l. Such ORS do not interfere with milk clotting in the abomasum. Abbreviation: [SID3] = $[Na^+] + [K^+] - [Cl^-]$; [Atot] = $7.6 \times [\text{albumin}]$ or $3.6 \times [\text{total protein}]$.

9.15 PARTLY DISPLACEMENT OF MILK PROTEIN BY SOY PROTEIN IN MILK REPLACER ALTERS RNA METABOLISM IN SMALL INTESTINAL MUCOSA OF GOAT KIDS

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Feeding pre-ruminants with soy protein instead of milk protein leads to an alteration in the intestinal structure and absorption of digested nutrients. Dietary nucleosides as precursors for nucleic acid (NA) synthesis are important for rapidly dividing cells as gut epithelial cells, since gut epithelial cells have limited capacity for de-novo purine and pyrimidine synthesis. The present study was conducted to determine whether a soy protein-based diet or a soy protein-based diet supplemented with indispensable amino acids (IAA) changes the relative need for exogenous precursors for RNA biosynthesis in the small intestinal mucosa when compared to casein-fed goat kids, and whether these changes affect mucosal cell growth. Twelve 2-wk old male goat kids were fed comparable diets based on cows' milk, in which 50% of crude protein was either casein (CAS), soy protein (SP) or soy protein supplemented with IAA (SPA) for 34 d (n=4/group). Samples from proximal, medial and distal jejunal mucosa and blood were collected 5 h after feeding ¹⁵N labelled yeast RNA (6 mg/kg body weight) with the diet to measure villus heights and crypt depth, mucosal protein, RNA and DNA contents, activities of xanthine oxidase (XO; EC 1.2.3.2) and alkaline phosphatase (ALP; EC 3.1.3.1), ¹⁵N enrichment of mucosal RNA and urea in plasma, and plasma concentrations of free AA. Data were analysed by GLM using diet as fixed effect. In medial jejunum, villus height and villus height/crypt depth ratio were higher (P<0.05) in CAS than in SP. RNA content and RNA/DNA ratio were lower (P<0.05) and protein/RNA ratio was higher (P<0.01) in CAS than SP. In medial jejunal mucosa, ¹⁵N enrichment of RNA and activities of XO and ALP were higher (P<0.05) in CAS than in SP and SPA. Activity of XO in medial jejunum was negatively correlated to ¹⁵N enrichment of mucosal RNA in medial jejunum (r=-0.75, P<0.05) and positively correlated to ¹⁵N enrichment of urea in plasma (r=0.57, P<0.1). The ¹⁵N enrichment of urea in plasma was negatively correlated to the ¹⁵N enrichment of mucosal RNA in medial jejunum (r=-0.65, P<0.1). Total AA concentrations in plasma showed no group differences, but Gly was lower (P<0.05) in CAS than in SP and lower (P<0.1) than in SPA.

In conclusion, morphological changes in the intestinal mucosa induced by partial displacement of milk protein by soy protein are accompanied by a reduced re-utilization of dietary RNA precursors for RNA biosynthesis and an increased activity of key enzymes involved in NA breakdown. Feeding a soy protein diet supplemented with IAA results in a slight not-significant stimulation of mucosal growth and no impact on salvage of exogenous RNA precursors for mucosal RNA biosynthesis.

10 HERD HEALTH MANAGEMENT

10.1 DAIRY HERD HEALTH MANAGEMENT: CURRENT STATE AND PERSPECTIVES

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Abstract

Herd health programmes concentrate on the prevention of diseases hence the reduction of economic losses and production costs, and the performance of the dairy herd. Modern animal health care requires excellent nutrition, good housing facilities and a close cooperation between a competent veterinarian and a skilled farmer/manager. The goal of herd health programmes is to improve animal health and welfare and to guarantee a high quality (safety) and wholesomeness of foods from animal origin. If medicines are to be used, they must be administered under strictly controlled conditions. Cost effectiveness of a herd health programme is essential. It has been shown that the net return to the farmer of money spent on such a programme is in the order of 400 %. In the future risk management programmes, based on HACCP (Hazard Analysis Critical Control Points) principles must be developed and be integrated into herd health programmes.

Introduction: animal health and welfare

The industrialization of animal farming comprised the concentration of animals in large units and the minimization of investment costs for animal facilities. During the last years this process has been intensified by increasing competition between food producing countries. Import barriers and high tariffs have partly vanished, resulting in more free trade and competition regarding farm products.

In addition to these developments, considerable attempts have been made to improve individual productivity by selected breeding. This resulted in extremely high producing cows. However, as an implicit consequence of this forced selection some adverse effects have arisen such as a lower fertility and a higher susceptibility to different diseases.

All these developments have resulted in production units where large numbers of high yielding cows are kept under minimal conditions, while at the same time management quality falls behind. It is therefore easy to understand that diseases are a common phenomenon in such herds. Typical examples of encountered diseases are mastitis and lameness.

Health status is influenced by several – strongly management related - factors, the most important being housing conditions, nutrition, hygienic measures, infections, animal breeding and selection. The etiology of most herd health and welfare problems is complex and multifactorial. For example the etiology of foot lameness, involves genotype, nutrition, housing and behaviour, and – in infectious cases - hygiene

It can be stated that pushing animals to the limits of their productivity is a matter of real concern. The physiological demands are extremely intense. As it is not realistic, because of demands for cheap daily food and of increasing competition, to expect that animal health problems will vanish gradually one has to accept the presence of impaired health and thus the necessity of veterinarians and medicines. Even if animal health and welfare improve in the future, large production units will remain, providing a good opportunity to a large number of diseases to affect the animal's health and welfare. Thus, strategies have to be developed to improve animal health and welfare, and to reduce the amount of residues in food of animal origin (de Kruif 1998).

Consequently, the questions are: how to keep animals in good health and how to improve animal welfare on the one hand and how to produce safe food for a reasonable price on the other hand. It is clear that both questions are linked and that the veterinarian faces a major challenge and responsibility on these issues. To this end herd health programmes have been developed. The key is that they need to be effective and practical.

In many European countries farmers have to comply with quality assurance programmes where participation in a herd health programme is compulsory. This means a new challenge for bovine practitioners and will stimulate farmers to pay more attention to public health, animal health and welfare.

The veterinarian's role in herd health management

Because there is such a close relationship between animal health and production, health management has already a long history. Originally it emphasized the eradication of contagious diseases such as brucellosis and foot-and-mouth disease. Later on, the emphasis was on the individual cow affected with a clinical disease. About 30 years ago subclinical disease in its broadest sense was recognized as the major cause of economic loss. Good examples are subfertility, subclinical mastitis and claw lesions. It turned out that regularly scheduled visits to farms to examine health and production status of the herd were effective in improving the status. Herd health programmes were developed. It is now generally agreed that diseases, many of which cause no recognizable clinical signs, are the most important contributors to reduced productivity. Each dairy herd presents a unique combination of factors contributing to suboptimum performance. It is the veterinarian's main task to implement an integrated animal health and production management system in order to prevent clinical and subclinical diseases. The final goal is to eliminate production inefficiencies which are caused by

factors that impair animal health and animal welfare, or at least reduce substantially their impact.

As the biological or chemical agents which may cause food poisoning accompany the animals from the stable via the dairy-factory or slaughterhouse to the “table”, any attempt to maintain a high level of production of consumer goods without taking into account what is happening in the stable is doomed to failure. There is a graving need to track animals and their products entering the food chain. For this reason herd health surveillance programmes have been developed and are currently implemented. The key person in these programmes is also the veterinarian. He has to ensure that the animals or animal products entering the food chain (dairy, slaughterhouse) are free from disease c_q microbiological contamination c_q chemical contaminations and residues.

Hence a herd health programme can be considered as a total quality assurance system. It is a combination of regularly scheduled veterinary activities and good herd management designed to achieve and maintain optimum animal health and production. Future herd health programmes should not only focus on fertility or recommendations for the control of disease, but should include recommendations on nutrition, housing, genetic improvement, cash-flow, animal welfare, the use of medicines and food quality including public health and food safety.

As a consequence of these developments the traditional role of the veterinarian as the healer of individual sick cows has been complemented by the delivery of health programmes concentrating on the prevention of diseases and the performance of the dairy herd. Such management programmes will lead to the most efficient economical and profitable production of dairy products, taking into account the demands of the modern consumer (de Kruif *et al.* 1998; Radostits 2001).

Requirements for a herd health programme

There are 4 requirements for a successful herd health programme:

- 1 a competent professionally acting veterinarian
- 2 a farmer who is committed to the programme
- 3 a good data recording system.
- 4 be cost-effective

Livestock producers perceive dairy cow practitioners as knowledgeable and skillful in the diagnosis and treatment of sick animals and reproductive performance, but they also perceive that the practitioners knowledge of nutrition, herd management and economics is weak. To be involved in integrated herd health the veterinarian has to improve his knowledge and skills. He has to be a cattle specialist who can provide a comprehensive economically-based health and production management veterinary service such as needed by the farmer. Moreover, the practitioner must have good

communicative skills, must master marketing principles and must understand farm management.

The success of a herd health programme depends heavily on the farmer's skills and ability to comply with the recommendations of the veterinarian. A bond of confidence between the farmer and the veterinarian is extremely important. The farmer should be motivated to start a herd health programme and to keep the programme going.

Educating the farmer and farm staff requires a common-sense approach. One way to get the message across is to make a list of costs associated with disease and leave it in the farm office where it can be perused by all concerned. This can then be used as the basis for discussion at a later date. Communication is essential in all aspects of herd health management. The relationship between the bovine practitioner and the farmer is a symbiotic one. Regular veterinary involvement on a farm may be the difference between a farm staying in or going out of business (Borsberry, 2002).

The data recording system should be as simple as possible. It is one of the most important components of any herd health programme (Fetrow 1993, de Kruif *et al.*, 2007). Many different systems are available and the types of records used vary considerably. The simplest and most common form, used in dairy herds is the manual handling of records. Manual systems have been proven satisfactory for 50-100 cow herds. As herds become larger manual methods are less satisfactory. This has led to the use of the computer. The computer is able to store a large amount of data, can prepare action lists, analyse data and provide a summary of up-to-date performance. However, modern systems of computerized data recording are often too complex resulting in too time consuming entering and analysis of the provided data.

The challenge for the veterinarian is to determine what services are needed by the dairy farmer and how these services can be delivered economically. The farmer must be convinced of the high merit of the programme and of its cost-effectiveness. If necessary the programme can be started on a partial basis. For example a programme to improve reproductive performance or milk quality. Later on the other parts of the programme can be implemented.

Ideally a herd health programme related to prevention consists of the following parts : fertility, udder health and milk quality, nutrition and metabolic diseases, control of infectious diseases, lameness, housing, health of calves and heifers, animal welfare, the use of medicines and food safety. An integrated approach to the dairy herd instead of focusing on separated disciplines e.g. fertility or milk quality alone has many advantages. It permits a total overview of the dairy herd and should always be advised (de Kruif *et al.* 1998 ; Radostits 2001).

Benefits and costs of a herd health programme

Cost-effectiveness of a herd health programme is essential. There is little information on the total benefits and costs for health management. Enhancement of the profitability of the farmer is the primary objective of every herd health programme. Health problems cause financial losses. The calculation of these losses is very difficult. The outcome of calculations often differs very much, even for similar farm and price conditions. Financial losses can be attributed to one or more of the following factors:

- less efficient production.
- reduced animal production (milk, calves)
- veterinary costs
- mortality
- reduced slaughter value
- lost future income owing to disposal

When calculating the total loss per farm, these factors must be added.

Huirne et al. (2002) calculated losses due to reproductive failure, mastitis and lameness. The average was respectively US\$ 80, 88 and 27, in total US\$ 195 per average present cow per year. Considering all other diseases not yet included, total average losses in dairy cattle may increase to – at least – US\$ 300 (230 Euro) per cow per year. This corresponds to almost 30% of the farmer's return to labour and management.

It will not be possible – nor profitable – to avoid all calculated losses. However big differences among farms do exist, easily exceeding the calculated average loss. Moreover, the best 20% of farms prove to realize only half of the calculated losses on the average farm. So, there is reason to expect that considerable economic improvement can be achieved, especially for farms with higher than average losses (Huirne et al., 2002). The goal for a farmer could be to belong to the best 20%.

If this goal can be reached by implementing a herd health management programme an average farmer will experience a profit of 115 Euro per cow per year. This means a benefit of the programme of 11,500 Euro per year for a 100 cow herd.

Veterinary herd health programmes have many costs. The most important are costs for the services on the farm, the time required for the analyses of data and the preparation of reports and advices, and the costs of medicines and vaccines. For a 100 cow herd the costs of a total dairy herd health programme may be estimated of being 2,000-3,000 Euro per year depending on the time required and medicines and vaccines used.

The cost-benefit analysis of this case shows that the net return to the farmer of money spent on such a herd health programme is in the order of 400-600 %. The impact of health management on the economic performance of a dairy herd has been the subject

of only a few studies that showed a substantial increase in profitability beyond the extra veterinary costs (Young et al., 1985; Hogeveen et al., 1992).

However the perception by many farmers that veterinarians are not production oriented has been one of the constraints of more widespread employment of veterinarians as health management advisers (Radostits 2001). The main reason that herd health programmes are only applied in a minority of the farms is that farmers are not convinced of its cost effectiveness. This phenomenon can be explained by behavioural economic principles (van Egmond et al., 2006). Many programmes are too complicated and especially the amount of administration is too high and thus too time consuming. The majority of the farmers is willing to pay for work. To get talking or writing paid is much more difficult! As a consequence the veterinarian should always combine work and advice and try to minimize the administrative work load.

Some benefits may be difficult to quantify. The satisfaction of having a healthy herd, reducing animal suffering and human health risks and minimising the use of antibiotics are some examples of such benefits (Huirne et al, 2002). The latter is also explained by behavioural economics.

Also the impact of disease on farm staff morale should not be underestimated, as a severe outbreak can have an adverse effect on the whole business and reduced attention to detail will allow other disease complexes to reach unacceptable levels (Borsberry, 2002).

In many practices a dairy herd health programme is charged using a contract based system of charging. The farmer and practice work out in advance the degree of inputs for the farm for the year and then an animal or hourly fee is calculated for herd health visits and routine preventive medicine work, as well as for follow-up activities.

Protocol of a herd health programme

A herd health programme is based on regularly scheduled farm visits, the monitoring of animals and their environment, the recording and analysis of animal health and production data, the provision and coordination of advice by the veterinarian, good farm management by the producer, and follow-up for evaluation.

The major objective of the programme is to support farmers in reaching their targets of performance and farm goals. During the first visit these targets should be discussed and set e.g. calving interval or milk quality. The objectives are set in the light of risk assessment and a cost-benefit analysis of what can realistically be achieved. Objectives will be different for each farm and will depend on a range of factors relating to herd health status, animal welfare, housing and nutrition.

The objective of each of the following visits is to determine the actual performance of herd health and production (assessment of strong and weak points), compare it with

targets of performance and farm goals and decide which performance index is normal resp. abnormal. The veterinarian then analyses the problem and attempts to determine the cost of the shortfalls in health status and to formulate cost-effective corrective action based on the risk factors involved. Before each of the following visits the veterinarian accesses the specific database for up to date information about the herd in question. Each visit will involve a clinical examination of the animals and at the end of the visit a herd management meeting (de Kruif 1998; Radostits 2001, de Kruif *et al.* 2007).

The farm visit is also designed to detect or predict health and production problems before they become economically significant and to indicate the corrective action necessary. Disease prevention should be a major item at each visit. Each herd management meeting should provide a summary of the herd health and production status, the diagnoses made and the reasons for failure to achieve the preset goals and recommendations for corrective actions. Furthermore general advice is given. This advice is directed at the prevention of specific diseases e.g. Infectious Bovine Rhinotracheitis or milk fever (de Kruif *et al.* 2002, 2007). Effects of previously given advice are evaluated at subsequent visits.

Perspectives of herd health programmes

The strengths and accordingly also the perspectives of a herd health programme are related to prevention of diseases or/and production inefficiencies, improving animal welfare and food safety. The following items need special attention in herd health programmes:

1. *Reproduction.* Activities related to reproduction become increasingly important because of the declining fertility of high producing dairy cows. Nearly all dairy farmers find veterinary intervention useful to enhance reproductive performance. Reproductive failure is associated with many risk factors, e.g. excessive loss in body condition, poor housing facilities and lameness. A shortfall in reproductive performance needs a thorough investigation and depending on the riskfactors involved control strategies have to be formatted.

2. *Udder health and milk quality.* Udder health and mastitis control is also one of the major reasons for which the dairy farmer finds veterinary intervention very useful : detailed mastitis control measures have been outlined and described (Radostits 2001, de Kruif *et al.* 2007). With proper implementation these programmes cause a decrease in the prevalence of common contagious mastitis pathogens. Herds that have implemented a comprehensive mastitis control programme also need to develop strategies to control infection with environmental micro-organisms and need to use an effective monitoring system for the early detection of new infections.

3. *Lameness.* Lameness is considered to be the third most economically important disease after reproductive failure and mastitis. Most cases of lameness are due to

lesions of the feet. These are associated with herd-level or individual cow-level risk factors. Herd-level factors include housing, hygiene, nutrition and foot trimming. The most important individual cow factors are stage of lactation, claw angle, horn quality, age and social status.

4. *Control of infectious diseases* : Infectious diseases can result in major economic losses. The control of these diseases must be considered in any dairy health programme. Outbreaks of diseases that are on list A of the « Office International des Epizooties (OIE) », for example foot- and mouth disease, are especially feared for they have the potential for rapid spread. Some other diseases have been eliminated by official governmental eradication schemes : brucellosis, leucosis and tuberculosis. The most important infectious diseases affecting dairy cows are IBR, bovine viral diarrhoea-mucosal disease (BVDV), Johne's disease, leptospirosis, salmonellosis, fascioliasis, infectious bovine keratoconjunctivitis and those causing mastitis, lameness and problems in young cattle. Prevention, control and eradication are used in association with infectious disease control. In the prevention of many infectious diseases, for example salmonellosis or mastitis, risk factors play an important role.

For certain infectious diseases the prevalence of infection can be monitored over time by regulatory laboratory examination of a small, random sample of the population, for example BVDV. Based on a sero-epidemiological profile the presence of several diseases can be followed.

For most of the common infectious diseases the complete elimination of disease is not necessary because there may be a level of disease in the population below which the cost of further expenditure on elimination would be greater than the benefits derived. To eliminate some specific pathogens and hence disease from a herd, eradication is used e.g. BVDV, Johne's disease and leptospirosis

To prevent the spread of infectious agents from infected animals to susceptible animals or to prevent the introduction of infected animals into a herd, vaccination and/or biosecurity management practices can be used. The decision to use a vaccine for the control of any disease must be based on considerations of the prospects for its control by other techniques, such as removing or reducing the effects of risk factors. When vaccination is used it must be established that an immune response actually can protect against the disease in question.

It must be stressed that biosecurity management practices are extremely important in preventing the spread of infectious diseases. A closed dairy herd system, in which producers do not purchase animals and do not take cows to shows or fairs, is very beneficial and should be advised. It is technically possible and is economical. It can prevent the introduction of infectious agents, such as IBR, BVDV, paratuberculosis and salmonellosis and can be a good starting point for eradication of these infections from the herd when present (Van Schaik et al . 1998). If animals are to be introduced into a herd they should be quarantined in an isolation facility for a specified period and possibly tested.

Risk analysis should be performed before designing a biosecurity plan. This should include an assessment of which diseases are of relevance, the likelihood of the disease entering the unit, the consequences of disease entry, which measures can be taken, the likely efficacy of the biosecurity measures and the costs.

The disease status of the herd should be established prior to setting up the biosecurity plan and disease monitoring should be carried out at regular intervals in order to detect disease early on (Grove-White, 2004).

5. *Nutrition and metabolic diseases.* Many health problems of a dairy herd relate in some way to the feeding programme. Small changes in feeding may bring about large changes in health and productivity. For this reason veterinarians must become actively involved in the herd's feeding programme including feeding management.

If a veterinarian is providing nutritional consulting services then a routine monitoring service is essential. There are many parameters that can help to determine the adequacy of a feeding programme, for example milk production and milk components, fecal consistency, body condition and rumen filling.

Diseases commonly viewed as metabolic diseases include ketosis, hepatic lipidosis, milk fever, displaced abomasum, rumen acidosis and retained placenta. Most of these diseases can be avoided if attention is paid to the fundamentals of feeding dairy cows including dry cow rations (Mulligan et al. 2006, de Kruif et al. 2007).

6. *Housing.* The goal in housing design is to provide an environment for the dairy cow that has a positive influence on the cow's health, welfare and production. This includes proper stalls and beddings, ventilation, access to feed and water and walking surfaces.

Many diseases are associated with housing : diseases of the respiratory tract, mastitis, lameness, parasitism and behavioural abnormalities (teat sucking).

The effects of housing and environment are complex and there is usually no simple solution to a particular problem. Agricultural engineers should be consulted. Decisions about replacement farm facilities must be preceded by a conscious determination of the management style and practices . New facilities must be designed to meet the requirements of the cows (Goodger 1996 ; Radostits 2001) reflected in good cow comfort.

7. *Health of young stock/replacement heifers.* One of the most important aspects of a dairy enterprise is to raise sufficient quantities of well grown heifers to calve by 24 months of age. These animals are the investment in the future and deserve full attention.

8. *Culling and genetic improvement strategies.* The increasing of cow longevity is receiving growing attention in the dairy industry. Monitoring the reasons cows are

culled for helps to identify health management problems that may be impairing the profitability of the herd. A culling rate between 20 to 25 % is the economical optimum (Rogers et al. 1988). Genetics has proved to be a powerful strategy for improving productive efficiency of dairy cattle. However the improvement in production has come at the cost of some loss in virtually every other aspect of dairy cow function. It is imperative that health and reproductive performance be given increased emphasis in genetic improvement programmes. Genetics can be used as a tool for preventive medicine. Genetic programmes for health traits and genetic improvement for disease resistance can become effective adjuncts to the other methods of preventive medicine.

9. *Animal welfare* .Animal welfare is central to profitable milk production. Neglect of welfare will inevitably lead to falling profitability. A herd health programme should also provide the farmer with the opportunity to set objectives for achieving an optimal animal welfare classification.

A welfare definition has been proposed for first analysis of all the factors likely to influence the welfare of farm animals, whether on the farm itself, in transit or at the point of slaughter (Webster 1997). This definition encompasses freedom from thirst, hunger and malnutrition, from discomfort, from pain, injury and disease, from fear and distress and freedom to express normal behaviour. These five freedoms have been considered as too vague to be handled at farm level (Bracke et al, 2001). These authors have deduced the biological needs from these five freedoms. The most important biological needs can be found in the practical concept of cow comfort (Noordhuizen et al., 2005) Cow comfort comprises practical parameters in 4 domains:

- housing and climate
- feeding
- health
- specific behaviour

Most of these domains have been addressed above.

For example in the dairy cow potential contributions to poor welfare are: hunger or acute metabolic disease due to imbalance between feed availability and requirements; chronic discomfort through bad housing; chronic pain through lameness or metabolic exhaustion after prolonged high production.

One has to realize that freedom from disease means that if diseases or infections develop, an immediate treatment must be started preventing unnecessary suffering of the animals. In that way medicines and vaccines do play an important role in improving animal welfare. The food animal practitioner is considered by those outside the profession as the ultimate advocate for animal well being. This position requires that practitioners provide leadership in the development of acceptable humane standards in farm animal agriculture (AVMA at work 1994). Cow comfort is an example.

However, it is very difficult to apply a cost-benefit analysis of all farm procedures which weighs the cost to the animal in terms of suffering, against the likely benefit to society. Obviously the higher the cost, the higher the need for justification. In particular cases the animals need the protection of new law that seek to balance any costs to the animal against the potential real benefit to man.

10. The use of medicines and food safety. In the past, the far most important tasks of the veterinarian were the control of the herd health status and the cure of animals. The prevention of health problems and the application of treatments remain quintessential, but under consumer and political pressure food safety issues are increasingly important.

If it is necessary to use pharmaceuticals either for prophylactic and/or curative treatment then clear (written) instructions have to be provided, allowing a proper and selective use of pharmaceuticals. This includes also the consideration of adequate withdrawal periods to prevent residues in foods of animal origin. Thus, residue levels exceeding the MRL values (maximum permissible residue level) can be prevented by proper management.

It is an illusion to believe that all food from animal origin can be produced by low yield farming without the proper use of pharmaceuticals. Antibiotics, anti-parasitics, vaccines and other medications are indispensable, but must be used under strict conditions in search for an optimal policy against specific contagious diseases. The veterinarian who is responsible for herd health surveillance will also be responsible, together with the farmer, for the prevention, surveillance and monitoring of residues of these pharmaceuticals in milk and beef. The practitioner should fully understand and establish a client's drug use protocol and residue avoidance plan (de Kruif 1998).

Veterinarians are the first line of defense on food safety and are capable of making the informed decisions, necessary to protect human food safety while providing comprehensive health management advice to animal owners (Radostits 2001).

Overall, the following constraints can be noticed regarding the introduction and implementation of herd health programmes. First, veterinarians are not able to convince farmers about the benefits such programmes will have. This is a communication and marketing problem. Secondly, farmers are not aware of the knowledge and skills of veterinarians in areas like housing, climate control, claw health, productivity and calf rearing. This, again, is a marketing problem of the veterinarian. Thirdly, veterinarians tend to fall back to technical handlings instead of conducting problem analysis, broad farm monitoring and discussion, they are reluctant to recording and reporting. These are features of non-professionalism.

Only if these constraints can be eliminated, a herd health programme can be properly implemented. Sometimes this means that veterinarians have to acquire knowledge and skills through professional short courses.

During the examination of animals a survey of contagious diseases is carried out to identify outbreaks at the earliest possible stage. Procedures in ongoing eradication programmes will be carried out during the visit. Also during the visit, a number of samples (blood, urine, faeces etc.) can be taken for laboratory analysis to monitor the health status of the herd in relation to epizootic and zoonotic diseases of importance. Controlling of zoonosis by a herd health programme is sometimes easy, for example brucellosis and leptospirosis in cows, but is sometimes very difficult, for example salmonellosis and Verocytotoxin-producing *E. coli* (VTEC) infections. New disease monitoring strategies and more research are required to reduce the number of infected herds and animals, and to reduce its (minimal but hazardous) contamination potential for humans.

Future developments

In the future, farmers will have to produce according to preset quality assurance system demands, as e.g. in the EU.

Quality is now defined in a broad sense: not only the product is involved but also the production method and the production unit surroundings. Instead of only the end product, the whole production process needs to be controlled. First of all, a general change in attitude and mentality of the farmer is needed, based on a Good Agricultural Practice (GAP) code (OIE 2006, FAO 2003). Elements of this code refer to hygiene practices and to standard operating procedures. A veterinarian who wants to serve in a quality assurance system needs to act according to a Good Veterinary Practice (GVP) code.

A herd health programme can be incorporated in different concepts of quality management, hazard analysis critical control points (HACCP) and ISO-9000 series. For the application of quality management to animal health care, the HACCP concept is preferred and suggested by the EU (Noordhuizen and Welpelo, 1996, Cannas da Silva et al., 2006).

HACCP emphasizes prevention to avoid of food safety problems. HACCP combines common sense with an evaluation of risks, in order to identify the points along the production process where possible hazards may occur (critical control points (CCP)), and then to strictly monitor and manage these points to make sure the process is under control. Examples of CCP on a dairy farm are bacteria counts per ml milk and milking machine cleaning water temperature. It is common sense and obvious that any quality assurance system based on the HACCP system is most successful in keeping food safe when it is used throughout the entire food production chain from farm to table (Blaaha 1997). The concept of quality assurance is as simple as considering what can go wrong in production that could cause a quality defect and hence economic losses and figuring out how to prevent it from going wrong.

Currently, various activities for developing a HACCP-based quality risk management programme are ongoing; field tests as well have been conducted. Examples can be found in Cullor (1995), Mansfeld (1998), Lievaart et al. (2005). Moreover, a website is available where HACCP-like templates for dairy farms can be downloaded (www.vacqa-international.com) and at the end of 2007 a book with HACCP-like applications on dairy farms will be available (Wageningen Academic Publishers). All this material is meant to support the practitioner at implementing quality risk management programmes in the future.

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10.2 LARGE DAIRY HERDS HEALTH MONITORING

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The metabolic and mineral disturbances increase the risk of a health loss and negatively influence the milk yield. The aim of the study was to evaluate the influence of two-month interval laboratory monitoring of representative groups of cows on herd health. The study were performed on two dairy farms: A-450 heads, the milk yield 10700 kg/lactation (high milk yield strategy); B-180 heads, the milk yield 6500 kg/lactation (cost reduction strategy). Six times at 2 month intervals jugular vein blood was taken from the cows of representative groups consisted of: 10 cows between 14th and 150th day after delivery, 10-between 150th day and 14th day before the end of lactation and 10-between 14th day after drying and 14th day before parturition. There were estimated: acid-base balance, the levels of Ca, Ca⁺⁺, Mg, P (inorg.), K⁺, GOT, urea, serum albumin, alpha-, beta-, gamma-globulin, fibrinogen and haptoglobin. In urine pH and ketone bodies were estimated. BCS of the representative groups of cows was assessed. Veterinary inspection of the herd was made during each sampling. Within 2 weeks after each monitoring the results were discussed with the owners and the appropriate recommendations were formulated. At the 1st monitoring of farm A alkalosis, hypocalcaemia and significant differences in cow's individual BCS were found. At the 2nd and 3rd examinations normalization of the mentioned parameters occurred. At the 4th examination alkalosis and hypocalcaemia appeared again, although the ionized calcium level was normal. At the 5th examination the levels of mentioned parameters tended to normalize, but the albumin level decreased, and GOT level was slightly elevated. At the 6th examination a significant increase of the urea level was found in early lactation group and in all examined cows the level of P increased. In herd B at a start of the study an alkalosis and elevated level of serum gamma-globulin were found in lactating cows. An important problem in this herd was too high BCS of dry cows and a rapid loss of body condition in early lactation cows. The levels of Ca, P and Mg were in normal range. At the 2nd examination the decreased levels of albumin, Ca and urea occurred. Ca level also decreased at the 3rd examination, but other parameters started to normalize. At the 5th examination the most of the cows showed corrected levels of mentioned parameters, but the increase of GOT was found. At the last examination alkalosis occurred again and the level of Ca was high in dry cows. Decreased means and rates of elevated levels of acute phase proteins indicated improvement of health in both farms during the study. Analysis of laboratory parameters helps in improvement of dairy herds health. The disturbances observed were caused by failures in program execution by the owner.

10.3 VETERINARY HERD CONTROLLING SYSTEM - CONTROL AREA 'METABOLIC HEALTH'

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The Veterinary Herd Controlling System (VHC-System), introduced by MANSFELD et al. (2002), is a pyramidal, dynamic quality assurance system for dairy herds. It allows optimization of process-quality and product-related quality. Individual farm goals are pursued through farm specific strategies and target-performance comparison. By means of a search of international literature a quantity of direct (directly related to animal health) and indirect (related to factors affecting animal health) critical control points (CCP) and control points (CP) and their corresponding indicators was collected. The indicators are quantitative or semi quantitative criteria allowing to evaluate a herd or a group of animals and to set goals. Suitable CCPs and CPs were implemented in a flow chart to be used by veterinarians as a tool for the status quo assessment as the first step as well as for regularly performed procedures. In the control area 'metabolic health' determination of 'incidences of metabolic disorders', 'body condition score', 'analysis of the milk recording' and 'control of feeding calculation' were fixed as CCPs on the lowest level. If corresponding indicators deviate from normal range, the VHC-System provides further logical steps to identify reasons for variations and to countermand them. On the next level the factor 'feeding' has to be evaluated concerning energy, fiber and mineral supply. If there is an insufficient supply by an inadequate feed intake, checks of the factors 'housing' (water supply, feeding area, grouping) and 'management' (feedstuff processing, feedstuff distribution) are necessary. The described quality assurance system designed as a flow chart represents an efficient tool to control 'metabolic health' on dairy farms.

10.4 MONITORING CALF HEALTH IN NORWEGIAN DAIRY HERDS

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Introduction: The Norwegian Cattle Health Recording System (NCHRS) has existed since 1975/76, and is well established for recording of common diseases in dairy cows. During the last 10 years, the Norwegian Cattle Health Services (NCHS) has put great effort in improving calf health recordings in this system. Accurate recordings on calf health could be useful in both herd health preventive work, as well as for genetic improvement work through breeding. The aims of this study were to improve calf health recordings and to identify the treatment rate for calf diseases in Norwegian dairy herds.

Material and methods: During 2004 through 2006 a survey on calf health in Norway was performed. The study included 261 randomized herds, where of 170 were dairy operations. Each herd participated for one year. Report calf diseases and treatments on calves up to 180 days of age were included.

Results: A total of 8212 calves were born during the project period. All together 2537 events were reported, of these 808 (31.8 %) were disease treatments and 1729 (68.2 %) were events of preventive therapy. Of the 808 treatments, 323 (40.0 %) were gastritis/enteritis, 301 (37.3 %) respiratory disease, 69 (8.5 %) arthritis, 32 (4.0 %) abscesses/phlegmones, 13 (1.6 %) indigestions, and 70 (8.6 %) other diseases. 25 % of all treatments for enteritis occurred before 9 days of age, and 25 % occurred after 45 days with a mean age of 21 days. For respiratory diseases the corresponding age limits were 24 days, 95 days and 53 days as median. The preventive treatments included 1508 (87.2 %) cases of dehorning, 126 (7.3 %) parasite treatments, 77 (4.5 %) bovine ringworm vaccinations, 9 (0.5 %) malignant edema vaccinations, and 9 (0.5 %) treatments for vitamin- or mineral deficiency. 91 herds (53.5 %) had reported cases of dehorning. All together, 4461 (54.3 %) of all calves born, and 515 (63.7 %) of the 808 disease treatments were from herds reporting dehorning.

Discussion: The most frequent diseases in calves in Norway are enteritis and respiratory disease which make up about $\frac{3}{4}$ of all diseases reported. The disease risk for all calves is approximately 4 % both for enteritis and for respiratory disease. It has to be taken in consideration that Norway is free from many of the serious infectious diseases, like IBR and BVDV, and that bovine ringworm is close to eradication. These figures could therefore be an indication of disease occurrence for countries with similar infectious status. In our study we found a higher relative proportion of disease recordings from herds which also report dehorning. Reports of dehorning could be used as an indicator of a well functioning reporting system.

10.5 NEW OPPORTUNITIES IN MONITORING AND EARLY DIAGNOSTICS AND CHALLENGES IN MANAGEMENT AND PREVENTION OF INDIVIDUAL DAIRY COWS

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Introduction

As a consequence of price and structural development in the dairy industry, the average herd size has increased rapidly in many countries and e.g. in Denmark it has increased to above 100 cows. This structural development is expected to continue and the large dairy farms should be considered as enterprises rather than traditional farms. In these larger units the farmer or farm staff has to look after an increasing number of animals for disease, reproduction, production and welfare. This, together with the high labour cost or lack of qualified staff, consumer and society demands on product quality, animal welfare, and concerns about the environmental effects of livestock production calls for automated precision management. Such automated precision management should combine advanced technologies and biological knowledge to organize dairy production (breeding, feeding, management, monitoring, early diagnostics, prevention and treatment strategies) in ways that: 1) results in low disease incidence and severity, 2) secures low impact on the environment, 3) results in the requested product quality, 4) secures optimal production and reproduction and 5) are profitable for the producer. This will allow the balance between animal health and production to be optimized, ultimately on a cow by cow basis.

The objective of this article is to give examples of new opportunities in monitoring and early diagnostics and challenges for future prevention and management of individual dairy cows.

Towards status-oriented strategies for in management and prevention

The incidence risk of the production diseases and reproductive problems is still substantial despite decades of effort to prevent the diseases (Ingvarstsen et al., 2003; Friggens, 2003; Ingvarstsen, 2006). This is not to say that the preventive measures up till now have been useless. The preventive measures have most likely improved the health in many problem herds. But why is the incidence of production diseases still high? This is probably because the improvements in prevention have been outweighed by a worsening incidence of production diseases due to e.g. selection for higher production and altered production systems and conditions. Further, the preventive

measures have generally focused on implementing changes at the herd level, for instance in feeding, rather than at individual cow level.

Figure 1 illustrates how genotype, stage of production, nutrition, management and environment may influence the risk of disease through effects on the organ function including epithelial status, metabolic and immune status. By epithelial status we mean the ability of the epithelia to resist physical stress, pathogen invasion, and transmission of for instance endotoxins from the rumen to the circulation during rumen acidosis. The condition of the epithelium of the rumen is of importance for the cow's metabolic and immune status. Metabolic and immune status are important for the organ function, e.g. the function of the alimentary tract. Figure 1 also illustrates that the metabolism and the immune system communicate, and that the animals' immune status plays a central role in their disease resistance. There are many examples of interactions between the physiological and immunological system, as well as the influence of nutrition and stress on the immune function (Ingvarstsen et al., 2003; Carroll and Forsberg, 2007).

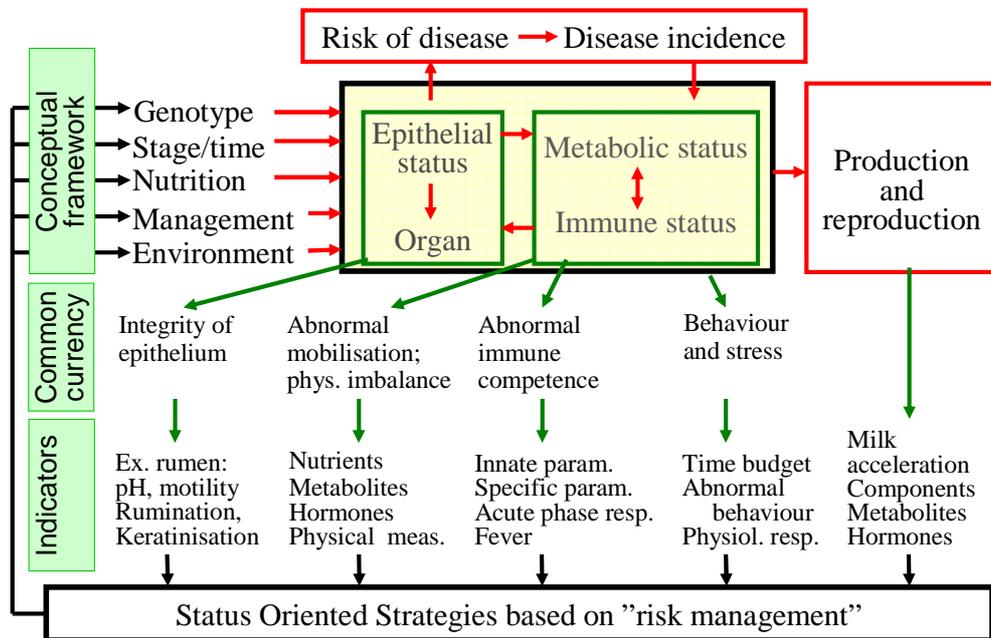


Figure 1. Status Oriented Strategies for optimising dairy cow health, reproduction and production. Status Oriented Strategies aim at securing a desirable status of epithelial integrity, metabolism and immune function, behaviour and stress and production through surveillance and health and production management based on indicators and risk assessment (Modified from Ingvarstsen et al., 2003).

A number of production diseases and reproductive problems have “common currencies” that offers the possibility to generalise and thus simplify diagnosis of poor health and reproductive problems. We have chosen organ function, abnormal mobilisation and physiological imbalance, abnormal immune competence, behaviour and stress and finally milk yield as common currencies (Figure 1). Utilizing the common currencies requires that they can be either directly measured or, more likely, be readily monitored by specific indicators. There are several areas in which farmers could alter their management if they had good indicators and interpretation of the resulting data. Changing nutritional management to maintain a normal metabolic and immunological status that will secure low disease susceptibility and a high productivity and efficiency, is an immediate option.

Generally farmers have used information to make changes on the herd level. However, in our opinion this is hardly the way to reduce morbidity in herds with an “average” morbidity. A greater focus on the prevention of disease in the individual animal, or at least in homogeneous groups of animals that have similar status, is necessary in order to reduce welfare threatening production diseases, reproductive problems and suboptimal production. This calls for automatic monitoring systems that can identify “risk animals”, i.e. animals with an increased risk of disease, reproduction problems and suboptimal production as a result of poor status. Such systems need to be based on biological and biometric models that can use and integrate status-oriented indicator data and turn that into real-time preventive suggestions based on risk management.

New opportunities in monitoring and early diagnostics – examples

Prototypes now exist of advanced automatic equipment that are monitoring in-line and real-time on milk to give a status on mastitis, ketosis, reproductive status and urea that will give new opportunities in future dairy cow management. The basis for the prototypes has been developed in a project called Biosens. Biosens is an interdisciplinary collaborative project established in 2001 between Lattec I/S, the Danish Cattle Research Centre (KFC) and our Faculty (DJF). Biosens carries out research on the relationships between physiological/immunological parameters in body fluids (particularly milk) and tissues and the status (disease, reproduction, nutrition) of the individual cow. Our research also provides tools for predicting cow status. DJF has been responsible for the experimental, biological, and biometric development and results from this project have created the biological, biometric and management basis for the development of the prototype of a proactive herd management solution called “Herd Navigator”. The system gives the farmer real-time information for proactive herd management concerning reproduction, mastitis, ketosis and urea. In the following a brief description is given of the reproduction model, and models that estimates the risk of mastitis and ketosis in individual cows.

Reproduction: The biological model developed had the aim to predict reproductive state on the basis of in-line milk progesterone measures (Friggens and Chagunda, 2005). However, a number of additional inputs were incorporated to make use of other

known effectors of reproductive performance that are not reflected in progesterone levels. These are: days from calving, breed, parity, signs of behavioural oestrus, insemination dates, pregnancy determinations, energy status, body fat status, milk urea content and reproductive disorders associated with calving. The model is designed to run each time a new trigger input (progesterone, behavioural oestrus, inseminations, pregnancy determinations) occurs using the current and previous values. The model can be used in the absence of the additional inputs. The milk progesterone values are smoothed using an extended Kalman filter before being processed in the biological component of the model. The model predicts the reproductive status of the cow, which can be one of three states: postpartum anoestrus, oestrus cycling, and potentially pregnant. The other model outputs are all reproductive status specific with the exception of days to next sample (DNS). DNS is designed to feedback to the sampling system so that the frequency of milk sampling (i.e. progesterone measurement) can be varied according to the predicted likelihood of a future reproductive event, such as onset of oestrus cycling. The other model outputs are: risk of prolonged postpartum anoestrus, risk and type of ovarian cyst, onset of oestrus, likelihood of a potential insemination succeeding, and likelihood of being pregnant (following oestrus). The model was evaluated using three simulated datasets consisting of a time-series of progesterone and it was shown that it has the potential to provide the basis for a useful reproductive management tool. Further testing on commercial farms is ongoing.

Mastitis: For early detection of mastitis the enzyme L-lactate dehydrogenase (LDH) has been chosen (Larsen, 2005) which in mastitis cows is highly correlated with cell count (Chagunda et al., 2006b). A dynamic deterministic biological model was developed that generates, for a given cow on a given day, a value for her risk (value between 0 and 1, 0 = no risk; 1 = full blown mastitis) of having mastitis (Chagunda et al., 2006a). The model combines real-time LDH measured in milk with additional factors that are other known risk factors of mastitis but that are not reflected in the indicator. The additional factors incorporated in the model are days from calving, breed, parity, milk yield, udder characteristics, other disease records, electrical conductivity, and herd characteristics. The model is designed to run each time a new LDH value is recorded and can run in the absence of the additional factors. Electrical conductivity measurements and disease records, where available, also trigger the model to run. As an input, milk LDH activity values ($\mu\text{mol}/\text{min per L}$) are multiplied by milk yield (L) to produce the amount of LDH ($\mu\text{mol}/\text{min}$) and are then smoothed using an extended Kalman filter before being processed by the biological model. The output comprises a risk of acute mastitis and a relative degree of chronic mastitis. The model also produces a days-to-next sample value that allows sampling frequency to be either increased or reduced depending on the risk of mastitis. The model functionality was investigated using simulated data, and real-farm data of naturally occurring mastitis were then used to validate the model. The results demonstrated that the model is robust to sampling frequency and random noise in the LDH measurements. It was able to detect mastitis reasonably well: Using a threshold mastitis risk of 0.7, sensitivity for detecting clinical mastitis was 82%. Specificity, that is, the ability to avoid misclassifying healthy observations as mastitis, was 99%. Recent test results indicate

that the model is able to detect mastitis cases up to 4 days before the cow would normally be treated. Thus, the model allows early treatment.

Ketosis: Despite the incidence of clinical ketosis being generally low, sub-clinical ketosis is important as it occurs more frequently and may increase the risk of other diseases, involuntary culling and impair production and reproduction (Ingvarlsen, 2006). We have described the rationale, structure, and functionality of a biological model to predict risk of ketosis in individual cows using in-line measurements of the ketone body β -hydroxybutyrate (BOHB) in milk (Nielsen et al., 2005). The model also uses acceleration in milk yield, body fatness at calving, diseases in current lactation, and incidences of ketosis in earlier lactations as additional risk factors for ketosis. However, the model is designed to function on the basis of milk BOHB in the absence of other data. Values of milk BOHB are smoothed before these are used in calculations in the biological part of the model. The model is designed to be updated each time a new BOHB measurement or a disease occurrence is available and then uses previous and current data. Outputs of the model are the risk of ketosis (value between 0 and 1, where 0 = no risk and 1 = clinical ketosis) and how many days until the next milk sample should be taken and analyzed for BOHB. Test examples from cows for which BOHB has been measured extensively were used to show the functionality of the model. The model performed equally well when reductions in sampling frequency were applied, and it was also relatively robust to the addition of up to ± 2 residual SD of random noise in the BOHB values. This model has the potential to provide the basis for a useful disease monitoring and management tool. However, thorough validation and testing of the model under a variety of on-farm situations remains to be done.

Challenges in management and prevention of individual animals

A large number of indicators has been suggested in e.g. Figure 1 and in the literature (Hamann and Kromker, 1997; Mottram et al., 2002), as have automated sampling (Godden et al., 2002) and measurement of components in milk (Delwiche et al., 2001; Pemberton et al., 2001; Mottram et al., 2007) have been suggested and developed. These systems have so far generally been based on just one analyte. The prototype for which we have briefly described the underlying models are clearly unique in the sense that it concurrently can give a real-time status of mastitis, reproduction and ketosis in individual dairy cows. The fact that the status is given immediately after milking makes it possible for the farmer to conduct proactive management and prevention.

The degree of proactive management that can be obtained from such a system depends on the degree of biological understanding and technical and sensor capability. For well defined activities, e.g. oestrus detection, the opportunities are obvious and already solved in the Herd Navigator system. Here information from the Herd Navigator attention list (a list of cows that has a high risk for heat, reproductive problems, mastitis or ketosis) together with other observations and Standard Operation Procedures gives suggestions to cows that needs special attention, insemination or treatment.

We have previously hypothesised that disease risk could be reduced by minimizing the physiological imbalance in cows, and that such a reduction at the same time would improve the production and reproduction of the cows (Ingvarsten et al., 2003; Ingvarsten, 2006). Ingvarsten (2006) defined cows in physiological imbalance as cows whose parameters (reflecting the function of the digestive tract, metabolic state, and immune state) deviate from the normal, and who consequently have an increased risk of developing production diseases (clinical or subclinical) and reduced production and/or reproduction. Put in another way it is situations where the regulatory mechanisms are insufficient for the animals to function optimally leading to a high risk of disease. The hypothesis has been based on the finding that the between cow variation in physiological parameters are very large particularly when compared to the average changes that will occur in these parameters when e.g. the nutrition of the animals are altered on a group basis.

And so what? Let us take the example of ketosis that generally develops over a period of time. The large between-cow variation in e.g. BOHB calls for proactive prevention of ketosis by, for instance, adjusting the feed composition of “high risk cows”. Their diet should be altered to bring down the physiological imbalance and thereby the risk of the subclinical state turns clinical. We now have the capability to automatically determine the risk of ketosis but how do we get to the position where we automatically change nutrient supply to the animals to prevent the imbalance and thereby the risk of disease and suboptimal production and reproduction? In our opinion we lack biological understanding, and particularly quantitative understanding, of individual animal response to changes in feeding and nutrient supply. Potentially we also lack more specific biomarkers and sensors to take on the challenge of building future automatic and proactive feeding strategies that will keep the frequency of cows in imbalance low and thereby prevent disease and concurrently optimize reproduction and production. The major task will be to better understand the biological basis of the imbalance measured and thus to be able to predict individual animal responses to changes in e.g. nutrient supply or management to overcome the imbalance (Ingvarsten, 2006; Friggens and Newbold, 2007).

Some insight into disease aetiology has been gained through the research up till now, but the knowledge of the disease aetiology and pathophysiology of many diseases remains scarce. A continuous effort in this field is very important in order to uncover the multifactorial complexity of the diseases and identify indicators that can be used in early diagnosis and identifying the causal factors of production diseases, i.e. in the subclinical phase.

The prevention of certain diseases is problematic as it can be difficult to diagnose the diseases by traditional means, in particular in the subclinical phase. With our prototype models we have shown that this issue can be resolved to the advantage of the farm manager by considering the underlying “degree of infection” to be a continuum rather than a binary classification. The degree of infection can be indexed by the relevant physiological indicator measured in blood, milk or urine (e.g. hyperketonaemia,

hypocalcaemia, hypomagnesaemia), although some subclinical conditions are more difficult to diagnose in practice (e.g. rumen acidosis and fatty liver). In these situations other non-invasive methods will probably improve the insight into the extent of fatty liver and furthermore render it possible to recognize and document risk factors. It is vital to recognize the problems at an early stage before the disease, e.g. rumen acidosis, causes permanent damage and manifests itself clinically.

Achieving this type of individually targeted early identification of risk cows together with timely intervention is greatly facilitated by accepting the “degree of infection” concept. This together with the frequent measurement technology becoming available will allow Status-oriented strategies based on “risk management”.

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10.6 APPLICATION OF POOLED SAMPLE METABOLIC PROFILES AS A HERD SCREENING TOOL

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Methodologies used in metabolic profiling have ranged from mean analysis of multiple analytes to proportional analysis of single analytes. Periparturient metabolic disease is a result of the cow's inability to maintain coordinated metabolism between lipid, glucose and amino acids. Use of pooled samples was evaluated as a method to collect usable information on herd metabolic status encompassing multiple parameters without the high cost of individual sampling. Aim of this study was to determine if diagnostic interpretation guidelines can be established for pooled metabolic profile samples. Blood samples were collected from 113 cows on 15 different farms for three defined time periods relative to calving (Early Dry, Close-up Dry, Fresh). Pooled samples (n=48, 16 per period) containing between 5 and 12 individuals were randomly composited by blending equal volumes (0.1 to 0.5 ml) of individual serum. Metabolic profiles were performed on individual samples and pooled sample of individuals and included urea nitrogen (SUN), glucose (Glu), albumin (Alb), aspartate aminotransferase (AST), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), total cholesterol (Chol), β -hydroxybutyrate (BHB), and nonesterified fatty acids (NEFA). A population of healthy cows (no disease events; n=49) was used to define reference analyte values (median, standard deviation [SD]). For each analyte measured, arithmetic mean of individuals or pooled sample value were subtracted from a reference median value and divided by the analyte's SD. Laboratory reference criteria were used to determine individual abnormal values. Regression and ANOVA were used to relate number of SD the mean individual and pooled sample values deviated from reference population median to percent of abnormal values within test samples. Deviation of sample arithmetic mean or pooled value from reference population median was linearly related to percent abnormal values within a pool. Pool size did not seem to influence this relationship. All models within Fresh period were significant ($r^2=.26$ to $.81$, $P<.04$ - $.0001$), except Cl. Close-up period models were significant ($r^2=.36$ to $.79$, $P<.01$ - $.0001$) for Alb, Chol, Cl, Mg, Na, K and NEFA. Across all analytes, mean or pooled values deviated $.26$ SD from reference population median for every 10% abnormal values within a pooled sample. This relationship was analyte-specific and ranged from $.11$ (Glu) to $.6$ (BHB). Multiples of these regression slopes can be used to generate analyte concentration criteria for interpreting pooled samples. Pooled samples must be interpreted relative to deviation from an expected population mean. The greater a pooled sample deviates from the expected population mean, the greater the abnormal values within the sample.

10.7 ASSOCIATION OF PREPARTUM RUMP FAT THICKNESS AND NON-ESTERIFIED FATTY ACIDS WITH SUBCLINICAL KETOSIS IN HOLSTEIN DAIRY COWS

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Introduction: Excessive adipose mass and/or a high rate of adipose mobilization have been associated positively with peripartum disease risk in dairy cows. Serum NEFA concentrations can be used as estimators of adipose mobilization rate, but are very dynamic and subject to short-term fluctuations. Adipose mass may be estimated by subcutaneous fat thickness, which is much more stable than are serum NEFA. The objective of this study was to determine the association of subcutaneous rump fat thickness (RFT) and serum NEFA concentrations with the occurrence of subclinical ketosis in dairy cows.

Materials and methods: Forty cows in the last month of gestation were selected randomly from a herd of 3000 Holsteins. At weekly intervals until calving ultrasound images of rump fat (for RFT) and coccygeal-vessel blood samples (for NEFA) were collected. After calving, blood samples were taken weekly for four weeks for beta-hydroxybutyric acid (BHB). Cows were retrospectively classified as sub clinically ketotic (SK) or normal(N) based on serum BHB above or below 1000 μ mol/L, respectively. The effects of outcome (SK vs N), time, and time x outcome interactions on RFT and serum NEFA were assessed by repeated-measures ANOVA. The effect of RFT and serum NEFA on post-partum SK risk was assessed at each pre-partum sampling time by multivariable logistic regression. The initial independent variables in the model were RFT (mm), NEFA (mmol/L), parity (1,>1), cohort group (1,2,3,4), days to calving, calving difficulty (low, high), and all 2-way interactions. Variables entered the model if $p \leq .25$ and stayed in the model if $p \leq .1$. SK was the dependent variable.

Results: Of the 40 cows, 21 developed SK. RFT means for SK cows were greater than for N cows at all 3 prepartum sampling times ($P < .05$). Serum NEFA in SK cows tended to be higher than N cows ($P = .1$), and was significantly higher ($P < .05$) at one week pre-partum. Pre-partum sampling time significantly affected serum NEFA ($P < .003$). RFT yielded the only significant odds ratio, with respective values of 1.24 ($P < .05$), 1.25 ($P < .02$), and 1.52 ($P < .01$) at -3, -2, and -1 weeks relative to calving. Odds ratios are based on an incremental increase in RFT of 1mm. These results demonstrated that larger RFT in the last three weeks of gestation is associated positively with the risk for SK: this association is greater as time to gestation approaches.

Conclusions: Measurement of RFT during last three weeks before calving may be a quick and reliable method of estimating SK risk in Holstein cows. Furthermore, these results suggest that RFT may be a more sensitive estimator of SK risk than serum NEFA, especially at more than one week prepartum. Additional studies in multiple herds are needed to substantiate these results.

10.8 OBSERVATIONS ON CHRONIC ANEMIA IN CROSSBRED CATTLE OF PUNJAB STATE IN INDIA

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In order to understand the etiology of chronic anemia in crossbred cattle (Holstein Friesian X Sahiwal) from rural dairy units of Punjab state in India, a total of 180 cows of 2 to 9 years of age were sampled. Cows having Hb 8gm/dl or below were categorized as anemic. The anemic cows were found to be clinically healthy, had no history of fever, anorexia, any evidence of external blood loss, discoloration of urine and/or jaundice. Blood samples from jugular vein were collected in sodiumEDTA for estimation of hemoglobin, total erythrocyte count, packed cell volume and for the presence of protozoa/rickettsial infection. Heparinized blood samples were collected to determine iron metabolism analytes (total plasma iron, total iron binding capacity, transferrin saturation). Fresh faecal samples were examined for ova/cyst and acid-fast bacilli (AFB). Urine samples were examined for microscopic hematuria. Tick load was estimated by counting the number of ticks in elbow and groin regions of each cow. Thirty per cent of these cows were anemic and the highest prevalence (36.7 per cent) was in 2 to 4 years of age. Anemia was normocytic, normochromic and of moderate degree (Hb- 4.30 to 7.90 g/dl). Wright and New Methylene blue stained blood smears did not show any evidence of bone marrow regeneration. Tick load was three times higher in anemic cows. However, regression analysis revealed that it accounted only for 6.22 per cent fall in the Hb. Sub-clinical (1-4 egg/slide) gastrointestinal parasitism (strongyle and amphistomes) was observed in 9.3 per cent of anemic and 3.2 per cent of non-anemic cows. A mild (3-7 per cent of RBC) but asymptomatic infection of *Theileria annulata* was detected in two anemic and three non-anemic cows. None of these cows, however, had present or past history of clinical theileriosis. Anemia was concomitant in 84.6 per cent of AFB shedder cows. The prevalence of anemia was also three times higher in herds with one or more AFB shedder cows as compared to the herds with no AFB shedder cow. Recurrent microscopic hematuria was detected in 3.7 per cent cows. However, iron homeostasis parameters were unaffected in these anemic cows. The present study suggested that tick infestation was a contributory etiological factor of chronic anemia and there was an association between chronic anemia and faecal shedding of AFB.

10.9 DEVELOPMENT OF FORAGE QUALITY AND DAIRY HERD HEALTH IN SAXONY DURING THE LAST 15 YEARS

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The objective of this study is the development of forage quality (grass and corn silage) and dairy herd health in Saxony during the last 15 years. The milk yield has remarkably improved from nearly 4,000 kg per cow to 8,600 kg per cow between 1989 and 2006. In the same period the fat content decreases from 4.49% (peak in 1993) to 4.10% (2006). The protein content slightly decreases from 3.49% (peak in 1995) to 3.42% (2006). Today 36 (of 1,038) farms produce of more than 10,000 kg per cow and 35 dairy cows have a life production of more than 100,000 kg milk. The development in 2007 shows the same tendency. Although the milk yield has considerably increased during the last 15 years, the milk urea content has remained stable (between 230 and 260 mg/l). This shows that farmers do not generally feed more protein with higher milk production. Unfortunately, while the content of somatic cells (indicator of udder health) has decreased from 403,000/ml to 230,000/ml between 1992 and 2000 it has risen again during the last six years (up to 283,000/ml). The development of fertility is not satisfactory. The herd replacement has increased from 18% (1992) to 39% (2006). The calving interval has risen from 371 days to 412 days. The age of first calving has dropped down from 30.4 months (1994) to 26.7 months (2006). The age of culling has decreased from 5.7 years (1994) to 4.9 years (2006). The average useful life was only 31.6 month or 2.4 lactations per cow in 2006. In contrast to the general development of herd health in Saxony, the top 50 dairy herds (with a milk production level between 9,000 kg and 12,000 kg) show some better health, fertility and reproduction data than the average. This demonstrates that there is no necessary tight junction between higher milk production and health worsening. The average energy content and the content of nutrients in grass silages show no improvement between 1998 and 2006 and no large variation. The development of successful preservations is remarkable: In 1998 only 78% of all samples show good or excellent preservation success. In 2006 more than 90% of all samples show good or excellent preservation success. The medium energy concentration and the content of nutrients in corn silages show the same small variation over the last years. The preservation success of corn silages normally is on a very high level (> 96% good and excellent). No strong correlation could be found between general forage quality and herd health. We argue, based on our experience, that other factors such as ration calculation, feeding system, herd management, breeding and housing system are have a much stronger impact on herd health.

10.10 STRONG ION DIFFERENCE THEORY EXPLAINS THE RELATIONSHIP BETWEEN URINE PH AND NET BASE EXCRETION IN CATTLE

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Introduction

A clinically valuable insight into acid-base balance in healthy cattle is provided by measuring urine net base excretion (NBE). This is because the kidney plays a central role in acid-base homeostasis by adjusting urine electrolyte excretion in order to maintain constant blood pH. Measurement of urinary NBE provides a sensitive and clinically useful method for evaluating acid-base balance in cattle because it provides an estimate of endogenous acid production and the magnitude of dietary acidification when a low dietary cation-anion difference (DCAD) diet is fed. Ingestion of a low DCAD diet induces a strong ion (metabolic) acidosis (Constable, 1999), as indicated by a decreased plasma strong ion difference and bicarbonate concentration, a decreased urinary pH, and decreased NBE (Tucker et al., 1988; Vagnoni and Oetzel, 1998). The term net acid excretion (NAE) is commonly used in studies of renal physiology in humans, other omnivores, and carnivores where urine pH is typically acidic when compared to normal plasma pH (7.40). The term net base excretion (NBE) is more appropriate in cattle and other herbivores because urine pH is usually alkaline, with NBE providing an estimate of endogenous base production. It should be recognized that $NBE = -NAE$, with both being measured in mEq/l.

Measurement of NBE is time consuming and difficult to perform on the farm. In comparison, measurement of urine pH provides a simpler “cowside” evaluation of acid-base balance. Urine NBE is the preferred technique in northern and eastern Europe for evaluating systemic acid-base balance in cattle, whereas urine pH is widely used in North America to monitor the effectiveness of feeding low DCAD diets in the late dry period in order to decrease the incidence and severity of hypocalcemia. Three important questions need to be answered: 1) what is the physiological background for the NBE equation?; 2) what are the major determinants of urinary NBE and urine pH in cattle?; and 3) can urine pH be used as a proxy for NBE in cattle, and if so, under what conditions?

Measurement of urinary net base excretion in cattle

A number of techniques have been used to measure urinary NBE in cattle. The most commonly used method (Jørgensen-Kutas) employs titration to a standardized endpoint where temperature = 37°C, Pco₂ = 0 mm Hg, and pH = 7.40 (normal plasma pH), and involves pH measurement of urine, acidification to pH < 4.0, heating, cooling, and alkalinization back to pH = 7.40 in order to determine measured titratable acidity (TA_{measured}) (Constable et al, 2007). Titratable acidity is defined as the number of mEq of NaOH that must be added to a bicarbonate free urine sample in order to increase the pH to 7.40. Formaldehyde is then added to the urine sample and the sample titrated with NaOH back to pH = 7.40 in order to determine the ammonium concentration ([NH₄⁺]) (Jørgensen, 1957; Kutas, 1965). Net base excretion is calculated as:

$$(1) \quad \text{NBE} = - (\text{TA}_{\text{measured}} + [\text{NH}_4^+]).$$

Accurate results are obtained with the technique if urine is anaerobically stored at -20°C for up to 30 days and thawed at room temperature for 2 hours (Chan, 1972), or anaerobically stored at 4°C for < 3 days (Bender and Staufenbiel, 2003). The specific steps of the most commonly used method for urine from cattle (the Jørgensen-Kutas method) are described below (Constable et al, 2007).

1) Obtain a freshly voided urine sample and immediately exclude air and measure urine pH. Exclusion of air is important because urine Pco₂ decreases by 17 mm Hg within 5 minutes when urine is kept in an open container (Gonzalez et al., 2004). Immediate pH measurement is preferred so that the temperature approximates 37°C, there is minimal loss of CO₂ to the atmosphere, and there is minimal microbial metabolism of urea to ammonium (Bender and Staufenbiel 2003).

2) The urine sample is well mixed and acidified to pH < 4.0 in an open glass beaker by adding a suitable volume of 1 M HCl. Mixing is required to resuspend the sediment in the urine sample (Bender and Staufenbiel, 2003). Acidification results in the conversion of carbonate to bicarbonate, and bicarbonate to CO₂. Acidification also dilutes the urine sample and assists in dissolving any phosphate salts in the sediment.

3) Heat the acidified urine sample to a slow boil for at least 2 minutes. Boiling increases the rate of CO₃²⁻ and HCO₃⁻ conversion to CO₂, facilitates mixing, and permits escape of CO₂ from the urine sample into the atmosphere.

4) Cool the urine sample back to 37°C in order to ensure that a standardized temperature is obtained for back titration. A standard temperature is required because pH is temperature dependent.

5) Back titrate the urine sample to a pH of 7.40 at 37°C by adding sequential volumes of 0.1 M NaOH. Calcium phosphate may precipitate during titration; however, the sequential addition of acid and alkali appears to dilute the urine sample sufficiently so that precipitation is not observed (Chan, 1972).

6) The measured titratable acidity (TA_{measured}) is the difference between the moles of HCl and NaOH added to the urine sample when $\text{pH} = 7.40$.

7) Add 8% formaldehyde (HCHO), which reacts with the ammonium ion (NH_4^+) to release H^+ and lower the pH below 7.40, such that: $\text{HCHO} + \text{NH}_4^+ \leftrightarrow \text{HC(OH)NH}_3 + \text{H}^+$. Back titrate the sample to a pH of 7.40 at 37°C by adding 0.1 M NaOH. The number of moles of NH_4^+ present equals the number of moles of NaOH added (Jørgensen, 1957).

8) Calculate NBE using the following equation: $\text{NBE} = -(\text{TA}_{\text{measured}} + \text{NH}_4^+)$.

There are variations on the commonly used Jørgensen-Kutas method. A simpler version adds 8% formaldehyde before acidification to $\text{pH} < 4$; this means that $\text{NBE} = -\text{TA}_{\text{measured}}$ and that the concentration of bicarbonate and ammonium are not specifically measured. A more complicated and infrequently used method involves measurement of urine bicarbonate concentration using one of 2 methods. In the first method, urine Pco_2 is measured using an anaerobic sample of urine and a blood gas analyzer (urine pH is usually outside the range of the instrument). The urine bicarbonate concentration is then calculated from the measured pH and Pco_2 values and known values for the apparent dissociation constant (pK_1') and the solubility of CO_2 (S) using the Henderson-Hasselbalch equation (Gonzalez et al., 2004). In the second method, urine is acidified to $\text{pH} < 4$, and then back titrated to the original pH before being titrated to $\text{pH} = 7.40$; titration to the original pH provides an estimate of the urine bicarbonate concentration (Lin and Chan, 1973).

The value for $\text{TA}_{\text{measured}}$ obtained in equation (1) is actually the value of the difference between actual titratable acidity (TA) and the bicarbonate concentration ($[\text{HCO}_3^-]$), such that $\text{TA}_{\text{measured}} = \text{TA} - [\text{HCO}_3^-]$ (Chan, 1972). A slightly more complicated equation, equation (2), is preferred whenever urine $[\text{NH}_4^+]$ and $[\text{HCO}_3^-]$ have been measured:

$$(2) \quad \text{NBE} = -(\text{TA} + [\text{NH}_4^+] - [\text{HCO}_3^-]).$$

The Jørgensen-Kutas technique has been applied to studies in healthy adult cattle (Kutas, 1967; Fürll et al., 1994; Vagnoni and Oetzel, 1998; Bender and Staufenbiel, 2003; Enemark et al., 2004) and sick cattle (Buscher and Klee, 1993). Net base excretion for healthy pasture or silage fed cattle is usually +90 to +210 mEq/l (Kutas, 1967; Fürll et al., 1994; Vagnoni and Oetzel, 1998; Bender and Staufenbiel, 2003; Enemark et al., 2004). Net base excretion for grain fed cattle with subclinical rumen acidosis is -100 mEq/l (Lachmann et al., 1986).

Strong ion difference theory

Strong ion difference theory offers a novel insight into the pathophysiology of mixed acid-base disorders and is mechanistic. The strong ion approach to acid-base balance reduces the chemical reactions in plasma to that of *simple ions* in solution (Constable, 1997). This assumption can be made because the quantitatively important plasma cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) and anions (Cl^- , HCO_3^- , protein, lactate, sulfate, ketoacids) bind each other in a salt-like manner. Plasma ions (such as Cu^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , Co^{2+} , and Mn^{2+}) that enter into oxidation-reduction reactions, complex ion interactions, and precipitation reactions, are not categorized as simple ions but are assumed to be quantitatively unimportant in determining plasma pH, primarily because their plasma concentrations are low.

Simple ions in plasma can be differentiated into 2 main types, *non-buffer ions* (strong ions or strong electrolytes) and *buffer ions* (Constable, 1997). Strong ions are considered to be fully dissociated at physiologic pH and therefore exert no buffering effect. Strong ions do, however, exert an electrical effect because the sum of completely dissociated cations does not equal the sum of completely dissociated anions. Stewart termed this difference the *strong ion difference* (SID) (Stewart 1983). Because strong ions do not participate in chemical reactions in plasma at physiologic pH, they can be regarded as a collective unit of charge (SID), with units of mEq/L.

The Bronsted-Lowry theory defines an acid as any substance that can donate protons. The dissociation reaction for a weak acid (HA) - conjugate base (A^-) pair is:



An apparent weak acid dissociation constant (K_a) can be calculated for a non-volatile buffer when the dissociation reaction for the weak acid – conjugate base pair reaches equilibrium by applying Henderson's law of mass action:

$$(4) \quad K_a = a_{\text{H}^+}[\text{A}^-]/[\text{HA}]$$

where a_{H^+} represents the hydrogen ion activity, and [HA] and $[\text{A}^-]$ represent the plasma concentrations of weak acid and conjugate base of the buffer, respectively.

As mentioned previously, simple ions in plasma are categorized as *non-buffer ions* (strong ions) or *buffer ions*. In turn, buffer ions are categorized as *volatile* (bicarbonate) or *non-volatile* (non-bicarbonate) buffer ions. The $\text{p}K_a$ for weak acid must lie within the range of $\text{pH} \pm 1.0$ in order for the weak acid to act as a buffer and produce a non-volatile buffer ion in a closed system. On this basis, non-volatile buffers in plasma possess a $\text{p}K_a$ between 6.4 and 8.4. This requirement means that non-volatile buffer ions derived from weak acids possessing a $\text{p}K_a < 6.4$ or > 8.4 act as strong (non-buffer) ions at physiologic plasma pH (7.4).

An additional assumption in strong ion difference theory is that the acid-conjugate base pair HA and A⁻ in equation (3) do not take part in plasma reactions that result in the net destruction or creation of HA or A⁻. The sum of [HA] and [A⁻] (called A_{TOT}) therefore remains constant through conservation of mass (Stewart 1983), such that:

$$(5) \quad [A_{TOT}] = [HA] + [A^-]$$

The final assumption in the simplified strong ion model is that plasma ions act as either *strong ions, volatile buffer ions* (HCO₃⁻), or *non-volatile buffer ions* (A⁻). In other words, plasma contains 3 types of charged entities; SID, HCO₃⁻, and A⁻. The requirement for electroneutrality dictates that at all times the SID equals the sum of bicarbonate buffer ion activity (HCO₃⁻) and non-volatile buffer ion activity (A⁻), such that (Constable, 1997):

$$(6) \quad SID - HCO_3^- - A^- = 0$$

By combining 4 equations, consisting of equations for conservation of mass, conservation of charge, and 2 apparent dissociation equations (K_1' for H₂CO₃ and K_a for plasma weak acids), a logarithmic equation relating plasma pH to 3 independent variables (P_{CO_2} , SID, A_{TOT}) and 3 constants (K_a , K_1' , S) was developed (Constable, 1997).

$$(7) \quad pH = \log \frac{2SID}{K_1' SP_{CO_2} + K_a A_{TOT} - K_a SID + \sqrt{\{(K_1' SP_{CO_2} + K_a SID + K_a A_{TOT})^2 - 4K_a^2 SIDA_{TOT}\}}}$$

A number of clinically important ramifications arise from the simplified strong ion equation (equation 7). Because acid-base derangements result from changes in P_{CO_2} , SID, and concentrations of individual non-volatile plasma buffers (albumin, globulins, phosphate), 6 primary acid-base disturbances can be distinguished, instead of the 4 primary acid-base disturbances (respiratory acidosis, respiratory alkalosis, metabolic acidosis, metabolic alkalosis) differentiated when using the traditional Henderson-Hasselbalch approach (Constable, 1997). Acidemia results from an increase in P_{CO_2} and non-volatile buffer concentration, or from a decrease in SID. Alkalemia results from a decrease in P_{CO_2} and non-volatile buffer concentration, or from an increase in SID. Strong ion difference theory has been applied to acid-base studies involving cows (Constable, 2002), calves (Constable et al, 2005), humans (Constable, 2001; Staempfli and Constable, 2003), horses (Constable, 1997; Staempfli et al 1999), dogs (Constable and Staempfli, 2005), cats (McCullough and Constable, 2003), and pigeons (Staempfli et al, 2006).

Application of strong ion difference theory to bovine urine

Bovine urine contains 6 strong ions (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻) and 4 quantitatively important buffers over the physiologic range for urine pH (4.7 to 8.7); two *volatile buffers* (bicarbonate, pKa = 6.1; ammonium, pKa ≈ 9.0) that are effective over a wide range of pH and two *non-volatile buffers* (monobasic phosphate, pKa₂ = 6.8; creatinine, pKa₁ = 4.9, pKa₂ = 9.3) that are effective whenever pH = pKa ± 1.0.

Application of strong ion difference theory (Constable, 1997) provides the following general electroneutrality equation for bovine urine involving urinary strong ion difference (SID = difference between strong cation and strong anion concentrations), urine pH, and the concentration of ammonium ($[\text{NH}_4^+]$), bicarbonate ($[\text{HCO}_3^-]$), creatinine ([creatinine]), phosphate ([P]), and organic buffer anions (OBA) in urine (Constable 2007), whereby:

$$(8) \text{SID} + [\text{NH}_4^+] - [\text{HCO}_3^-] + \{[\text{creatinine}](10^{4.9-\text{pH}} - 10^{\text{pH}-9.3}) - [\text{P}]/(1 + 10^{6.8-\text{pH}}) - \text{OBA}^-\} = 0.$$

When urine is titrated to pH = 7.40 in the Jørgensen-Kutas method, SID = NBE (net base excretion) and titratable acidity (TA) is the sum of the last 3 terms. Substitution for SID into the general electroneutrality equation for urine (equation (8) and algebraic rearrangement produces equation (2). *In other words, the net base excretion equation for bovine urine (equation 2) can be derived by applying strong ion difference theory to urine.*

When a urine sample is appropriately collected and stored, the general electroneutrality equation can be simplified for clinical use in cattle when urine pH is between 5.9 and 8.0, such that (Constable et al, 2007):

$$(9) \quad \text{pH} = 6.12 + \log_{10}([\text{Na}^+] + [\text{K}^+] + [\text{Mg}^{2+}] + [\text{NH}_4^+] - [\text{Cl}^-] - [\text{SO}_4^{2-}])$$

Urine pH in cattle is therefore primarily dependent on urinary SID, which is the difference between the urinary strong cation (K^+ , Na^+ , Mg^{2+} , Ca^{2+}) and strong anion (Cl^- , SO_4^{2-}) concentration. Pasture based diets are rich in potassium, which is excreted in high concentrations in bovine urine. High urine potassium concentrations secondary to high potassium intakes are therefore the main reason for alkaline urine in pasture fed cattle. Ingestion of acidogenic salts (such as HCl or H_2SO_4) results in acidic urine because of an increase in urine strong anion concentration (Cl^- or SO_4^{2-}) without a concomitant increase in urine strong cation concentration. The effect of ingestion of HCl or H_2SO_4 is therefore a decrease in urine SID and aciduria. Ingestion of ammonium chloride acidifies the urine because the gastrointestinal tract absorbs chloride to a greater extent than the ammonium ion. This is because at equilibrium urinary chloride and ammonium concentrations reflect their absorption efficiency; differential absorption of NH_4Cl will result in decreased urine pH (Constable et al, 2007).

Conclusion

Urine NBE provides the most accurate insight into acid-base homeostasis in healthy cattle. However, when urine pH is between 6.3 and 7.6, urine pH provides a clinically useful insight into acid-base homeostasis. The urinary potassium concentration has a profound effect on urine pH, with high urine potassium concentration producing an alkaline urine, and low urine potassium concentration producing acidic urine. Because

urine potassium concentration decreases when potassium intake (and therefore dry matter intake) decreases, urine pH provides a clinically useful and practical means for assessing dry matter intake and whole body potassium status in sick cattle (Constable et al, 2007). Cattle with low urine pH that are not being fed an acidogenic diet should be assumed to have decreased potassium intake. Such cattle should be orally supplemented with potassium.

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10.11 ACUTE PHASE PROTEINS AS MONITORING TOOLS IN FARM ANIMALS

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Introduction

The acute phase proteins (APP) are blood proteins the measurement of which provides a means to assess the innate immune system's response to disease (Ceron et al., 2005; Murata et al., 2004; Petersen et al., 2004). By definition, the APP serum concentration increases or decreases by > 25% in response to inflammation, infection and trauma. They can be used as quantitative markers for prognosis and monitoring responses to therapy, for general health screening as well as for diagnosis of disease. The APP are highly sensitive for the presence of pathological lesions although they have a low specificity for a particular disease as they are elevated in numerous conditions.

However, there are major differences between species in the APP response in disease (Table 1). In any one species positive APP have been found that have either major, moderate or minor responses. A major APP has a low concentration in the serum of healthy animals, but with the concentration increasing over 100 or 1000 fold on stimulation, reaching a peak 24-48 hours after the insult and falling rapidly during recovery. A moderate APP is present in the blood of healthy animals but on stimulation the concentration will increase 5-10 fold, reach a peak concentration 2-3 days after stimulation and decrease more slowly than the major APP. A minor APP shows a gradual increase of 50-100% of the resting level. Negative APP have also been identified, which fall in concentration during the response, though apart from the hypoalbuminemia of infection and inflammation the measurement of these proteins has not yet been fully exploited by clinical pathology laboratories.

Table 1 Acute Phase Protein: Major and Moderate Responders in Various Animal Species

Species	Major APP	Moderate APP
Cat	SAA	AGP, Hp
Dog	CRP, SAA	Hp, AGP,
Horse	SAA	Hp,
Cow	Hp, SAA	AGP
Pig	CRP, MAP, SAA	Hp,

Acute Phase Proteins in ruminants

Perhaps the most significant species difference in the acute phase response in ruminants is that haptoglobin (Hp) is a major APP whereas in many other species it is a moderate APP. In healthy cattle the serum concentration is below 20 mg/L but it can increase in concentration to over 2 g/L within a couple of days of infection. In cattle, Hp is an effective marker (Table 2) for the presence, severity and recovery of animals with mastitis, enteritis, peritonitis, pneumonia endocarditis, endometritis and for monitoring processes such as tail docking and surgical castration (Murata et al., 2004; Petersen et al., 2004). Elevations have also been reported in cows with fatty liver syndrome, at parturition, during starvation and following the stress of road transport (Kato and Nakagawa, 1999; Nakagawa et al., 1997; Uchida et al., 1993).

In cattle SAA has been identified as a marker of inflammation being elevated more in acute rather than chronic conditions (Horadagoda et al., 1999). It was raised also by experimental infection with *Mannheimia haemolytica*, with bovine respiratory syncytial virus and in experimental and natural cases of mastitis (Eckersall et al., 2001; Gronlund et al., 2003; Heegaard et al., 2000; Horadagoda et al., 1994).

The mammary isoform of SAA (M-SAA3) is secreted in milk from mammary glands of dairy cows with mastitis (Eckersall et al., 2001; Jacobsen et al., 2005; Nielsen et al., 2004; Winter et al., 2003) is also found in milk from ewes with this condition (Winter et al., 2003). The discovery that this isoform of SAA along with Hp is synthesised in the mammary epithelia during mastitis suggests a potential role as a biomarker for this condition.

Table 2 Bovine diseases where an acute phase response has been described (Petersen et al., 2004).

Acute Phase Protein	Disease/Condition
Haptoglobin	<i>Mannheimia haemolytica</i> <i>Pastuerella multocida</i> Bovine viral diarrhea virus Bovine respiratory syncytial virus Foot and mouth disease virus Mastitis Clinical respiratory tract disease Castration Metritis Uterine bacterial contamination Hepatic lipidosis
SAA	Mastitis <i>Mannheimia haemolytica</i> Bovine viral diarrhea virus Bovine respiratory syncytial virus
AGP	Hepatic abscesses Metritis Mastitis Respiratory tract disease

As the APP are raised in a variety of infectious diseases, a role in ante or post- mortem inspection in assessment of animal health and welfare and as an aid to meat inspection has been suggested. The APP could be used as a screening tool for monitoring the general health and welfare of animals at slaughter the APP could be valuable and could be adapted to assays of the required accuracy, precision and robustness for use in this scenario. Initial studies of the APP at slaughter have given encouraging results. Six fold increases were found in Hp concentration comparing dairy cows with infectious, metabolic or traumatic disease at slaughter to those with only minor lesions (Hirvonen et al., 1997). A more recent study showed a 40 fold increase in Hp and a 7 fold increase in SAA concentration between healthy beef cattle and dairy cattle culled with acute pathological lesions (Tourlomousis et al., 2004).

The APP in other ruminants have not been studied in as much detail as cattle, but it appears that the acute phase response is similar. Study of an experimental model of ovine caseous lymphadenitis, showed that an initial rise in Hp and SAA concentration reached a peak within one week and had returned close to normal within 14 days. This was followed by a lower and more lasting increase in AGP which was still evident 4 weeks after infection. Therefore, although a pathogen may cause a chronic rather than an acute reaction, the APP can still be stimulated. A further recent study has shown that vaccination of lambs causes an acute phase response that reaches a peak within 24-48 h and could have a role to play in the assessment of vaccine efficacy.

Acute phase proteins in pigs

In the pig, as in man and in the dog CRP is a major APP. Serum CRP concentration increased in pigs following aseptic inflammation (Lampreave et al., 1994) and with experimental infection with *Actinobacillus pleuropneumoniae*. In this experimental model plasma levels of CRP correlated with clinical findings and were reduced following antibiotic treatment (Lauritzen et al., 2003). Porcine CRP has also been found to rise in experimental models of *Mycoplasma hyorhinis*, *Toxoplasma gondii*, *Streptococcus suis* and porcine reproductive & respiratory syndrome virus infection. Raised Hp concentrations are also found during the porcine acute phase response. Increases in Hp were found associated with clinical signs of lameness, respiratory disease, diarrhea, tail bite and ear necrosis. At slaughter, increased Hp has been found to be related to the presence of lesions and chronic abnormalities. Experimental or natural infection with *Actinobacillus pleuropneumoniae*, *Mycoplasma hyorhinis*, *Toxoplasma gondii*, *Bordetella bronchiseptica*, *Pasteurella multocida* and Porcine Reproductive and Respiratory Syndrome virus lead to increased Hp concentration in serum (Petersen et al., 2004).

During the acute phase response in pigs a major acute phase protein (pig MAP) can be detected (Lampreave et al., 1994) and has been identified as porcine inter-alpha-trypsin inhibitor heavy chain 4 (Gonzalez-Ramon et al., 2000). Increases in pig MAP have been shown during infection with *Actinobacillus pleuropneumoniae* (Heegaard et al., 1998), in post weaning multisystemic wasting disorder (Segales et al., 2004) and following transport (Saco et al., 2003). This APP was also increased in experimental models of *Mycoplasma hyorhinis*, *Toxoplasma gondii*, *Bordetella bronchiseptica*, *Pasteurella multocida* and Porcine Reproductive and Respiratory Syndrome virus.

In the pig AGP has been the subject of contrasting experience. AGP concentration was shown to be raised in pigs with naturally occurring pneumonia and meningitis (Itoh et al., 1993) but in studies where aseptic inflammation caused an acute phase reaction, the AGP concentration was not significantly affected (Eckersall et al., 1996; Lampreave et al., 1994). Furthermore, an experimental model of porcine reproductive and respiratory syndrome virus showed no increase in serum concentration of AGP while Hp was increased (Asai et al., 1999). Raised levels of AGP have been found in neonatal piglets taking about 20 weeks to fall to adult levels (Itoh et al., 1993) so it is important to interpret AGP levels in the pig with regard to age.

Acute phase proteins in animal production

Measurement of the APP can detect or confirm the presence of infection or pathological lesion but a major role for these analytes could be in monitoring the health status of animals in production. The APP can detect the presence of sub-clinical disease which is the cause of reduced growth rate and lost production. The pro-inflammatory cytokines that stimulate the production of the APP also cause the anorexia/cachexia of associated with infectious and inflammatory conditions and

identification of the presence of sub-clinical disease is a pre-requisite for taking corrective measures. Use of an acute phase index, by combining the results of both positive and negative APP has been suggested as a means to increase the sensitivity of detection of sub-clinical disease (Toussaint et al., 2000).

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www.gla.ac.uk/vet/research/apph/genesandproteins/acutephaselab/.

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10.12 ACUTE PHASE PROTEINS AS MARKERS OF HEALTH STATUS OF PIG HERDS. APPLICATION IN CERTIFICATION PROGRAMS

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Introduction: The pork market is increasingly concerned with the quality concepts of production. The consumers are demanding more control on the quality and safety of the meat they eat, and certifying the whole productive process 'from the farm to the fork' may in a near future not only provide an added value for the final product but also became a must. In this context, acute phase proteins (APP) appears as new interesting parameters for the certification of meat production chains. APP are blood proteins which respond to disease and stress modifying their concentration, and can be used as general markers of health and welfare. The APP assay can identify herds where poor hygiene, lack of surveillance or poor vaccinations responses is leading to immunological stress resulting in a reduction of feed efficiency. **Aim:** The usefulness of the APP Pig Major Acute Phase protein (pig-MAP) and haptoglobin as herd health status markers was evaluated in farms of different health status.

Materials and methods : To determine baseline values, Pig-MAP and haptoglobin concentrations were determined in sera samples from clinically healthy pigs of different ages (4, 8, 12, 16, 20 weeks of age), obtained in 8 commercial farms (10 animals per farm and age). Reference values for each age were established. In a second study APP were analysed in farms of different health status, including farms with respiratory diseases and prevalence of PRRS, *Actinobacillus pleuropneumoniae* or *H. parasuis*, a farm with diarrhoea outbreaks in piglets, as well as high health status farms. In each farm serum samples were obtained at several ages, from animals randomly selected.

Results: Reference baseline levels. Pig-MAP mean concentration in piglets was around 1 mg/mL, being lower in the fattening period (0.7-0.8 mg/mL). Haptoglobin concentration increased with time, from around 0.6 mg/mL at 4 weeks of age to 1.4 mg/mL at 12 weeks. Mean values of around 0.9 mg/mL were observed in the fattening period. Different health status farms. In the high health status farms APP concentration remained in the baseline levels. In the rest of the farms significantly elevated levels of APP were determined in some of the sampling time points, indicating the period where the infections were developing.

Conclusions: Measuring Pig-MAP concentration may be used to determine the health status of farms and to determine critical points in the productive chain. Implementing a program of APP assay may help to optimise the productive process. The APP assay might be incorporated in certification programs to assess the general health status of farms.

10.13 FEED CONVERSION RATIO & AVERAGE DAILY GAIN AS KEY DRIVERS OF THE ECONOMIC BENEFIT IN PIG PRODUCTION.

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Today, most farmers take decisions based on financial indexes. In this, cost of production is often regarded as the most important factor and long-term driver in pig business. Nevertheless, the decision process to increase or lower costs is more complex and needs also to involve other production parameters.

The objective of this article is to examine the economic impact of vaccine induced improvements of Feed Conversion Ratio (FCR) and Average Daily Gain (ADG) applying the Return On Investment (ROI) concept. The effect and costs of ileitis were evaluated in a before-after comparison of the aforementioned production parameters between batches of pigs vaccinated against ileitis. Ileitis is a widespread but often undiagnosed enteric disease caused by the intracellular bacterium *Lawsonia intracellularis* (LI). Infections with LI lead to a thickening of the intestinal epithelium which result in disturbed nutrient absorption of the pig. Since early 2006 the first vaccine against LI (Enterisol® Ileitis, Boehringer Ingelheim) is available in Europe, providing lifelong protection with a one time oral vaccination around weaning. Situated in France the case-study was performed on an 80 sow farrow-to-finish unit. On this specific farm the presence of ileitis caused by LI was considered to be one of the major causes for potential economical losses in the past and Enterisol® Ileitis was introduced to counteract these problems. The vaccine was administered by trough at 26 days of age. The benefit of vaccination was analysed comparing the results of a 6 months period without vaccination versus a 6 month period with vaccination; Jan.-Jun. 2005 versus Jan.-Jun. 2006 respectively. Records over the life period 8-115 Kg showed that FCR and ADG improved substantially. FCR improved by 0.19 points from 2.60 to 2.41 kg/kg. ADG increased by 45 g/day from 704 to 749 g/day. The calculated economic benefit is 2.84 and 2.29 euro per pig for FCR and ADG respectively. This improvement by 7.3% in FCR and increase of 6.4% in ADG lead to an improved gross margin of 5.1 € per pig. Deducing vaccine costs of 1.25 €/ pig from the gross margin leads to a positive ROI of 4:1, i.e. every € invested into a vaccination program against LI generated an extra profit of 3.88 € in this respective case study.

In conclusion, a single focus on costs may exclude investments to improve ADG and FCR and therefore lead to suboptimal management decisions. Applying the ROI concept instead may raise the input cost initially but may also open up new ways to improve long term profitability by further exploiting the genetic potential of a pig herd through vaccination.

10.14 DETECTION OF CANDIDA CATENULATA AND CANDIDA SLOOFFIAE IN FAECES OF ECOLOGICALLY FED PIGLETS

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Problem: As yet no assured knowledge exists on the detection of yeasts in the intestine of pigs. In human beings particular yeasts are thought to be of clinical importance. In face of that, the presence of selected yeasts in pigs is essential to know and may provide the basis for future discussions of possible detrimental effects. Faeces are easy to obtain and may reflect the microbiota of the terminal gut, at least in principle. **Aim:** The aim of this pilot study was to investigate whether yeasts are present in the faeces of piglets under practical conditions of ecological agriculture.

Material and methods: In total, 399 faecal samples from ecologically fed piglets (Piétrain x Crossbreed German Landrace/Large White) housed in two different farms were quantitatively investigated for grown yeasts. Samples were taken 7 (n=40), 8 (n=89), 9 (n=153), and 10 (n=117) weeks post natum (pn). Up to 7 weeks pn, piglets were suckled from the sow. In addition, they received a milled mixed feed created for sucking piglets from 5 weeks of age. From 8 weeks pn, piglets were weaned and slowly converted to a milled mixed feed for growing piglets. Two and 4 different mixed feeds for sucking and growing piglets were used, containing similar concentrations of proximate nutrients. After hygienic investigation, the feeds were classified as suitable for feeding. Faecal samples were subjected to serial dilutions and cultured on Sabouraud agar plates for 3 days at 30°C and 37°C. Grown yeasts were identified by use of chromogenic candida agar plates (Oxoid: Chromogenic Candida Agar), assimilation tests and sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA. The concentration of yeasts was given in 1 gram of faeces.

Results: *Candida (C.) catenulata* and *C. slooffiae* were detected after 7 weeks pn in the range of 1.0×10^3 to 2.2×10^5 colony forming units (cfu)/g (16 samples) and 1.0×10^3 to 1.7×10^5 cfu/g (20 samples), respectively. One week later, *C. catenulata* (1.0×10^3 to 7.1×10^4 cfu/g) and *C. slooffiae* (1.0×10^3 to 1.3×10^5 cfu/g) were isolated from 4 and 12 samples. At the 9th week pn, both yeasts grew from a total of 19 and 55 samples (1.0×10^3 to 1.25×10^5 cfu/g and 1.0×10^3 to 6.9×10^5 cfu/g). After 10 weeks both yeasts were detected in 10 (1.0×10^3 to 4.2×10^5 cfu/g) and 39 samples (1.0×10^3 to 2.3×10^5 cfu/g), respectively.

Conclusions: Our results suggest that yeasts like *Candida catenulata* and *Candida slooffiae* may reach remarkable concentrations in the terminal gut of ecological managed piglets from 7 to 10 weeks of age. In addition, *Candida slooffiae* seems to be present more often than *Candida catenulata*. Environmental effects don't seem to affect the results. Although the experimental design of this study was not made to investigate such effects.

10.15 DIAGNOSTIC MONITORING OF HEALTH IN DAIRY COWS HERD IN PERIPARTURIENT PERIOD

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On dairy farms with high milk production there are much health problems. One of the most important period, the critical time of metabolic stress in dairy cows is calving. Whole periparturient period is very important as well because it has a great influence on future health, fertility and milk productivity. In controlling and resolving these problems the great role have the farmers and the veterinarians. There are many varied methods of dairy farms supervising. Diagnostic monitoring in herds of dairy cattle has a fundamental role in controls an acceptable level of health, productivity and welfare in the cows and their progeny. Being familiar with the internal environment of the animal enables early detection of any dangers to the health of the animal and enables one to react before any clinical signs appear. Thus good management in periparturient dairy cows is necessary for prevention of many disturbances which can occur early during or after delivery. The aim of the study was to compare the values of selected blood parameters in dairy cows shortly pre and post partum. The materials consisted of 30 clinical healthy cows tested 3-7 days before calving and 3-5 days after calving in this same good environmental and nutritional conditions in separated, labor sector. Selected various biochemical and morphological parameters of the blood similar to standard metabolic profile were included in the study. Significant differences ($\alpha=0.05$) were noted in total bilirubin, SGOT, inorganic phosphorus, calcium, chloride and haptoglobin. Parturition is an important period in cows from the point of view of the physiological changes taking place which in turn produce measurable, significant changes in the diagnostic parameters of the blood. These differences are presented in the paper.

10.16 VETERINARY HERD CONTROLLING SYSTEM - CONTROL AREA 'REPRODUCTION'

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The Veterinary Herd Controlling System (VHC-System), introduced by MANSFELD et al. (2002), is a pyramidal, dynamic quality assurance system for dairy herds. It allows optimization of process-quality and product-related quality. Individual farm goals are pursued through farm specific strategies and target-performance comparison. By means of a search of international literature a quantity of direct (directly related to animal health) and indirect (related to factors affecting animal health) critical control points (CCP) and control points (CP) and their corresponding indicators was collected. The indicators are quantitative or semi quantitative criteria allowing to evaluate a herd or a group of animals and to set goals. Suitable CCPs and CPs were implemented in a flow chart to be used by veterinarians as a tool for the status quo assessment as the first step as well as for regularly performed procedures. In the control area 'reproduction' determination of 'AI-balance', 'pregnancy rate' and 'incidences of reproductive disorders' (ovarial cysts, retained placenta, dystocia, endometritis, abortions and milk fever) were fixed as CCPs on the lowest level. If corresponding indicators deviate from normal range, the VHC-System provides further logical steps to identify reasons for variations and to countermand them.

Further checks are concerning the factors 'management' (management of AI, management of heat detection, analysis of the reproductive efficiency), 'feeding' (check of the energy balance and protein supply), 'housing' and 'breeding'. The described quality assurance system designed as a flow chart represents an efficient tool to control 'reproduction' on dairy farms.

10.17 METHODS TO CONTROL THE PERIPARTAL PERIOD IN DAIRY COWS

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The peripartal period is a critical phase of the re/production cycle. There occur a number of interrelated diseases, the so-called syndrome of periparturient crisis of dairy cows, as well as associated diseases during this period. As a part of the health state checking during the peripartal period, it was the aim to evaluate: 1) clinical index of dairy cows, 2) methods of biochemical control, 3) use of the F/P parameter in milk on the 1st yield check, 4) effect of additive products Fortis Adam and Fortis Eve, administered in TMR in the dose of 500 g. Three groups of Holstein cows (n=17) on the 1st to 4th lactation within a 500 cows herd with the yield of 10 600 kg were evaluated. Group I was control, Groups II and III were supplied with the Fortis Adam 21 days prior to and 7 days after delivery, animals of group III were extra given the Fortis Eve 7 days post partum. The clinical index (higher value speaks for worsening state of health) was determined on the basis of BCS, temperature, urine examination, digestive, reproductive and locomotion apparatus evaluation and mammary gland check. Blood was collected on days 1, 10 and 20 p.p. Serum samples were examined for total proteins, Ca, P, Mg, AST, total bilirubin, NEFA and BHB. Analyses were performed using the automatic analyser DCP Konelab 20i and Cobas Mira.

Comparing dairy cows from group III with those from group I, following significant differences were found ($p \geq 0.05$): clinical index 4.76 vs. 9.00 points, on the first blood sampling AST 1.51 vs. 1.86 $\mu\text{kat/l}$, bilirubin 6.44 vs. 9.62 $\mu\text{mol/l}$, on the second blood sampling total proteins 83.68 vs. 76.23 g/l , phosphorus 2.40 vs. 1.90 mmol/l and bilirubin 4.35 vs. 9.64 $\mu\text{mol/l}$, on the third blood sampling bilirubin 3.41 vs. 5.26 $\mu\text{mol/l}$ and NEFA 0.32 vs. 0.44 mmol/l , number of inseminations necessary for pregnancy 2.06 vs. 2.93. Milk yield until the first control were nonsignificantly higher - 40.69 vs. 38.98 kg, F/P lower - 1.14 vs. 1.29 and a shorter service period - 99.15 vs. 113.00 days in group III. The clinical index of dairy cows, biochemical control on days 1, 10 and 20 p.p. and F/P value of milk on the first check make it possible to evaluate in an objective way the health status of cows during the peripartal period. Use of products Fortis had a positive effect on some parameters studied.

Relations between the clinical index, biochemical parameters, re/production parameters and the development of cows in the subsequent peripartal and lactation period are the subject of our further preventative medical studies. This study was supported by Grant MSM6215712403.

10.18 RECONTROLLING OF REFERENCE VALUES OF HAEMATOLOGICAL AND CLINICAL-CHEMICAL PARAMETERS IN HEALTHY SOWS OF A HIGH PERFORMANCE HERD

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The breeding of new races of slaughter pigs with higher meet percentage, changed conditions in fattening regime and housing as well as new laboratory measurement methods require a control of the existing reference values for swine.

Therefore, this study is designed to recontrol the existing reference values for blood count and metabolism of healthy high performance gilts and sows in terms of the reproduction cycle and season, and to recommend a correction if necessary. Blood samples were taken from 60 sows of one high performance farm, divided seasonally into four groups, each consisting of five gilts and ten sows, beginning in May 2005. The samples were collected in each group three days before, four weeks and fourteen weeks after insemination as well as one, seven and fourteen days after parturition. The blood count was determined in EDTA blood by means of the haematology automat ADVIA® 120. Creatine kinase (CK), aspartate aminotransferase (AST), alkaline phosphatase (AP), glutamate dehydrogenase (GLDH) activities and cholesterol, total protein, albumin, urea, creatinine, calcium and phosphate concentrations in serum were measured with the analysis automat Hitachi 912. The AP activity decreased from 68.0 ± 20.1 U/l during gestation to 49.2 U/l ($p < 0.05$) at the first day after parturition and decreased again to 50.2 ± 28.9 U/l ($p > 0.05$) in the following two weeks. During gestation the AST activity decreased from 31.9 ± 7.9 U/l to 25.4 ± 10.6 U/l ($p > 0.05$), followed by an increase to 29.2 ± 7.4 U/l ($p > 0.05$) the first day after parturition. The cholesterol concentration decreased from 1.9 ± 0.5 mmol/l to 1.6 ± 0.3 mmol/l ($p < 0.05$) two weeks later. The other parameters were always within the existing reference values, did not differ during pregnancy and lactation and showed no significant seasonal differences. The creatinine concentration was always above the former reference limit of $130 \mu\text{mol/l}$. The most likely reason is the breeding of new races with higher meet percentage. The lower AP activities can be explained with methodical differences. We used a standardized system, which is used in the human laboratory medicine as gold standard. Some reagents, pH values concentrations and wave length differ from the former methods. The reference values of these two parameters should therefore be corrected.

10.19 PREPARTUM SERUM CONCENTRATION OF FREE FATTY ACIDS AND POSTPARTUM DISEASES IN DAIRY COWS

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Hepatic lipidosis and ketosis are common health disorders in dairy cows during the first weeks after parturition.

The objective of this study was to determine the association between the prepartum blood serum concentration of free fatty acids (FFA) and the risk of postpartum diseases.

Forty Holstein cows, 6 to 15 days before parturition were divided on the basis of body condition score (BCS) into 3 groups: Group 1 (n =14) 3.2 to 3.6; Group 2 (n =14) 3.7 to 4.1 and Group 3 (n =12) 4.2 to 4.6. Blood samples were collected for the determination of serum FFA. The laboratory analysis and clinical examination were performed periodically to diagnose diseases until 30 days postpartum. The principal diseases were: metritis, subclinical ketosis and hepatic lipidosis. Of the total number of cows, 21 animals were sick, and 19 clinically healthy; 15 animals had a serum FFA concentration above 0.4 mmol/L and 25 cows below this value. Cows with a serum concentration of FFA above 0.4 mmol/L were postpartum sick in 93.3%, but also 28% of cows with FFA below 0.4 mmol/L suffered from the some disease ($P < 0.01$). There were no differences in serum FFA among three groups of cows with a different BCS ($P = 0.9$).

The determination of serum concentration of FFA 1 or 2 weeks prepartum enable to identify cows probably suffering from postpartum diseases as are: retained placenta, metritis, hepatic lipidosis and ketosis; therefore a value of FFA is the useful indicator in the prevention of these diseases.

10.20 SEROLOGICAL STUDY OF NEOSPORA CANINUM INFECTION IN CATTLE FROM THE SOUTHWESTERN OF IRAN

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Neospora caninum is a major pathogen of cattle and dogs, being a significant cause of abortion in cattle in many countries. Cattle infected with the parasite are three to seven times more likely to abort compared to uninfected cattle. The parasite may be transmitted to cattle through the ingestion of oocysts or by congenital infection from mother to fetus via the placental. The economic effects of bovine neosporosis may be due to abortion, stillbirth and neonatal mortality, early fetal death and resorption manifest as return to service, increased time to conception or infertility, increased culling, reduced milk production, and reduced value of breeding stock. In order to investigate the seroprevalence of *Neospora caninum* infection in cattle in Southwestern of Iran, blood samples were collected from Holstein (121 animals) and cross-breed (436 animals) cattle from three farms and seven area of Ahvaz, respectively. All of the Holstein cattle were >4 years old but cross-breed cattle from different age groups were selected at random and divided into four age groups (< 2, 2-4, 5-6 and > 6 years old). Sera were tested for the response of anti - *N. caninum* antibodies by commercial ELISA kit. Anti - *N. caninum* antibodies were detected in 117 (21%) sera out of 557 tested. Significant differences were found between Holstein (53.71%) and cross-breed (11.93%) cattle. Although there was not an age-dependent antibody response, but differences of *N. caninum* infection between Holstein farms or various area was significant.

In conclusion, the results which represent the first investigation carried out on cattle in Southwestern of Iran; emphasize to consider neosporosis as one of the possible causes of abortion in cattle in this area.

11 REPRODUCTIVE HEALTH

11.1 REDUCED FERTILITY IN MODERN HIGH YIELDING DAIRY COWS: ETIOLOGY AND PREVENTION

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Abstract

The fertility of high yielding dairy cows has been declining over the past 25 years. Worldwide, studies have clearly demonstrated that the resumption of ovarian cyclicity has been retarded, that there is a significant increase in the incidence of postpartal ovarian abnormalities and that calving rates have dropped significantly from 55 to lower than 40%. Consequently, the calving interval has increased from about 385 to over 410 days, and the percentage of cows culled because of fertility problems has risen over 8% per year. This decrease in fertility leads to a significant decline in the profitability of our modern dairy herds and may cause a yearly loss of 5000 euro or more in an average 100 cow herd. The subfertility syndrome is a multifactorial problem in which the negative energy balance (NEB) clearly affects endocrine signaling, follicular growth as well as oocyte and embryo quality. At the Ghent University, recently some research has been done to unravel some of the mechanisms by which the NEB negatively affects the fertility of the modern high yielding dairy cow. The next challenge however is to ‘translate’ all this knowledge into practice and to offer the people in the field possibilities and strategies to minimize the current fertility decline. Solutions for this complex problem are however difficult to achieve because they involve optimizing a whole series of critical managerial factors such as housing, nutrition during the dry, transition and lactation period, hygiene and care around parturition, heat detection, timing of insemination, claw health and the use of good quality semen. Only if management is on a very high level can high milk production and good fertility be a feasible combination!

Key words: high yielding dairy cow, fertility, negative energy balance

Introduction

urrently farmers, veterinarians, consultants and even researchers are very demanding for our dairy cows. Besides the demand to produce lots of milk containing high levels of protein, we want them to calve each year. The latter implies that we like every dairy cow to be pregnant or to be recovering from pregnancy and preparing for a new pregnancy at all times, which makes fertility a full time job for her. Getting pregnant

during the first 85 days after calving assumes, however, a supreme co-operation of the involuting uterus, the hypothalamus, the pituitary, and the ovaries, leading to an undisturbed involution of the uterus, to the resumption of normal ovarian cyclicity, to the expression of heat symptoms and finally to conception. It is clear that the most important events concerning reproduction occur while the cow is at peak production and experiences severe metabolic stress. At the Ghent University research is going on to reveal the effects of a high level of production on the fertility of the modern dairy cow and to detect the underlying causes of fertility disturbances. The current review summarizes some of the results of this research.

Decreasing fertility?

Many studies report a decrease in the fertility of modern dairy cows. Since these studies originate from regions all over the globe, this situation seems to be widespread and universally accepted. In the United States, for example, the conception rate has been reported to decrease by 0,45% per year over a twenty year period (1). In the UK this decrease has been in the order of 1% per year (2,3). As comparable results from several other countries continue to appear, these reports have provoked an alarm response that goes on unabated. While over the same time period, significant increases in the level of milk production were reported, people got tempted to blame the increase in milk production to cause the decrease in fertility. In Flanders, average milk production of Holstein Fresian cows increased from 7.496 liters in 1995 towards 8.440 liters in 2000. During the same time period, the calving interval increased from 399 towards 407 days, while the 56-day non-return rate remained relatively stable (69,7 in 1995 and 69,9 in 2000). Analyses of fertility data of the local AI center revealed that the prolongation of the calving interval was mainly due to a prolongation of the interval from parturition to first insemination, while the data expressing the ability of the cows to conceive (i.e. pregnancy rate after first and second insemination, number of inseminations per pregnancy) were not associated with the prolongation of the interval between two consecutive calvings. Hence, we came to the conclusion that at least in Flanders the decrease in fertility of dairy cows as expressed by a prolongation of the calving interval, was mainly due to the inability of the farmers to see their cows in heat at the moment they should inseminate them.

Based on the aforementioned knowledge, more research was set up to further elucidate the anoestrus problem in Flemish high-yielding dairy herds. In a detailed study examining fertility data of 3.108 lactations (4), in 1.291 (42%) of all studied lactations no heat was observed within 60 days after calving. Cows not seen in heat within 60 days after calving had an average increase in days open with 26 days (days open: 111 vs 85 days). Of the 1.817 cows which had been seen in heat during the first 60 days after calving, 622 (34%) had to be examined later on because they had not been seen in heat at the time they should be inseminated. The latter cows were stated to suffer from 'a cessation of observed heat symptoms', and had an average increase of 24 days in the interval from calving to conception (days open: 109 vs 85). Hence in total, 1.913 (62%) off all lactations were identified as having suffered from one or another kind of

preservice postpartum anoestrus. Both cows not seen in heat within 60 days after calving and cows suffering from 'cessation of observed heat symptoms' had a significantly increased risk of being culled in the current lactation.

Abnormal ovarian activity post partum

The aforesaid results led to the question whether the anoestrus problem is merely due to shortcomings in the management (e.g. failure to detect oestrus) or whether it is peculiar to the modern high-yielding dairy cow herself. Furthermore, when problems could indeed be designated as being inherent to the high-yielding dairy cow, the next question arises as whether the anoestrus problems are caused by a lack of expressing heat symptoms by the cow, or are caused by ovarian/uterine disorders leading to the symptom of anoestrus. In order to investigate this into more detail, further research was carried out based on the analysis of milk progesterone profiles (5). Although it is nearly impossible to compare the results of different studies because of different sampling protocols and the use of different definitions for both normal and abnormal progesterone profiles, authors nowadays come to very comparable conclusions. The first significant rise in progesterone is stated to occur on the average at 37 days after calving (5), indicating that the first postpartum ovulation in the modern-day dairy cow occurs around day 30 after calving. The very wide range and standard deviation mentioned, however suggested the presence of a lot of cows with ovarian abnormalities. The latter was confirmed by the same study in which 47% of the 448 examined progesterone profiles showed an abnormal pattern during the preservice postpartum period. The two most frequently recognized abnormalities were delayed cyclicity or anovulation (= no significant progesterone rise during the first 50 days after calving), and prolonged luteal phase (= a period of at least 20 days of positive progesterone levels without a preceding insemination). In comparison with moderate yielding Friesians, modern high yielding Holsteins showed an increased incidence of postpartum abnormal ovarian cycles (Table 1).

By means of regularly carried out rectal palpations we found that small, inactive ovaries and not cysts were the most important reason of delayed cyclicity. Searching for the causes of prolonged luteal phases, in almost half (48%) of these cows an abnormal uterine content could be palpated, in 3% a cyst-like structure on one of the ovaries was discernable, while on 49% no specific reason for this ovarian abnormality could be found (5).

Table 1. A comparison of postpartum reproduction parameters based on measurement of progesterone in milk twice weekly in two different studies using moderate yielding Friesians (6) or modern high yielding Holsteins (5).

Ovarian activity based on progesterone profiles	Traditional herds (Fagan and Roche, 1986)	Modern high yielding dairy herds (Opsomer et al., 1998)
Number of cycles	448	448
Number of cyclical patterns (%)	78	53
Delayed cyclicity (%)	7	20,5
Temp. cessation of cyclicity (%)	3	4
Prolonged luteal phase (%)	3	20,5
Short cycles (%)	4	0,5
Other irregular patterns (%)	4	1,5

Based on a multivariate analysis at farm level taking into account a number of relevant factors, we demonstrated that calving during the stable period, an extended length of the previous dry period, health problems during the first month of lactation and clinical parameters illustrating the appearance of a severe negative energy balance (NEB), significantly increase the risk for delayed cyclicity before service. Parity, problem calvings, health problems during the first month of lactation and a (too) early resumption of ovarian cyclicity after calving significantly increase the risk for prolonged luteal cycles before service (7). Hence, these field studies clearly confirmed previous carried out clinical trials in which the health status and the NEB of the animals shortly after calving were demonstrated to be the most important risk factors leading to delayed cyclicity and anovulation, while the occurrence of prolonged luteal cycles is not directly dependent on the energy balance of the animals but is mainly caused by puerperal disturbances. The latter is furthermore enhanced by the fact that cows in NEB do suffer from a reduced immunity by means of a decreased killing activity of the neutrophils, which renders them more susceptible towards different kinds of puerperal infections (8).

Association between negative energy balance and fertility problems

As the typical homeorhetic changes of several hormones and metabolites are known to act as specific markers for the adaptation of the cows to the metabolic challenge they face during the first weeks after calving, investigations have been done to see whether elevated or lowered levels of these metabolites may be seen as the link between the NEB and the fertility decrease we currently notice in the modern-day dairy cow. As elevated serum concentrations of non esterified fatty acids (NEFAs) are an important

characteristic of the cow in NEB, NEFAs have been tested to see whether they may have a negative impact on fertility.

At our department research has been done to investigate to what extent metabolic changes that occur in early postpartum high-yielding dairy cows are reflected in the follicular fluid (FF) of the dominant follicle (>8mm) (10). Nine blood samples were taken per cow from nine high-yielding dairy cows between 7 days before and 46 days after parturition. From day 14 post partum on and together with blood sampling, FF samples of the largest follicle were collected from the same cows by means of transvaginal follicle aspiration. Serum and FF samples were analyzed using commercial clinical and photometric chemistry assays for glucose, β -OH butyrate (β -OHB), urea, total protein (TP), triglycerids (TG), NEFA and total cholesterol (TC). All cows lost body condition during the experimental period, illustrating a NEB during the experimental period. In FF, glucose concentrations were significantly higher and the TP, TG, NEFA and TC concentrations were significantly lower than in serum. The concentrations of glucose, β -OHB, urea and TC in serum and in FF changed significantly over time ($P < 0.05$). Throughout the study, changes of all metabolites in serum were reflected by similar changes in FF (Figures 1-3). Especially for glucose, β -OHB and urea, the correlations were remarkably high. The results of that study confirm that the typical metabolic adaptations which can be found in serum of high-yielding dairy cows shortly post partum, are reflected in FF (Table 1) and, therefore, may affect the quality of both the oocyte and the granulosa cells (10).

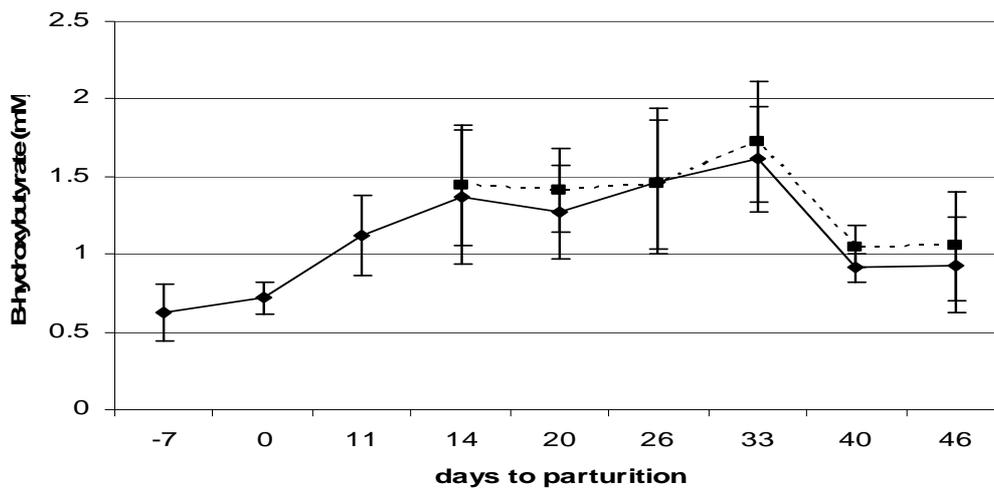


Figure 1. Average (\pm SEM) β -hydroxybutyrate concentrations (mM) in serum (full line) and follicular fluid (dotted line) of 9 high yielding dairy cows during the experimental period.

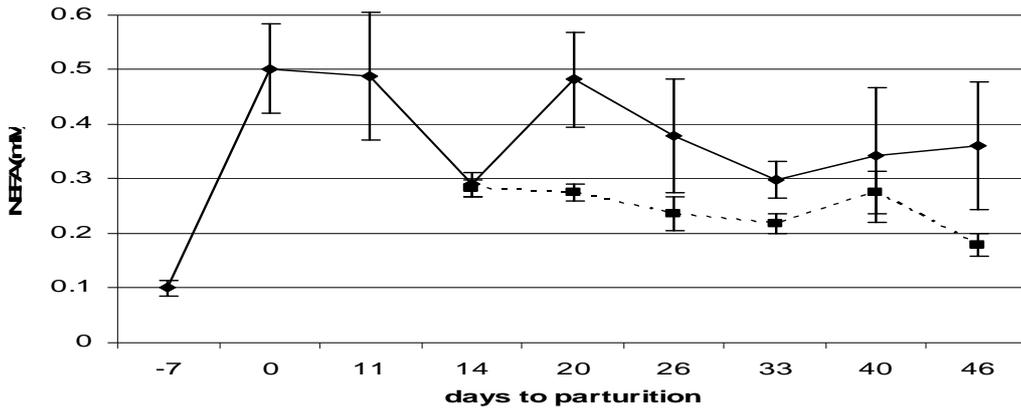


Figure 2. Average (\pm SEM) Nefa concentrations (mM) in serum (full line) and follicular fluid (dotted line) of 9 high yielding dairy cows during the experimental period

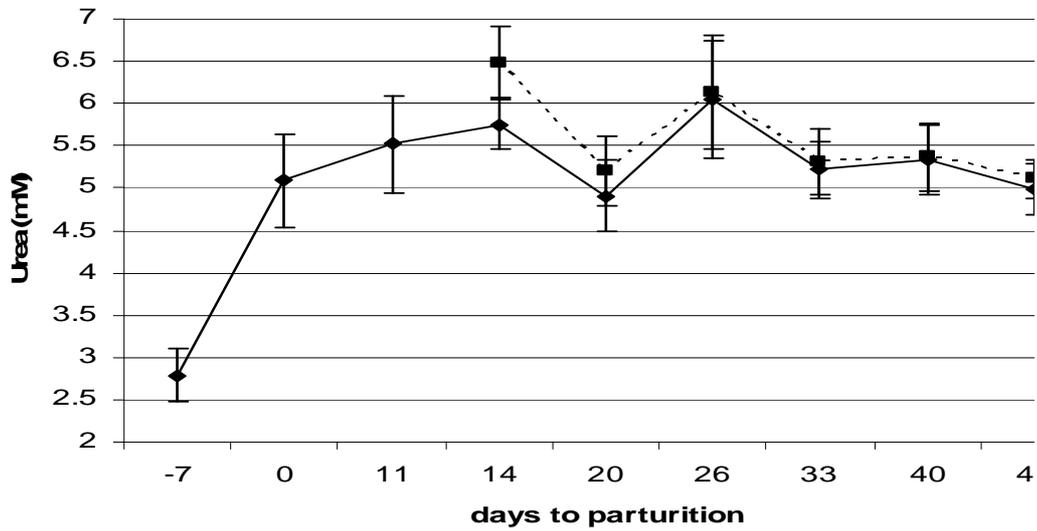


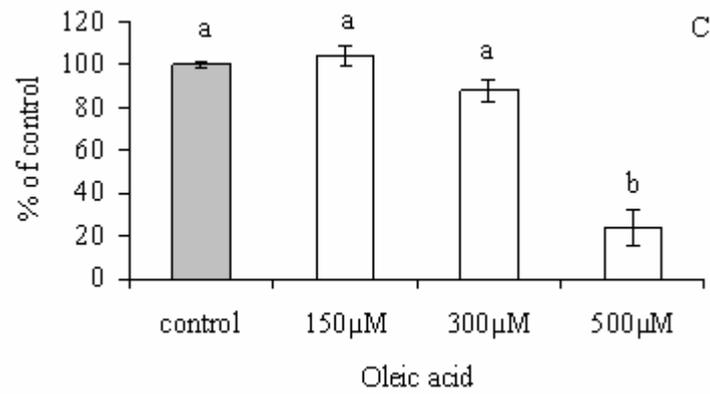
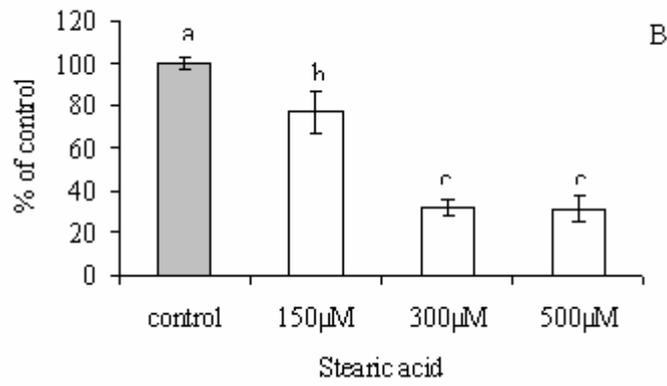
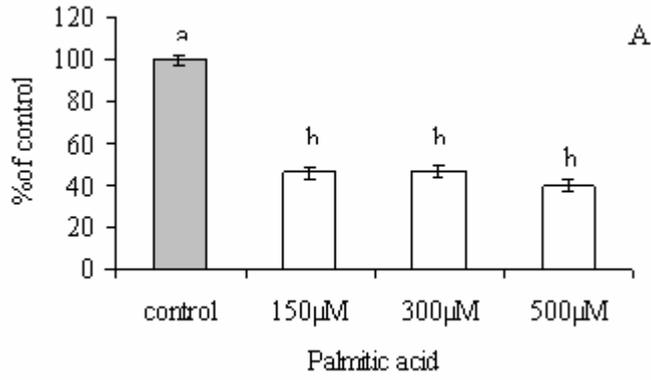
Figure 3. Average (\pm SEM) Urea concentrations (mM) in serum (full line) and follicular fluid (dotted line) of 9 high yielding dairy cows during the experimental period.

Table 2. Correlation coefficients (r 's) between metabolite concentrations in follicular fluid and serum per experimental session in nine dairy cows.

Correlations (r)	Glucose	β -OHB	Urea	Total Protein	Triglycerides	NEFA	Total Cholesterol
14 days pp	0.834*	0.996*	NS	NS	0.892**	NS	NS
20 days pp	0.788*	0.972*	0.929*	NS	NS	NS	0.787*
26 days pp	0.733*	0.992*	0.987*	NS	0.872**	NS	NS
33 days pp	0.925*	0.976*	0.990*	NS	0.710*	0.845*	0.918**
40 days pp	0.916*	0.971*	0.973*	0.860*	NS	NS	0.862**
46 days pp	0.901*	1.00**	0.782*	NS	NS	0.908*	0.948*

Values are presented for significant correlations (* $P < 0.05$; ** $P < 0.01$; NS: not significant).

Based on the study of Leroy et al. (10), we knew the concentration of several metabolites like NEFA in the FF. In a next study (11) we tested the effect of the most abundant NEFA (oleic- (OA), C18:1; stearic-(SA) C18:0 and palmitic acid (PA) C16:0) on granulosa cell proliferation using the concentrations which were measured *in vivo* by Leroy et al. (10). Granulosa cells were harvested through repeated aspiration of follicular fluid from large follicles (>8mm) on slaughterhouse ovaries. Cells were cultured for 48h under serum free conditions with 1 ng/ml FSH and 10 ng/ml insulin. Cells were treated with 0, 150, 300 or 500 μ M of the individual fatty acid or 450 μ M of a 1:1:1 combination of all three fatty acids. At the end of culture, granulosa cell numbers were determined spectrophotometrically. Both PA and SA had a significant inhibitory effect on granulosa cell proliferation at the three concentrations tested ($P < 0.01$). This effect was not dose dependent for PA ($P > 0.05$) since all three concentrations reduced cell numbers evenly (52,9 to 60% reduction). Stearic acid on the other hand had a more severe negative effect on cell proliferation at 300 μ M and 500 μ M than at 150 μ M ($P < 0.01$). Oleic acid only inhibited cell proliferation significantly ($P < 0.01$) at the highest concentration of 500 μ M (66,5% reduction). The combination treatment also reduced cell numbers significantly ($P < 0.001$) compared to controls (34,7% reduction). It could be concluded that *in vitro*, NEFAs reduce cell proliferation and/or survival of bovine granulosa cells. The latter indicates that elevated NEFA concentrations may affect ovarian cells and hence ovarian functioning contributing to the decrease in fertility which is currently mentioned in high yielding dairy cows (11).



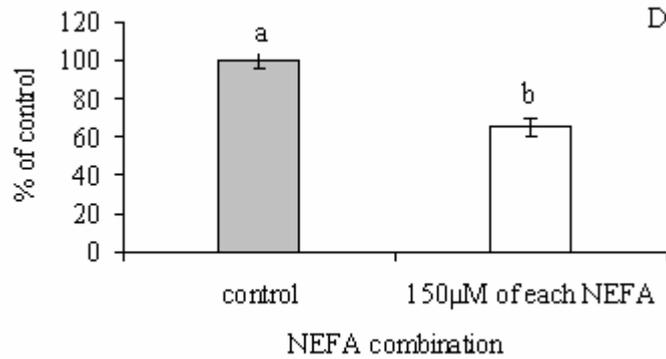


Figure 4. A-D. Effects of different concentrations of palmitic (A), stearic (B) and oleic (C) acid alone or combined (D) on granulosa cell proliferation (cells per well; mean \pm SEM) after 48 h of culture. The NEFA-combination (D) contains 150µM of each of the fatty acids. Data are expressed as percentage of controls. Means with different superscripts differ significantly ($P < 0.05$).

In a more recent study, we furthermore investigated the effect of elevated NEFA levels on the oocyte quality, testing their effect on fertilisation rate, cleavage and subsequent blastocyst formation using an in vitro model (12) (Tables 3 and 4).

Table 3. Effect of stearic acid (C18:0) added to the maturation medium on maturation and fertilization rate, cleavage rate (\pm SEM) at 48h after fertilization (pi) and number of blastocysts (\pm SEM) at 8 days pi relative to the number of bovine oocytes put in culture or relative to the cleaved zygotes

	Negative control	Positive control	Stearic acid (C18:0)
Maturation rate (%)			
Metaphase I	9.2 ^a	18.6 ^{b*}	26.0 ^{b*}
Ana-/Telophase ¹	16.1 ^a	11.6 ^a	18.4 ^a
Metaphase II	74.8 ^a	67.8 ^a	54.0 ^b
Fertilization rate (%)			
Metaphase II	10.7 ^a	8.8 ^a	23.4 ^b
2 Pronuclei	69.7 ^a	72.2 ^a	55.6 ^b
> 2 Pronuclei	12.5 ^a	12.1 ^a	12.5 ^a
Cleavage rate at 48h pi (%)	76.9 \pm 3.2 ^a	77.4 \pm 2.7 ^a	57.9 \pm 3.6 ^b
% blastocysts from oocytes	33.3 \pm 3.6 ^a	34.4 \pm 2.1 ^a	21.3 \pm 3.5 ^b
% blastocysts from cleaved	43.1 \pm 4.3 ^a	44.4 \pm 2.1 ^a	39.6 \pm 7.0 ^a

^{a,b}Data within a row marked with different superscripts, differ significantly ($P < 0.05$).

* $P = 0.1$, ¹ Significant interaction term "treatment X replicate".

Table 4. Effect of palmitic acid (C16:0) added to the maturation medium on maturation and fertilization rate, cleavage rate (\pm SEM) at 48h after fertilization (pi) and number of blastocysts (\pm SEM) at 8 days pi relative to the number of bovine oocytes put in culture or relative to the cleaved zygotes.

	Negative control	Positive control	Palmitic acid (C16:0)
Maturation rate (%)			
Metaphase I	9.1 ^a	12.5 ^a	24.1 ^b
Ana-/Telophase	15.9 ^{a,b}	10.5 ^a	19.9 ^b
Metaphase II	75.0 ^a	77.1 ^a	63.2 ^b
Fertilization rate (%)			
Metaphase II	21.6 ^a	20.2 ^a	33.5 ^b
2 Pronuclei	64.0 ^a	59.2 ^a	43.4 ^b
> 2 Pronuclei ¹	7.0 ^a	5.8 ^a	11.6 ^a
Cleavage rate at 48h pi (%)	76.6 \pm 2.3 ^a	74.5 \pm 2.6 ^{a,b*}	66.6 \pm 3.2 ^{b*}
% blastocysts from oocytes	22.4 \pm 2.0 ^a	24.6 \pm 1.5 ^{a§}	17.2 \pm 3.0 ^{a§}
% blastocysts from cleaved	29.1 \pm 2.4 ^{ab§}	33.2 \pm 1.8 ^a	22.7 \pm 4.1 ^{b§}

^{a,b}Data within a row marked with different superscripts, differ significantly ($P < 0.05$).

¹ Significant interaction term "treatment X replicate".

* $P = 0.07$ § $P = 0.06$ § $P = 0.12$

It was concluded that high levels of NEFAs during a period of NEB may influence fertility of high yielding dairy cows by hampering the oocyte maturation as expressed in lower fertilisation rates and subsequent lower cleavage and blastocyst development. Using a new lipid analysis technique to evaluate the lipid content of single bovine oocytes and embryos we were furthermore able to demonstrate a significant increase of the lipid content of in vitro produced embryos, after culture in the presence of serum .

How to translate this knowledge towards practice?

The biggest challenge for practitioners is to 'translate' this knowledge into practice and use it to help the herds they have in their herd health control program to reach an acceptable level of reproduction. As modern herd health control programs should focus on taking preventive measures rather than on increasing curative treatments (14), not only modern cows but also their 'coaches' have to adapt to the current levels of milk production. This adaptation has to do with an optimization of the management! While reproduction is a full time job for the dairy cow, coaching her to reproduce well takes no less time. Based on the above it is clear that implementing a dairy herd fertility control program should definitely be more than putting our arms in cows' rectums to examine problem cows. Giving advice upon the management of the dairy 'top athletes' to prevent fertility problems for sure needs at least the same amount of energy. The challenge is to integrate the current knowledge into nutritional management, production medicine, and reproductive management procedures taking

into account the specific obstacles each individual herd has to face, to finally optimize fertility of the herd (15). In the absence of such a holistic approach, the response to traditional veterinary therapies and herd health programmes may become increasingly diminished.

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11.2 EXPRESSION AND LOCALISATION OF MATRIX DEGRADING AND APOPTOTIC FACTORS IN THE BOVINE CORPUS LUTEM DURING INDUCED LUTEOLYSIS

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Anoestrus is a great problem of cows with a high milk production. The persistence of a corpus luteum (CL) on the ovary might be one cause for the blocking of the oestrous cycle. To get further information about the regression of the CL, we investigated the mRNA expression localisation of the matrix degrading metalloproteases (MMP) -1, -2, -14, tissue inhibitor of MMP (TIMP) -2 and the apoptotic factors TNF α , its two receptors, FAS and FAS-Ligand. We used a model of induced luteolysis in cows. 500 μ g Cloprostenol was injected during the midluteal phase and the CLs were collected at 0.5, 2, 4, 12, 24, 48 and 64h after PGF 2α injection. The ovaries (n=5/group) were collected by transvaginal ovariectomy and the mRNA expression was examined by realtime PCR. Immunohistochemistry was performed for MMP-1, -2, and -14. We found the following mRNA expression pattern: MMP-1 was increased by 256-fold from 2h to 64h, whereas MMP-2 was only increased from 24h to 64h by 3-fold. MMP-14 was constantly up-regulated from 12h onwards. TIMP-2 was constantly decreased from 24h to 64h. For TNF α a significant increase during all time points was seen with the highest up-regulation from 0.5h to 4h after PGF 2α injection. TNFR1 was not regulated whereas the expression of TNFR2 was increased from 2h to 64h on a constant level. FAS revealed a constant increase from 2h onwards till 64h. FAS-Ligand was up-regulated during the whole time of luteolysis with the highest increase at 12h. Immunohistochemistry for MMP-1, MMP-2 and MMP-14 showed an increased staining for MMP-1 and MMP-14, which was seen in large luteal cells beginning 24h after PGF 2α application. MMP-2 showed a strong increase in staining in endothelial cells at 48h. These results show that mRNA expression of the MMPs and the apoptotic factors are highly increased at the beginning of the functional and structural luteolysis, which seem to be necessary for the following apoptosis and degradation of luteal and endothelial cells during structural luteolysis. The increase of the MMP proteins during structural luteolysis in luteal and endothelial cells might lead to apoptosis by isolating these cells from their environment. Further investigations are necessary to prove if these processes are disturbed in cows with a high milk yield leading to a corpus luteum persistens.

11.3 VALUES OF UREAGENESIS INDEX IN COWS BETWEEN 70-90 DAY POST PARTUM AS PREDICTING FACTOR OF LOW FERTILITY

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An increase of the amount of ammonia in reticulorumen and portal circulation blood leads to the intensification of ureagenesis in liver, which results in a rapid increase of urea concentration in blood. Despite the intensification of ureagenesis, the ammonia concentrations in blood increase proportionally to the amount of free ammonia in rumen. An increase in the amount of ammonia nitrogen in relation to urea nitrogen (ureagenesis index) in blood is not proportional to the level of ureagenesis intensification but results from the increase of ammonia concentration in blood, which would suggest liver failure or inhibition of urea cycle activity. The aim of the study was to determine the values of ureagenesis index in cows on 70-90 day after parturition depending on the number of parturitions and the relation of elevated values of this index to fertility. The study was carried out on five farms with the herds of 160-300 cows. The observations involved 126 dairy cows (13 primiparous and 113 multiparous). The average milk production in previous lactation in cows was 8,305 kg/305 days. Blood samples were collected 80.8±7.5 days after parturition, four hours after morning feeding. Chemical analysis of blood samples included determination of urea nitrogen in serum and ammonia in plasma. In the examined population higher values of the ureagenesis index were noticed in 9.52% of cows (including 2 primiparas). The index was significantly higher ($p < 0.002$) in cows after four or more parturitions ($1.0242 \pm 0.5754\%$) in relation to younger cows ($0.7438 \pm 0.1357\%$) and controls ($0.3754 \pm 0.1033\%$). The increase in the value of this index resulted from very low concentrations of urea nitrogen in blood ($p < 0.001$) and elevated concentrations of ammonia ($p < 0.03$). Low concentration of urea nitrogen was confirmed also in milk ($p < 0.05$). It was demonstrated that the period between pregnancies was longer in cows with higher values of the index and in the postpartum period endometritis and ovarian cyst were more often present. The pregnancy index and number of inseminations per pregnancy did not differ in the studied groups of cows. The concurrence of low concentrations of urea nitrogen in blood in the early postpartum period (14-30 day) and on 70-90 day after parturition suggests that elevated values of ureagenesis index are the result of prolonged period of liver regeneration after metabolic effects of negative energy balance and lipolysis. Lower fertility is related to metabolic disturbances and liver pathology, which is indicated by higher percentage of metritis and ovarian cyst in cows with elevated values of ureagenesis index.

11.4 RELATIONSHIPS BETWEEN HEALTH DISORDERS AND PRODUCTION AND REPRODUCTION TRAITS OF HOLSTEIN COWS IN THE CZECH REPUBLIC

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Problem: The increase in milk production is accompanied by an increase in health problems and, subsequently, a decline in fertility and secondarily in a marketable production. In addition to a decrease in production, health and fertility as well as herd replacement costs are increased. Field data concerning the incidence of health disorders are not systematically recorded and collected in the Czech Republic, unlike in some other European countries.

Aim: The objective of this study was to evaluate the relationships among several health disorders: milk fever (MF; hypocalcemia and parturient paresis), retained placenta (RP; retained fetal membranes), metritis (ME; endometritis and pyometra), ovarian cysts (OC; follicular and luteal cysts), clinical mastitis (CM), and lameness (LS; foot and leg problems) on the productive and reproductive performance of dairy cows. **Material and method -** The dataset of 1432 Holstein cows calved between January 2000 and April 2004 from 4 commercial dairy herds was analyzed by the linear regression model which included the effects of herd-year-season of calving, parity, incidences of studied health disorders, and regression to the first calving age within parity. The fixed effect of breeding value level for milk yield of cow was added to the model for ANOVA of production traits and effect of FCM yield level was added to the model for the analyze of reproduction traits.

Results: MF had a significant influence ($P < 0.01$ and $P < 0.05$) on milk yield in 100 days of lactation (100d-Mkg) and on FCM yield in 305 days (305d-FCM), ME occurrence affected ($P < 0.001$) milk yield in 305 days of lactation (305d-Mkg) and 305d-FCM, OC had a significant effect ($P < 0.01$ and $P < 0.0001$) on 100d-Mkg and 305d-Mkg, or 305d-FCM, respectively. It was verified that health disorders relating to parturition, such as RP and ME had a significant influence ($P < 0.01$ to $P < 0.0001$) on the evaluated reproduction parameters, i.e. days from calving to the first AI service (DAI), open days (OD), and the number of inseminations needed for conception (NAI). Significant effect ($P < 0.0001$) of OC on all reproduction parameters was also found. Mastitis and lameness occurring during lactation had significant effects on OD and NAI ($P < 0.05$ to $P < 0.0001$). MF occurrence significantly related only to a longer period to the 1st AI ($P < 0.01$).

Conclusion: Occurrence of health disorders, such as milk fever, metritis, and ovarian cyst negatively affected milk yield of cows. The higher protein and lower fat content in milk were found in cows with clinical mastitis. Cows with health disorders were later inseminated and they conceived later after their previous calving compared to healthy cows, and need more inseminations for conception.

11.5 EFFECT OF PREOVULATORY INTRAVENOUS GLUCOSE-INFUSIONS ON THE SECRETION OF LUTEINIZING HORMONE AND THE OVULATION TIME IN DAIRY COWS

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Problem: Over the past decades, the reproductive performance of lactating dairy cows has been declining as milk production has increased. One of the reasons discussed is the delayed ovulation, which represents a major problem for the fertility of dairy cows. Reports about its incidence vary between 17 and 46 % of the examined cows. An insufficient energy supply at the time of ovulation may contribute to this effect. In a recent study a significant correlation between the energy supply at the day of insemination and the incidence of delayed ovulation could be detected. The correlation between the energy balance and the pulsatile secretion of Luteinizing Hormone (LH) has been described previously. In a preceding study we demonstrated a shortening of the oestrus cycle by intravenous infusion of glucose at day 19 of the cycle. **Aim:** The aim of the present study was to test the effect of this treatment on the preovulatory patterns of LH-secretion and the time of ovulation in dairy cows.

Material and methods: Ten pluriparous, lactating Holstein Friesian cows, which were kept in tie-stalls in the clinic, were examined every two days and from a diameter of the dominant follicle over 10 mm on daily via transrectal ultrasonography for patterns of follicular growth and ovulation time. From the seventeenth day of the oestrus cycle until the seventh day after ovulation blood samples were collected to determine serum concentrations of progesterone, estradiol-17 β and glucose. The cows were randomly allocated into two groups: Group 1 (n = 5): Infusion of one litre of a 5% glucose-solution on day 19 of the oestrus cycle; Group 2: Infusion of 0.9 % saline-solution on day 19 of the oestrus cycle. Blood samples for the determination of the preovulatory LH-concentrations were taken every three hours from the end of the treatment until ovulation. The determination of LH was carried by means of a species-specific 125I-radioimmunoassay.

Results: The peak-concentration of LH differed significantly ($p = 0.043$) between the two groups, being much lower in group 1 after the infusion of glucose (14.8 ± 9.1 ng/ml) than in the control-group 2 (43.1 ± 21.7 ng/ml). The interval between the infusion and the LH-peak was distinctly shorter in group 1 (25.8 ± 9.6 hours) than in group 2 (52.8 ± 35.7 hours), whereas the intervals between LH-peak and ovulation did not differ. No significant differences in the concentration of the glucose could be detected.

Conclusions: A short-term supply of glucose on day 19 of the oestrus cycle shortens the cycle length by an earlier induction of the preovulatory LH-peak. The preovulatory LH-secretion seems to be influenced by the availability of glucose.

11.6 CHANGES IN UTERINE BLOOD FLOW DURING THE FIRST TWELVE WEEKS AFTER PARTURITION IN HOLSTEIN FRISIAN COWS

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The pivotal factor for good fertility is an uncomplicated puerperal period. Monitoring the puerperal period means mainly the examination of uterine infections and involution. The latter one is in bovine medicine mainly based on transrectal manual palpation and B-Mode sonography of the uterus. The main problem of these methods is that they are not suitable during the first days after parturition, because the uterus is too huge to be delineated. In human medicine a non-invasive method to observe the uterine blood flow has been established. This technique is used to demonstrate pathological conditions in the puerperal period. The aim of this study was to examine uterine blood flow during the first 12 weeks after parturition in cows by colour Doppler sonography and to compare it with findings made by transrectal palpation and B-mode sonography of the uterus. Examinations were carried out on Days 1, 7, 14, 28, 56 and 86 after parturition (= Day 0) in 42 Holstein Frisian multiparous dairy cows (lactation number: $2,69 \pm 0,72$). Findings obtained by transrectal manual palpation were quantified by using scores with 6 grades (1 to 6) for uterine size (SUS). Using B-mode sonography the mean diameter using scores with 4 grades (0 to 3) of intrauterine fluid accumulation (IUF) within the uterine corpus was determined. Blood flow in both uterine arteries, ipsi- and contralateral to the former location of the fetus was quantified by the blood flow volume (BFV) and the pulsatility index (PI). SUS and IUF decreased between Days 1 and 28 from 5.16 to 2.34 (SUS) and from 2.57 to 0.05 (IUF) ($P < 0.05$) and remained at a constant ($P > 0.05$) level afterwards. BFV values decreased steeply from 3988.48 ml/min on Day 1 to 1053.94 ml/min on Day 7 ($P < 0.05$). Between Days 7 and 28 there was a moderate decrease to 164.08 ml/min on Day 28 ($P < 0.05$). From this time on there were no changes ($P > 0.05$) in BFV values until the end of the study. PI values rose from 1.54 on Day 1 to a peak value of 5.56 on Day 28 and decreased ($P < 0.05$) linearly to 3.13 on Day 86. The results show that transrectal colour Doppler sonography is a useful method for examining changes in uterine perfusion during the first twelve weeks post partum in cows. While uterine blood flow volume was an appropriate parameter for demonstrating changes during the early involutinal process, the Pulsatility Index was suitable to investigate alterations in uterine perfusion in the whole examination period. If these findings are relevant to fertility of the cows has to be proven in future studies.

11.7 COLOR DOPPLER SONOGRAPHY OF UTERINE BLOOD FLOW IN THE SECOND HALF OF GESTATION IN LACTATING HOLSTEIN-FRIESIAN COWS

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The main goal of the present study was to measure uterine blood flow volume (BFV) in the second half of gestation in lactating Holstein cows. Secondary goals were to determine whether there are individual variations in uterine blood flow and correlations between uterine blood flow, maternal weight and birth weight of the calf. Forty-four cows were examined via color Doppler sonography in weeks 21, 25, 29, 33 and 39 of pregnancy. The cows were divided into two groups based on the following variables: body weight (light < 575 kg, heavy > 575 kg), birth weight of the calf (light < 42 kg, heavy > 42 kg). The BFV was measured via transrectal Doppler sonography of both uterine arteries. There was a linear increase in uterine BFV throughout the study period with a 5.5-fold increase from $3,053 \pm 1,143$ ml/min to $16,912 \pm 5,793$ ml/min. Variation coefficients were used to quantify inter-individual variations, which ranged from 34 to 37%. There was a weak correlation between uterine BFV and birth weight of the calf in weeks 21 to 37 ($0.30 < r < 0.49$; $P < 0.05$); and a moderated correlation in week 39 ($r = 0.60$; $P < 0.0001$). Uterine BFV in week 21 was significantly ($P < 0.01$) higher in heavy cows (3.394 ± 1.119 ml/min) than in light cows (2.658 ± 1.064 ml/min). Compared with light cows, the increase in uterine BFV was 32% higher in heavy cows during the study period. In week 21, there was no significant difference ($P > 0.05$) in uterine BFV between cows producing a heavy calf (3.351 ± 1.130 ml/min) and those producing a light calf (2.796 ± 1.115 ml/min). Thereafter, the BFV increased and in week 39 was 43% higher in cows producing a heavy calf than in those producing a light calf. Because there was a positive correlation between maternal and calf weight, these two factors were analyzed independently. There was a positive correlation between calf weight and uterine BFV ($P < 0.05$) but not between cow weight and BFV.

In conclusion, there was a linear increase in uterine BFV in lactating Holstein cows in the second half of pregnancy with marked individual variations. In the second half of gestation, uterine BFV appeared to be affected primarily by fetal factors.

11.8 BACTERIAL COMPLICATIONS OF UTERINE INVOLUTION IN CATTLE: CLINICAL PATHOLOGY, PREVENTION, THERAPY

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Abstract

The bacterial contamination of the postpartum uterus is a frequent finding which by itself does not disturb the anatomical and histological restoration of tubular genital tract. In dairy cows, however, the improper balance between uterine infection and the intrauterine antimicrobial self-defence mechanisms often results in complications, such as puerperal metritis, clinical endometritis, pyometra and subclinical endometritis. This paper reviews the bacteriology of uterine involution, defines the different clinical forms, and summarizes the principles of their pathology, prevention and therapy.

Introduction

The anatomical and histological restoration of postpartum (pp) uterus (e.g. uterine involution) is frequently affected by inflammatory complications, such as *puerperal metritis*, *clinical endometritis*, *pyometra* and *subclinical endometritis* (Sheldon et al., 2006). These are the most common forms of genital diseases in dairy cows, which may delay the complete regeneration of endometrium, and disrupt the resumption of cyclic ovarian function resulting in postponement of the first insemination (AI), increasing the number of AI-s per conception, and thus prolonging the calving interval and decreasing the calving rate (reviewed by Hussain and Daniel, 1991a). After reviewing the bacteriology of uterine involution, and the predisposing factors for its bacterial complications, this paper defines the different clinical forms, and summarizes principles of their pathology, prevention and therapy.

Bacteriology of uterine involution. Metabolic predisposition for bacterial complications

Before calving the uterine lumen is sterile. At and post parturition bacteria may invade the birth canal and uterine cavity ascending from the environment as well as from the animals' skin and faeces (Messier et al., 1984; Noakes et al., 1991). A wide variety of bacteria can be isolated from almost all cows during the first 10-14 postpartum days. Bacterial presence in the uterus is usual at this time and can be detected more than 90%

of the cows, regardless of disease signs (Sheldon and Dobson 2004). In the first 10 days after calving mostly *Streptococcus spp.*, *Staphylococcus spp.* and *Bacillus spp.* were isolated from the uterus of cows without visible clinical signs of puerperal metritis, whereas *Arcanobacterium pyogenes*, *Escherichia coli* and different Gram-negative (GN) obligate anaerobic bacteria (*Fusobacterium necrophorum*, *Prevotella spp.* and *Bacteroides spp.*) were predominant in the uterus of clinically diseased animals (Huszenicza et al. 1999). Most of the clinical and reproductive consequences are attributed to the presence of *A. pyogenes*, either alone or in combination with other bacteria like *E. coli* and GN obligate anaerobes. The incidence and species of bacteria gradually decrease along with the pp days. Thus the presence of bacteria is sporadic on 28-35 days after calving, and the uterine cavity should be sterile thereafter (Olson et al. 1984; Paisley et al., 1986; Hussain, 1989; Hussain and Daniel, 1991a; Lewis 1997; Sheldon and Dobson, 2004; Sheldon et al., 2004b; Williams et al., 2005).

Presence of bacteria in the uterus of pp cows (*bacterial contamination*) does not always result in inflammatory uterine diseases. Despite, the intrauterine (i.u.) *bacterial infection*, which follows certain cases of contamination, implies adherence of pathogenic organisms to the mucosa, colonization or penetration to the epithelium and/or release of bacterial products (toxins, enzymes etc.) that may lead to establishment of uterine diseases (Sheldon et al. 2006). However, the i.u. bacterial infection as such, does not even necessarily mean a clinical manifestation of disease; this is dependant on the immune status of the host. The course of uterine involution may be considered as a “see-saw balance”: in a physiological situation the self-defence mechanisms of the uterus (such as pathogen-specific antibodies, phagocytosis of non-antibody-opsonized bacteria and myometrium contractility) are able to counteract the bacterial infection.

The invading pathogens (*A. pyogenes* and *E. coli*) evoke an antibody response that may help the uterus reduce the severity of subsequent infections (Radcliffe et al., 2005), showing the involvement of humoral factors in local antimicrobial self-defence of tubular genitals. However, the role of host's humoral immune response in disease remains poorly defined. Phagocytosis of non-antibody-opsonized bacteria by polymorphonuclear (PMN) neutrophils and other leucocytes seems to be the initial defence against the invading pathogens (Hussain 1989; Cai et al., 1994; Lewis, 1997; BonDurant, 1999; Kimura et al., 2002; Mateus et al. 2002; Lewis, 2004; Sheldon and Dobson, 2004). Any impairment in this mechanism may lead to bacterial complications in uterine involution, and also to mastitis caused by environmental pathogens. The peri-parturient decline in plasma levels of insulin-like growth factor-1 (IGF-1) and leptin, furthermore in bio-availability of IGF-I, is supposed to interfere with the innate immunity of the dam (Vangroenweghe et al., 2005; Kulcsár, 2007), modulating the apoptosis and functionality of PMN leukocytes especially in hyperketonaemic dairy cows. The increased plasma levels of non-esterified fatty acids (NEFA) and β OH-butyrate (BHB) impair the migration, phagocytic and killing activity and/or the oxidative burst of PMN and other leucocytes, enhancing the susceptibility of host to invading pathogens (Sartorelli et al., 1999 and 2000; Suriyasathaporn et al., 1999 and 2000; Zerbe et al., 2000). On d 1-3 after calving the elevated (≥ 1.00 mmol/l) BHB

levels predispose the cows for mastitis caused by environmental pathogens, and this form of mastitis coincides frequently with puerperal metritis (Jánosi et al., 2003). Metabolic disorders like hyperketonaemia (Reist et al 2003), and deficiency conditions (such as selenium, vitamin E and vitamin A/ β -carotene deficiency; Lewis, 1997; Sheldon and Dobson, 2004) may alter the competence of cellular self-defence mechanisms, and so can increase the risk for developing metritis. *Myometrium contractility* is the third component of self-defence mechanisms, since uterus contractions remove the uterine content (lochia or exudate) (Paisly et al. 1986). Early pp cows show a high variability in characteristics of uterine contractility, and the myometrial contractions significantly decrease with time (Bajcsy et al., 2005). Lack of exercise, lack of regular sucking and hypocalcaemia (parturient paresis) are the most frequent causes of decreased myometrium contractility, resulting in the stagnation and accumulation of lochia, and so increasing the risk of bacterial complications (Kamgarpour et al., 1999; Lewis, 1997; Sheldon and Dobson 2004). However, in a recent study of Bajcsy et al. (2005) there was no correlation between blood ionized calcium (Ca^{2+}) concentrations and any of the contractility parameters.

Retained foetal membranes (RFM), stillbirth, twinning and calving difficulties requiring manual assistance within the birth canal (dystocia) may increase the infection pressure and hence the likelihood of incidence of the disease, even if the uterine defence mechanisms are intact (Paisly et al. 1986; Hussain 1989; Hussain and Daniel 1991a; Lewis, 1997; Sheldon 2004; Sheldon and Dobson 2004). Unhygienic housing environment further worsen the situation. The hygienic conditions at calving are usually better in small family-operated dairy units than in large-scale herds, where the cows are kept inside year round and calve in continuously used maternity barns. The clinical incidence of bacterial complications is much higher in dairy cows as compared to suckling beef cows, and shows a wide variation between farms (Paisley et al., 1986; Hussain and Daniel, 1991b).

On a herd level, most important risk factors associated with the incidence of metritis are the herd size (higher incidence in large herds), season (higher incidence in cows calving between November and April), parity (higher incidence in 1st and >3rd parity cows), lack of grazing, dystocia and RFM (Bruun et al 2002; Sheldon and Dobson 2004).

In a recent study (authors' unpublished observation) healthy cows (n=10) and their metritis-affected counterparts (n=14) were sampled via endometrium biopsy during the early puerperium (on day 4 to 14) to determine the apoptosis index. Blood samples were taken simultaneously for BHB analysis. Paraffin-embedded tissue samples were immunofluorescence stained for apoptosis (nuclear fragmentation) detection, terminal deoxynucleotidyl-transferase mediated dUTP nick-end labelling (TUNEL). Nuclei were counterstained with fluorescence 4,6-diamino-2-phenylindole (DAPI). In the photographed sections total cell number was determined by DAPI-stained cells per field. TUNEL positive cells with concurrent DAPI-staining showed the apoptotic cell number. The *apoptotic index* was calculated by dividing the total number of apoptotic endometrial cells by the total number cells and multiplying by 100. Before

immunostaining one section from each biopsy sample was stained with haematoxylin and eosin for a histological evaluation. According to their uterine status and plasma BHB content, the cows were classified as (i) healthy controls (n=10), (ii) normoketonaemic-metritic (n=9), furthermore (iii) hyperketonaemic (BHB: ≥ 1.00 mmol/l) individuals with or without puerperal metritis (n=5). The apoptosis index of healthy controls (BHB: 0.45 ± 0.12 mmol/l), normoketonaemic-metritic cows (BHB: 0.42 ± 0.15 mmol/l) and hyperketonaemic animals (1.47 ± 0.32 mmol/l) was $50 \pm 6\%$, $52 \pm 1\%$ and $24 \pm 6\%$, respectively, with a close linear correlation ($R^2=0.78$) between apoptosis index and BHB content. It was concluded that during the earliest weeks of lactation hyperketonaemia decreases the occurrence of apoptosis in the endometrium, increasing the risk of necrotic degeneration of epithelial cells and the consequent inflammatory complications in dairy cows. So it seems to be an alternative way how hyperketonaemia, related to the postpartum negative energy balance, may interfere with postpartum uterine involution.

Clinical forms of bacterial complications

Uterine complications of early puerperium (*puerperal metritis*), the intermediate to late stage of involution (*clinical endometritis*, *pyometra*) and the service period (the same as in the intermediate to late stage of involution, plus *subclinical endometritis*) form a chain of pathological events developing from one to another in the above order (Paisly et al. 1986; Kennedy and Miller, 1993; Lewis, 1997; BonDurant, 1999; LeBlanc et al., 2002a; Kasimanickam et al., 2004; Gilbert et al., 2005; Sheldon et al., 2006).

Puerperal metritis.

Puerperal metritis is the bacterial complication of the early puerperium (Paisley et al., 1986; Kennedy and Miller, 1993; Lewis et al., 1997; BonDurant, 1999; Drillich et al., 2001; Sheldon 2004; Sheldon and Dobson 2004; Sheldon et al. 2006), which occurs during the first two weeks after calving (typically on 4-10 days pp), and is characterized (i) by a large amount of *foul smelling, reddish-brown, watery* (e.g. *putrid*) *exsudate* with some necrotic debris in the uterus and a thin uterine wall in the first half of this period, or (ii) by a limited amount of malodorous, purulent uterus exsudate and a thick (oedematous) uterine wall some days later. In more severe cases, local symptoms are accompanied by general signs, like fever (> 39.5 °C), loss of appetite, dullness, reduced milk production or diarrhoea with dehydration as a consequence (*toxic puerperal metritis*). From pathological point of view the puerperal metritis is an acute putrid inflammatory disease due to the massive bacterial infection of the uterus. The degenerative and infiltrative processes lead to an excessive damage of luminal and glandular epithelium and myometrial tissue. In the most severe cases the inflammatory process may extend to the entire thickness of the uterine wall, sometimes also to the serosa (*perimetritis*) and the suspensory ligaments (*parametritis*). Incidence of puerperal metritis varies from 2 to 37 % (Kelton et al 1998).

Although in the first 10 to 14 days after calving there may be a lot of other bacteria present in the uterus, the *A. pyogenes* and *E. coli* combined with certain GN anaerobic bacteria (*F. necrophorum*, *Bacteroides spp.* and *Prevotella spp.*) are considered the main responsible pathogens in this complication (Sheldon and Dobson 2004; Williams et al., 2005). *A. pyogenes* and GN anaerobes invade not only the endometrium, but usually also the submucosa, and occasionally the deeper layers of the uterine wall (Paisley et al., 1986; BonDurant, 1999). Stagnating lochia in the uterine cavity provides an excellent medium for multiplication of *E. coli* and/or other coliforms, resulting in extensive liberation of a lipopolysaccharide like cell wall component (endotoxin) of these microbes (Peter et al., 1987 and 1990; Dohmen et al., 2000; Mateus et al. 2003). The enormous quantity of endotoxin in lochia of cows with dystocia and/or RFM shortly after parturition enhances the development of uterine infection by *A. pyogenes* and GN anaerobes thereafter in the later pp period (Dohmen et al., 2000). Endotoxin in the lochia provides a positive chemotactic signal for leucocytes, enhancing their migration from the blood into the uterus. Furthermore, in the stimulated immune cells (mainly in macrophages) endotoxin induces the release of histamine and pro-inflammatory *cytokines*, such as tumor necrosis factor- α (TNF α) and interleukins (IL1 and IL6), and activates the phospholipase A₂, cyclooxygenase-2 and 5-lipoxygenase enzyme systems producing various *eicosanoids* (PGF_{2 α} , PGE₂ and also prostacyclines and thromboxanes) (Madej et al., 1984; Peter et al., 1987; Kindahl et al., 1992 and 1996; BonDurant, 1999; Mateus et al. 2003; Sheldon 2004; Sheldon et al., 2004b). One of these eicosanoids is the PGF_{2 α} , with known luteolytic and immunostimulative properties, which also increases the myometrial contractility in cattle (Hirsbrunner et al. 2003; Lewis, 2004). In the first 2 to 3 weeks increased plasma levels of PGF_{2 α} and its stable inactive metabolite (13,14-dihydro-15-keto-prostaglandin F_{2 α} , PGFM) were found. These high PGFM concentrations coincided with the fast regression phase of uterine involution. Significantly higher values were reported to occur in cows affected than in those not affected with puerperal metritis (Madej et al., 1984; Del Vecchio et al., 1992 and 1994). Plasma PGFM concentrations could be used as an indicator for following up the course of metritis (Del Vecchio et al., 1992 and 1994; Nakao et al., 1997; Seals et al., 2002; Mateus et al., 2003). PGE₂ is also an important uterine inflammatory mediator. Due to its immunosuppressive and luteotropic actions the high level of PGE₂ in the uterine content may enhance the degree of bacterial infection (Slama et al., 1991 and 1994).

When the pro-inflammatory cytokines (TNF α , IL-1) and other products of activated macrophages reach the central nervous system, these mediators initiate marked changes in secretory pattern and/or serum level of numerous neurotransmitters and hormones, such as cortisol and catecholamines, prolactin, growth hormone, IGF-1, pancreatic glucoregulatory hormones (insulin, glucagon), leptin, thyroid products, and gonadotrophs (LH) (reviewed by Huszenicza et al., 2004; Kulcsár, 2007). Simultaneously the production of acute phase proteins, such as α_1 -acid glycoprotein (α_1 -AG) and haptoglobin (HP) are increased (Hirvonen et al., 1999; Sheldon et al., 2004b; Williams et al., 2005). Cytokines and/or cytokine-mediated neural and endocrine changes play key role in induction of systemic symptoms, e.g. fever,

lethargy, loss of appetite (anorexia) and many catabolic changes in energy (lipid, carbohydrate), protein and mineral metabolism, resulting in rapid and intensive loss of body condition and weight. In more severe cases simultaneous alterations are also seen in cardiovascular, pulmonary and gastrointestinal functions (hypotension, tachycardia, ultimately causing decreased cardiac output, respiratory distress, diarrhea), as well as in blood cell counts and blood coagulation system (BonDurant, 1999; Sheldon 2004; Sheldon et al., 2004b). However, our related knowledge is still rather limited currently.

In one of our recent experiments (Kulcsár, 2007) development of puerperal metritis, and the related changes in plasma metabolites, metabolic hormones and certain cytokines and acute phase proteins were studied in $\geq 2^{\text{nd}}$ parity cows predisposed for this disease (RFM: n=10; dystocia: n=31). Vaginoscopy and rectal palpation were performed at 2-3 day intervals, starting on day 3 postpartum. Simultaneously blood samples were collected once a day until d 15, and thereafter 3 times a week until d 45 for assaying NEFA, BHB, IGF-1, insulin, leptin and TNF α levels and some acute phase proteins (HP, α_1 -AG). Also uterine swab samples were taken for bacteriology. Severe form of puerperal metritis (PM_S) with fever (rectal temperature > 40.5 °C) and depressed feed intake and milk production was observed in 7 cows, a mild form (PM_M) with putrid discharge only developed in 15 animals, whereas 19 cows remained healthy. At the beginning the parameters of PM_S, PM_M and healthy cows did not differ. Thereafter in metritis-affected cows the BHB, IGF-1, insulin and leptin decreased, whereas the NEFA and α_1 -AG increased, and remained altered for some weeks. Fluctuating elevations in plasma TNF α and HP were detected only in PM_S cows, and also their body condition loss was more severe. These tendencies related to the uterine complications, but were not altered by the uterine culture.

Development of puerperal metritis coincides with the recruitment of the first-wave cohort of follicles, followed by the emergence and selection of the first dominant follicle (DF). This first-wave DF produces 17 β -estradiol (E₂) and may ovulate in healthy non-suckled dairy cows, mostly in those which have been over the nadir of the negative energy balance shortly after calving (Butler, 2003; Diskin et al., 2003). Puerperal metritis was hypothesized to have a direct negative effect on ovarian function (Sheldon et al 2002). Endotoxin absorbed from the uterine cavity suppressed the preovulatory LH peak, and/or induced cystic degeneration of DF-s in pp cows (Peter et al., 1989; Lopez-Diaz and Bosu, 1992). Although all details of the neuroendocrine regulation have not yet been fully delineated in puerperal metritis, the endotoxin and/or cytokines of uterine origin can disrupt the peri-ovulatory progression of the first-wave DF, resulting in its atretic or cystic degeneration with a subsequent postponement in resumption of ovarian cyclicity (Peter, 2004; Sheldon, 2004). These ovarian consequences are well-documented by many clinical trials using progesterone profiles for monitoring the pp ovarian function (Huszenicza et al., 1987; Peter et al., 1987; Gilbert et al., 1990; Huszenicza et al., 1999). During the early weeks of lactation similar ovarian alterations were also reported to occur in mastitis with intensive endotoxin and cytokine release (Huszenicza et al., 2004 and 2005).

Diagnosis of puerperal metritis is quite straightforward and obtained on the basis of clinical signs. Time elapsed from parturition, malodorous, watery, reddish-brown uterine discharge, with or without systemic signs are enough for the diagnosis (Sheldon and Dobson, 2004; Sheldon et al., 2006). Although pyrexia statistically correlated with the presence of uterine pathogens and febrile animals have significantly higher plasma concentration of acute phase proteins, fever is an indicator of puerperal metritis but additional clinical signs are necessary to identify the disease (Sheldon et al. 2004b). The vaginal discharge usually becomes fetid soon (on day $\leq 3-4$) after calving in toxic puerperal metritis, but only later (on day 6-10) in the milder cases (Földi et al., unpublished data). This difference in the time of first appearance of pathognomic signs may result in false negative clinical finding in numerous cases, if in animals not affected by dystocia and/or RFM the course of involution is checked too early, on day 4-5 after calving (when a cow leaves the maternity unit in large-scale dairy herds).

Clinical endometritis and pyometra

Clinical endometritis occurs from the third post partum week (after pp day 14) onwards, characterized by presence of abnormal (*mucopurulent*: approximately 50% pus and 50% mucus, or *purulent*: estimated pus content $>50\%$) content in the uterine cavity, and the same quality of vaginal discharge expelled through the cervix still open. In endometritis only the endometrium is inflamed, the condition does not extend deeper than the stratum spongiosum. This superficial process is characterized by degenerative changes of surface epithelium, vascular congestion with stromal edema, and migration of neutrophil granulocytes and other inflammatory cells (lymphocytes, plasma cells) into the endometrium and the uterine cavity. Pus content of the uterine exudate is actually derived from the large amount of perished neutrophil granulocytes, phagocytosed bacteria and tissue debris. The endometrium becomes hyperaemic and congested, the superficial epithelial cells may desquamate and necrotize. The uterine infection may ascend also into the oviduct. In chronic forms of clinical endometritis the scar tissue may replace the functional endometrium, resulting in periglandular fibrosis, cystic degeneration and/or atrophy of uterine glands. (Lewis, 1997; BonDurant, 1999; Sheldon, 2004; Sheldon and Dobson, 2004). The reported lactational incidence of endometritis varies from 7 % to 62 % (Gilbert et al 2005). During the puerperal period the uterus of almost all of the cows is contaminated, and perhaps in 90 % of them a mild, non-pathological form of endometritis develops. In majority of cows the local antimicrobial defence mechanisms can eliminate the pathogens, and this mild non-pathological form of endometritis resolves within some days (Sheldon, 2004). However, when following the incomplete recovery of puerperal metritis the uterus remains infected with *A. pyogenes* and GN obligate anaerobs (*F. necrophorum*, *Prevotella* and *Bacteroides* ssp.), or re-infected with these pathogens from the environment, clinical endometritis may develop. Unlike the other uterine pathogens the incidence and importance of *E. coli* gradually decreases with time: the involvement of coliforms in pathogenesis of clinical endometritis is only secondary (Paisley et al., 1986; Hussain, 1989; Hussain and Daniel, 1991a and 1991b).

The bovine *pyometra* is a closely related inflammatory disease, which develops after the first ovulation in presence of an active (sometimes persistent) luteal tissue, usually from about the 20th - 21st day onwards. Due to the luteal progesterone production the cervix is closed i.e. not permeable, consequently the mucopurulent or purulent exudate accumulates in the uterine cavity (BonDurant, 1999; Sheldon and Dobson 2004; Sheldon et al. 2006). When clinical endometritis occurs after the first ovulation, simultaneously with the formation of luteal tissue *pyometra* may develop, due to the progesterone-regulated closure of cervix. In these cows the (muco)purulent exudate accumulates in the uterus (Lewis, 1997; BonDurant, 1999; Sheldon, 2004; Sheldon and Dobson, 2004). Concomitantly, the corpus luteum (CL) may persist (Olson et al., 1984; Huszenicza et al., 1999). However, it is unknown, whether CL persistency is responsible for the *pyometra*, or is a consequence of it, and what the real underlying mechanism is.

As potent chemotactic signals stimulating the influx of PMN cells into the inflamed uterus, the local production of TNF α , leukotriens and also other eicosanoids (PGF $_{2\alpha}$ and PGE2) may be relevant in pathogenesis (Hussain, 1989; Hussain and Daniel, 1991a; Kindahl et al, 1992 and 1996; BonDurant, 1999; Seals et al., 2002). However, the local release of these inflammatory products, and/or their absorption from the uterus remains limited. Consequently, usually neither systemic signs are generated nor production of acute phase proteins is induced. So cows affected by endometritis or *pyometra* are rarely systemically ill, as a direct consequence of the uterine condition (BonDurant, 1999).

It has been supposed for a long time that in ruminants the postpartum uterus is less sensitive to invasion by pathogens until the first ovulation, and during the follicular phase of the subsequent cycles. However the susceptibility to bacterial invasion increases when functional CL is present (Paisley et al., 1986; Hussain, 1989; Hussain and Daniel, 1991a). In a model study progesterone treatment for a few days increased the susceptibility of animals to intrauterine inoculation with *A. pyogenes* and *E. coli*, reduced the circulating PGF $_{2\alpha}$, and elevated the plasma PGE2. Ovariectomy increased and progesterone treatment decreased the cellular immune response as measured by concanavalin A-stimulated lymphocyte proliferation. It was concluded that progesterone makes the postpartum uterus susceptible to infection, ovariectomy allows the dam to remain resistant, and uterine prostaglandins may mediate the process (Lewis, 2003 and 2004). Intrauterine inoculation of *E. coli* shortened the luteal life span in cattle (Gilbert et al., 1990). This finding is associated with intrauterine liberation of endotoxin triggering a subsequent endometrial PGF $_{2\alpha}$ release directly (Kindahl et al., 1996), and/or through the activation of local TNF α -related PGF $_{2\alpha}$ producing paths (Okuda et al., 2002). These mechanisms are able to cause premature luteolysis, if a sensitive CL is present on the ovary, and thus shorten the oestrus cycle, as proven by both experimental (Peter and Bosu, 1987; Peter et al., 1987; Kindahl et al., 1996) and field studies (Huszenicza et al., 1987 and 1999). So the life span of CL may be either shorter (in mild endometritis) or even longer (in severe endometritis / *pyometra*) than a physiological cycle (Huszenicza et al., 1999).

Diagnosis of clinical endometritis seems not difficult, but considering that uterus size and quality of content may show a large variation between individuals and strongly depend on the time from parturition, we have to admit that clinical diagnosis is quite subjective. At the assessment the actual stage of uterine involution should always be considered: 3 to 5 weeks after calving the uterus is still enlarged, has not been fully involuted. Later, however, it seems to be often normal at rectal palpation. Before the first ovulation and formation of the first CL the cervix is open. So in endometritis at least a small amount of (muco)purulent cervical discharge exists, although this may not always be noticed in standing animals. No systemic signs occur, in most cases also the milk production is normal (BonDurant, 1999; Sheldon, 2004; Sheldon and Dobson, 2004; Sheldon et al., 2006). Several attempts were made and reported to investigate and validate objective diagnostic criteria for endometritis. Rectal palpation itself is not reliable enough for the diagnosis (Sheldon et al., 2006). Using a sterile metal or a disposable foil-lined cardboard vaginoscope, the visualization of discharge expelled through the cervix and external orifice is justified and obligatory for the correct diagnosis. According to the findings of LeBlanc et al. (2002a), on day 21 after calving the proposed definition of clinical endometritis was the presence of purulent or malodorous uterine discharge detectable in the vagina and/or the cervical diameter greater than 7.5 cm. After day 26 also the mucopurulent discharge was considered as abnormal and proof of endometritis.

Diagnosis of pyometra by rectal palpation and/or transrectal ultrasound echography (ultrasonography, US) is based on the well defined signs of enlarged uterus, high volume of the accumulated uterine content, closed cervix (i.e. no visible discharge) and CL on the ovary (BonDurant, 1999; Sheldon, 2004; Sheldon and Dobson, 2004; Sheldon et al., 2006).

Subclinical endometritis

The recently defined *subclinical endometritis* occurs any time after the histological completion of the uterine involution (i.e. on and after week 8), and is characterized by endometrium extensively infiltrated with neutrophil granulocytes, which can be recognized only by cytological examination of endometrium. Samples for endometrium cytology (collected by cytobrush technique, or by low volume uterine flush) are fixed and stained with a modified Wright-Giemsa staining. Subclinical endometritis is a chronic, unapparent inflammatory process of endometrium with a relatively high proportion of PMN leukocytes in the uterus, which suppresses the fertility of affected cows. There is no or only a minimum of exudate accumulated in the uterus, resulting in the complete lack of cervical discharge with pathognomic property (Kasimanickam et al., 2004; Gilbert et al., 2005; Sheldon et al., 2006). The further details are out of the scope of this review.

Principles of prevention and therapy

Management of uterine infections at first sight seems only a sophisticated term for the therapy, however, it has a broader meaning: beside therapy, it involves prevention at individual cow, as well as at herd level. Since the factors causing impairment of the balance between uterine infection and self-defence mechanisms are quite complex, the preventive measurements should also be comprehensive (Paisley et al., 1986; Hussain and Daniel, 1991b).

On large-scale dairies, a key point of successful therapy is the timely diagnosis, based on the systematic scheduled monitoring of pp cows by transrectal palpation and vaginoscopy. Ideally, we recommend doing these examinations at days 1-3, 6-10, 14-21 and 28-35 postpartum, however, if someone performs a routine check for subclinical metritis, an examination at pp. days 40-60 may also be desirable. In daily routine of those large farms, these requirements can be fulfilled by scheduling two "reproduction days" a week (e.g. Monday, Thursday). In small herds kept on grazing land this regular checking is not justified due to the lower pressure of infection (Paisley et al., 1986; Hussain and Daniel, 1991b).

The aim and practice of the treatment is somewhat different for the different conditions. In RFM and dystocia stimulation of myometrial contractility is recommended by means of repeated use of oxytocin or PGF_{2α}, to prevent intrauterine stagnation of lochia, and so the massive intrauterine infection. There are some data on the limited success of administration of antimicrobials in such cases (Paisley et al., 1986; Hussain and Daniel, 1991b). However, recently beneficial effect of antibiotic treatment on reproductive performance was also reported (Drillich et al. 2003). *E. coli* is a pathogen of particular importance during this period of the postpartum: its presence is highly correlated with prevalence of *A. pyogenes* and GN anaerobes at 14 days post-partum (Dohmen et al, 2000). Methods treat intra-uterine infections with *E. coli* in the immediate postpartum period would probably be the most efficient to reduce endometritis later. However, in the practice it is difficult to estimate the realistic efficacy of these procedures.

When *puerperal metritis* has already developed, application of antibiotics active against *A. pyogenes*, *E. coli* and GN anaerobes should be administered, in order to treat the massive uterine infection and to prevent the further bacterial and septic complications. Beside that, ingredients which increase the tonicity of myometrium can also be administered (Melendez et al., 2004).

In a recent survey in Hungary (authors' unpublished observation) we checked the minimal inhibitory concentration) (MIC) of the main pathogens isolated from the uterus of cows with puerperal metritis, against oxytetracyclin (OTC) and amoxicillin, as well as of some cephalosporins (cephapirin and cefquinome) just recently have applied in therapy of this disease. Uterine swabs were collected from Holstein-Friesian cows showing at least the local clinical signs of puerperal metritis in 5 herds. Isolation and identification of the bacteria were performed according to the conventional bacteriological techniques. The strains were identified up to species levels using

appropriate biochemical tests and Bio-Merieux ATB automatic identifying system. For the determination of MIC 90 μ l amounts from broth containing two-fold concentration increments of antimicrobial agents were added to 96-well microdilution trays. Each well was inoculated with 10 μ l bacterial suspension (the inoculum was 1.5×10^5 CFU/ml). The MIC was read as the lowest concentration of the antibiotic at which no growth was recorded. A total number of 33 swabs were collected and cultured. The main isolated pathogens were as follows: *A. pyogenes* (n=28), *E. coli* (n=30), *F. necrophorum* (n=4), bile-resistant *Bacteroides spp.* (n=9), bile-sensitive *Bacteroides spp.* (n=24), black pigmented *Prevotella and Porphyromonas spp.* (n=10). High MIC ($> 1.0 \mu\text{g/ml}$) was measured against OTC at more than half of *E. coli* and at almost all Gram-negative anaerobes (except for the few strains of *F. necrophorum*). Also about 25% of *A. pyogenes* strains were resistant to OTC. Almost all *E. coli* and all bile-resistant *Bacteroides spp.* were completely resistant to amoxicillin (MIC $> 1.0 \mu\text{g/ml}$ and MIC $\geq 4.0 \mu\text{g/ml}$, respectively). Other Gram-negative anaerobes showed variable MIC, while majority of *A. pyogenes* strains possessed relatively high *in vitro* sensitivity (MIC $\leq 1.0 \mu\text{g/ml}$) to amoxicillin. All the isolated pathogens, but one *E. coli*, proved to be sensitive (MIC $\leq 1.0 \mu\text{g/ml}$) for cefquinome, a 3rd generation cephalosporin derivate. *A. pyogenes* and Gram-negative anaerobes showed 100% sensitivity against cephalirin, however, 75% of the *E. coli* strains had of MIC $\geq 4.0 \mu\text{g/ml}$ and the remaining 25% were even around $1.0 \mu\text{g/ml}$. We conclude that increasing MIC of OTC and amoxicillin against the main uterine pathogens is remarkable, as compared to our previous findings. This tendency justify new ways in antimicrobial therapy of APE in dairy cows.

Local use of OTC is the most traditional way of antimicrobial therapy. However, due to the decreased susceptibility of uterine pathogens to OTC the efficacy of this therapy is decreasing nowadays, and also the locally irritative character of tetracyclines is emphasized. The MIC of OTC against the main uterine pathogens has been increased during the last 10-15 years, which would require very high (2-4 g/day) doses for 3-5 days. The expected *in vivo* efficacy of other traditional antimicrobials (amoxicillin, aminoglycosides) is questionable (Paisley et al., 1986; Hussain and Daniel, 1991b; Cohen et al., 1993, 1995 and 1996; Sheldon et al, 2004a; Pécsi et al., 2007b). Recently, therefore, extensive research has been and is being performed with new (third and forth) generation cephalosporins. Beside the susceptibility of the most important uterine pathogens (very low MIC values; Sheldon et al, 2004a) these ingredients have ideal pharmacokinetic properties with short withdrawal periods for milk and meat. Some experiences were already reported with systemic administration on cows with fever (Drillich et al., 2001; Chenault et al, 2004; Melendez et al., 2004), and also promising results have been achieved with intra-uterine use (authors' unpublished observation). Local versus systemic administration of antibiotics is a long debate. An intra-uterine treatment leads to high concentrations of the drug in the uterine cavity and the endometrium, and relatively small amount is absorbed into the systemic circulation (Masera et al. 1980). Systemic administration of antibiotics results in concentrations in the uterine tissue and lumen that are at best comparable to plasma levels, and which often do not reach minimal inhibitory concentrations against uterine pathogens. However, a disadvantage of parenteral treatment is the higher total quantities of

antibiotics needed to reach similar concentrations. Furthermore, a systemic antimicrobial treatment is not justified as long as the infection remains localised to the uterus. Systemic antibiotic administration (Chenault et al, 2004) has its primary importance in the treatment of the most serious cases of metritis, when the infection has some consequences beyond the reproductive apparatus. For these toxic potentially life-threatening cases, beside systemic antimicrobial therapy, treatment procedure improving the general condition (non-steroidal anti-inflammatory drugs, fluid plus electrolyte replacement) are also necessary (Paisley et al., 1986; Hussain and Daniel, 1991b).

Efficacy of therapy is improved, if simultaneously with administration of antimicrobials, also the predisposing metabolic disorders (like hypocalcaemia or hyperketonaemia) would be treated and compensated. This improvement would be especially important in large-scale dairy units, where cows calve year round, the animals are kept under total confinement conditions with no pasturing, and fed with total mixed ration. However, the “on-spot” identification of cows affected by subclinical forms of these metabolic malfunctions is not easy. Presence and degree of ketonuria is a simple, practical parameter for the detection of elevated ketone production in the decompensated stage of post partum negative energy balance in dairy cows. A field trial (Pécsi et al., 2007a) was carried out in Hungary to assess the effect of treated and non-treated ketonuria on the course and clinical cure of puerperal metritis (treated with a standard dose i.u. OTC), as well as on the reproduction performance afterwards. It was concluded that in metritic cows characterized by ketonuria and received no antiketogenic treatment, the course of uterine involution was slower, resulting in more prolonged recovery period. These cows required more antimicrobial treatment, and their reproductive performance was poorer, as compared to the metritic cows without ketonuria, or to those having ketonuria, but received proper antiketogenic treatment. However, no difference was observed between the latter groups.

At clinical and subclinical forms of *endometritis* treatment should finally be directed to improve fertility by elimination of pathogens from the uterus. In therapy of pyometra also luteolysis should be initiated. So currently preparations containing natural PGF_{2α} or its synthetic analogues have been widely used for treatment of endometritis and pyometra in most of the production systems for many years (Etherington et al., 1994; Nakao, et al., 1996; Feldmann et al., 2005). As antimicrobials the first-generation cephalosporins (cephapirin) can be the drug of choice. Efficacy of intrauterine treatment with cephalapirin has been defined in several large field trials, which demonstrated an improvement of reproductive performances (McDougall, 2001; LeBlanc et al, 2002b; Kasimanickam et al., 2005). Antibiotic treatment can lead to a more rapid clinical cure of the animal which helps to a return to normal of reproductive functions. However, also the importance of prevention should be underlined.

Conclusion

Uterine infections and the consequential diseases have detrimental effects on reproductive performance of dairy cows. Therefore, better understanding in aetiology and pathogenesis, improvement in diagnosis and therapy is of great practical and economic importance. We recommend a scheduled monitoring system for large-scale dairies to achieve timely diagnosis and thus, more efficient therapy. Despite the impressive results obtained during the past two decades, there is a substantial space for further research concerning subjects like the role of different inflammatory mediators in the pathogenesis and the precise description of host's immune response mechanisms. There are even more practical aspects to be improved as well, namely: less subjective diagnostic tools, as objective and uniform measurement systems to assess the real clinical and bacteriological efficacy of the different therapeutic protocols.

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11.9 THE INCIDENCE OF SUBCLINICAL ENDOMETRITIS DIAGNOSED BY INTRAUTERINE CYTOLOGICAL EXAMINATION IN GRAZING DAIRY CATTLE IN ARGENTINA

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Postpartum endometritis is one of the most common diseases in dairy cattle and has a negative impact on reproductive performance, followed by economic losses to the farmer. Recent studies have shown that even subclinical endometritis reduces conception rates and prolongs days open. Subclinical endometritis can be diagnosed by cytological examination of the endometrium. Using the cytobrush-method, the proportion of polymorphonuclear neutrophils (PMN) in the smear determines the presence of subclinical endometritis. Threshold values of PMN for the presence of subclinical endometritis are not defined consistently in literature and vary between 5 and 25%. Most of these studies, however, were conducted on dairy farms in North America and Europe. No studies on the occurrence of subclinical endometritis in South America have been published yet. It can be hypothesized that the situation in South America with regard to e.g. climate and pasture systems influences the incidence and impact of subclinical endometritis.

The objective of the study was to determine the incidence of subclinical endometritis in grazing cattle in dairy farms in Argentina. The study was conducted on 4 dairy farms in Buenos Aires Province, Argentina. All cows were examined for clinical signs for chronic endometritis, i.e. vaginal discharge. Cows with no vaginal discharge were enrolled in the study. Diagnosis of subclinical endometritis was performed by cytological examinations with the cytobrush-method. A small brush mounted on the tip of a metal rod and protected by a disposable catheter was inserted through the vagina into the uterus. Cells were collected by rotating the cytobrush while in contact with the uterine wall. The cytological smears were fixed, stained and evaluated under a microscope at 400x magnification. A total of 160 cytological samples were collected from 98 cows between 17 and 75 days postpartum. The interval between two samples was at least 14 days. Statistical analyses revealed a negative correlation between days postpartum and the proportion of PMN ($p < 0.05$). At the first cytological examination between 17 and 38 days postpartum ($n=76$), the proportion of cows with 0% PMN was 21.1%. The proportion of cows with $\geq 5\%$ PMN and $> 15\%$ PMN was 39.5% and 18.4%, respectively. These are the first data on the occurrence of subclinical endometritis in dairy cows in South America.

Further analyses will provide informations on the impact of subclinical endometritis on reproductive performance and help to define a threshold value of PMN for the presence of subclinical endometritis in grazing dairy cows.

11.10 INFLUENCE OF UTERINE TORSION WITH SURGICAL TREATMENT ON THE METABOLISM OF LIVER, KIDNEY AND MUSCULAR SYSTEM.

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Objective: The aim of this study was to investigate the influence of uterine torsion intra partum and necessary surgical treatment on the metabolism of liver, kidney and muscular system during the first 10 days postoperatively in contrast to surgical treated dystocia of different genesis or normal parturition respectively.

Material and methods: 45 bovines with surgical treated uterine torsion intra partum were included in the investigation. Blood samples were taken from V. jugularis externa, every 24 h before surgical treatment as well as afterwards, continuing for 10 days postoperatively. The activities of the enzymes GLDH, AST, LDH and CK and of the substrates β -hydroxybutyric acid, bilirubin, cholesterol, urea, creatinine and lactate were determined. Control groups were surgical treated bovines of other dystocia and bovines with normal parturition.

Results: During the first four days after surgery the activities of GLDH, AST and LDH of bovines with uterine torsion were particularly noticeable and significantly increased in comparison to bovines with dystocia of different genesis or to bovines with normal parturition respectively. The increase of enzyme activities reflects a distinctly affected liver metabolism during this time. The kidney function is less affected by uterine torsion in contrast to the flow of urine, which is apparently greatly affected.

Conclusion: Cows with surgical treated uterine torsion intra partum are substantial different from those with surgical treated dystocia of different genesis or normal parturition. Clinical relevance: Preoperative a substantial increased creatinine concentration at time of admission indicates a poor prognosis quoad vitam. After surgery a liver-protective therapy is recommended to bovines with surgical treated uterine torsion intra partum during the first four days.

11.11 EFFECTS OF GRINDING INTENSITY / PARTICLE SIZE OF THE DIET ON FECES QUALITY IN SOWS AT DIFFERENT STAGES OF REPRODUCTION

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Problem: Hard and dry feces are quite often a problem in pregnant sows, especially near to parturition. Obstipation is thought to be one of the most predisposing factors for development of MMA (mastitis, metritis, agalactia) in sows (1). Among other factors like low moving activity due to housing conditions and scant water supply a generally accepted factor is the feed (amount and chemical composition, esp. crude fibre content) offered to sows (2). **Aim:** The study was conducted to check influence of grinding intensity of the ingredients in diets for sows (identical chemical composition) on feces quality.

Material and methods: The study was performed under farm conditions in pregnant (ps), periparturient (pps) and lactating sows (ls). Housing of sows differed: ps were housed in a group, while pps and ls were housed individually (beginning one week before parturition till the end of lactation). Sows were fed once (ps) respectively two times a day (pps and ls). Feeds differed regarding to the stage of reproduction (common diets for pregnant and lactating sows were used). The diets were identical in botanical and chemical composition, but differed in grinding intensity. In ps three different grinding intensities (hole size of sieves in hammer mill: 2, 5 and 8 mm) were used, while in pps and ls only two variants were used (2 and 8 mm). Special emphasis was given to the water content and the consistency of feces.

Results: Lower grinding intensity of the feed caused significantly more moisture and softer feces as could be demonstrated by measurement of dry matter content and penetration depth of a cone of a penetrometer. The observed effect was greater after feeding the coarsely ground diet compared to the medium ground diet(3).

Conclusions: If problems concerning hard and dry feces occur under field conditions not only well known effects as crude fibre content in the diet should be taken into consideration but also grinding intensity of the components in the diet. It has to be mentioned, that the grinding intensity in pelleted feeds can not or only hardly be controlled optically. Influence of feed grinding intensity is worth mention not only regarding gastric ulcer but also regarding feces quality and consistency in sows.1) Martineau et al (1992), *Vet. Clin. North. Am. Food Anim. Pract.* 8, 6612) Tabeling et al. (2003), *J. of Anim. Physiol. and Anim. Nutr.*, 87, 116-1213) Warzecha, A. (2006), http://elib.tiho-hannover.de/dissertations/warzechaa_ws06.pdf

11.12 INFLUENCE OF DIFFERENT DOSE OF SELENIUM AND VITAMIN E ON METABOLISM, HEALTH AND COLOSTRUM QUALITY IN DAIRY COWS

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The aim of the work was to study the influence of different dose of parenteral administration of selenium and vitamin E in dairy cows prior to parturition on selected metabolic parameters, health and colostrum quality. A total of 19 dairy cows from a farm with selenium deficiency were included in the study. The cows were divided in 3 groups (C, E1, and E2). In group E1 (n=7) a product containing selenium and vitamin E (44 mg of sodium selenite and 500 mg of alpha tocopherol acetate) was administered once during the dry period (4 weeks prior to expected parturition) IM; E2-experimental group of 7 animals to which the injectable product was administered twice during the dry period (8 and 4 weeks prior to expected parturition), i.e. in the total dose of 88 mg of sodium selenite and 1 000 mg of alpha tocopherol acetate. Group C (n=5) consisted of control animals to which no product was administered. On the day of parturition samples of blood and first colostrum were collected for laboratory examination. Concentrations of selenium were determined in blood and that of vitamin E, thyroid hormones (T3 and T4) and activities of enzymes detecting muscular damage (CK, AST, LD) were determined in serum. Colostrum was analysed to determine the concentrations of selenium, vitamin E, immunoglobulins, as well as to determine its density. The occurrence of the disease during the first month after parturition was evaluated in all groups. Higher concentrations of selenium and vitamin E were found in the samples (experimental groups E1 and E2) collected on the day of parturition. Group E2 showed a significantly higher ($p<0.05$) T3 concentration compared to groups C and E1 (3.05 ± 0.42 nmol/l vs 1.88 ± 0.71 and 1.81 ± 0.30 nmol/l, respectively). The same pattern was confirmed for immunoglobulins concentrations in colostrum (34.08 ± 5.93 UZST vs 22.87 ± 5.41 and 21.38 ± 8.33 UZST, respectively). Compared to group C, cows in group E2 also showed significantly ($p<0.05$) higher concentrations of selenium in colostrum (45.43 ± 10.56 vs 29.29 ± 8.42 $\mu\text{g/l}$). The administration of selenium and vitamin E did not influence other parameters evaluated in the study. During the first 30 days of the postpartum period a trend of lower occurrence of mastitis was observed in group E2 compared to both group C and E1 (no case of mastitis compared to 5 and 4 cases of treated mastitis, respectively). It can be therefore concluded that the administration of selenium and vitamin E to pregnant dairy cows showed a positive effect on the some metabolic parameters (T3, selenium and vitamin E concentrations in blood), quality of colostrum, and health.

11.13 EFFECTS OF OXYTOCIN APPLICATION OF IN THE EARLY LUTEAL PHASE ON THE OESTRUS CYCLE LENGTH IN LACTATING DAIRY COWS

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Problem: There is some evidence in literature that oxytocin application in the first week of the oestrus cycle led to premature luteolysis between 8 and 12 days after ovulation. **Aim:** The aim of the present study was to verify, if repeated applications of 20 IU oxytocin influence the length of the oestrus cycle in lactating dairy cows.

Material and methods: Ten pluriparous, lactating Holstein Friesian cows, which were kept in tie-stalls, were examined every two days and from day 10 after ovulation on daily via transrectal ultrasonography for follicular growth, ovulation time and the development of the corpus luteum. From the tenth day of the oestrus cycle on blood samples were collected to determine serum concentrations of progesterone and estradiol-17 β . In group 1 for each cow three complete, non-influenced oestrus cycles were observed. During the fourth cycle (group 2), each cow received 20 international units (IU) oxytocin intravenously from day 2 until day 6 after ovulation. Cycle length, maximum diameter of the dominant follicle, concentrations of hormones and expression of oestrus behaviour were evaluated by means of single-factorial variance analysis.

Results: The mean oestrus length was 21.9 ± 1.6 days in group 1 and in 21.1 ± 2.1 days in group 2 ($p = 0.19$). The diameter of the preovulatory follicle was not influenced either by the application of oxytocin (15.9 ± 1.6 mm in group 1 and 16 ± 2.1 mm in group 2, $p = 0.98$). Also the concentrations of progesterone and estradiol-17 β and the expression of oestrus symptoms showed no significant difference between both groups ($p > 0.05$). **Conclusions:** The commonly used dosage of 20 IU oxytocin for the treatment of udder diseases does not influence different parameters of the oestrus cycle in dairy cows. In contrast to the elder bibliographical references we could not cause short oestrus cycles by application of oxytocin. To what extent variations of the treatment regime (dosage of oxytocin, period of the application, route of application) may cause different results has to be investigated in further examinations.

11.14 RELEVANCE OF ACUTE PHASE PROTEINS (HAPTOGLOBIN) FOR THE EARLY DIAGNOSIS OF FERTILITY DISORDERS

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Fertility of dairy cows has considerably deteriorated during the recent years. Research on reasons is therefore most important. As a rule disorders before and during calving as well as during puerperium precede conception problems. An early diagnosis and pathophysiologic characterisation are required for an effective pro-phylaxis. The acute phase reaction (APR) can be integrated into the diagnostic process. The major acute phase protein (APP) in cattle is the haptoglobin (Hp); it is stimulated by cytokines which can represent their impact. Besides, cytokines influence the energy metabolism (Insulin) directly, hence the analyses of Hp reflects the stress on the metabolism.

Objective: a) Are there any relationships between the peripartally measured Hp-concentrations and the fertility relevant functions b) Does Hp have any early diagnostic information for later fertility disorders? Experimental design: 969 cows and heifers (milk yield 9350 kg/year) were examined -clinically as well as clinical-chemically during one year at 28 and 10 days before parturition (a.p.) as well as 3 and 28 days after parturition (p.p.) From healthy cows (BFT < 25 mm, leukocytes < 10 G/l, no diseases up to 12 w.p.p.) as well as cows with various diseases and fertility relevant disorders, samples were obtained and bio-chemically analysed (Hitachi 912, Boehringer Mannheim). Hp was analysed using the test set by Tridelta, Dublin.

Results: Increases in Hp-concentrations have started to rise at approx. 1 to 2 weeks a.p. 10 days before parturition, Hp-concentrations are significantly higher in cows with premature birth (median = 0.83 g/l) and twins (0.40 g/l) as well as after parturition endometritis (0.33 g/l). 3 days after parturition, Hp-concentrations are significantly increased in cows with twins (1.86 g/l), premature birth (1.85 g/l), endometritis (1.52 g/l) retained placenta (1.51 g/l), ovarian cysts (1.22 g/l) as well as dystocia (0.92 g/l). This period of time reflects the time of the disturbed puerperium as shown in earlier studies. But 4 weeks after parturition, Hp-concentrations are physiological again. FFA information is comparatively more sensitive and lasts longer; however Hp indicates specific disorders in the energy metabolism via cytokine which potentially may be taken from the fat tissue.

Conclusion: Hp concentrations increase approx. 10 d a.p. in cows that later have endometritis and premature or twin births ($p < 0.05$). Hp concentrations are risen in calving and puerperal disorders 3 p.p. ($p < 0.05$). Hp especially correlates with FFA, but it is not such a long time changed. They mainly metabolic disorders persist after retained placenta and are dominant in cows with posterior ovarian cysts during early lactation.

11.15 RELATIONS BETWEEN NATAL COURSE OF MILK COWS AND HEIFERS WITH REGARD TO ENDOCRINOLOGICAL PARAMETERS

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Problem: It is known that heavy natal courses show endocrine differences compared to normal birth, however, if this due to an undeveloped endocrinium or to the stressful situation throughout birth is still under discussion. **Aim:** The aim of present study was to examine the endocrine situation of heavy natal course and normal birth in milk cows (n=15) and heifers (n=13) shortly around birth.

Material and methods: For this study 17 β -estradiol and cortisol concentration were measured in the blood of cows, heifers and their calves between 14 days ante partum (a.p.) to 14 days post partum (p.p.).

Results: The maximal concentration of estradiol and cortisol were apparent in all mothers at the time point of birth. However, multipare animals showed higher estradiol values than heifers (382 +/- 130 vs 255 +/- 59 pg/ml, p<0.01) at birth as well as significant higher levels from 7 d a.p. to 12 h p.p. (p<0.05). The multipare animals with heavy birth had higher estradiol concentrations than those with normal birth between 4 d and 1 d a.p. (325 +/- 80 vs 203 +/- 20 pg/ml, p<0.001), such as cortisol values. The milk cows and heifers with heavy natal courses had also higher cortisol levels compared to those with normal birth. Interestingly, the cortisol concentrations of newborn animals of heavy natal course were significantly higher than of calves with normal natal course (36.2 +/- 5.1 vs 20.7 +/- 4.82 ng/ml, p<0.001).

Conclusions: In summary, a significant increase of estradiol and cortisol levels is required for normal birth, however, exorbitant high levels seems to be related to heavy natal course in cows and heifers. If these extreme levels of stress hormone and female sex hormone levels within the mother and its calf will have an influence on postnatal development of the calf needs further clarification.

11.16 THE EFFECTS OF LEVAMISOLE ON REPRODUCTIVE QUALITY IN BOARS

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The aim of research was to establish if the application of immune system synthetic modifier levamisole will have an effect on nonspecific immunity and sperm parameters in boars. Blood samples were taken once a week for 7 weeks before and 7 weeks after test treatment with levamisole. Semen was collected twice a week and were monitored during 7 weeks before and after application of levamisole and physiologic solution. Its determined significant increase in number of leukocytes, monocytes, Beta - and Gamma-globulines, neopterin, erythrocytes, hemoglobin, hematocrit and albumines in the group treated with levamisole, while the C-reactive protein level was similar in all groups. In the treated group we determined significantly lower number of trombocytes and level of Alpha-globulines. Evaluating sperm parameters we determined significantly higher volume, concentration and total sperm doses number, as well as the sperm total density and sperm motility in the group treated with levamisole. It is concluded that different stressful situations that weaken immune response in boars which has been proven in periods of high collection frequencies could be avoided by application of levamisole. Based on this research we have also concluded that levamisole had an influence in purpose of improvement in boars reproductive quality.

11.17 PATHOLOGICAL FINDINGS IN OVARIES AND ENDOMETRIA FROM CATTLE WITH FERTILITY DISTURBANCES

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Fertility disturbances are common in high-performance dairy cows. In many cases, alterations of the endometrium are clinically not detectable. The first aim of this study is to survey pathological findings in ovaries and endometria of infertile or subfertile cows. The second aim is to verify if the endometrial biopsy is a useful tool for the diagnosis of endometrial alterations. For these purposes, the genital tracts of 85 cows, culled because of low fertility or infertility, were investigated macroscopically and histologically. From 23 of these cows, two endometrial biopsies were taken one to 3 days before slaughter. Findings in biopsies were compared to findings in post mortem investigations of the uterus. 32 % of the animals showed ovarian cysts, thereof 50 % graafian follicle cysts, 42 % luteinized cysts and 8 % epithelial inclusion cysts. In 11 % ovarian neoplasms (granulosa cell tumor and rete adenoma) were found which had not been detected by clinical methods. In 4 % of all cases, a purulent endometritis was diagnosed macroscopically, 16 % exhibited clear mucus within the uterus, and 80 % showed no macroscopically detectable uterine alterations. In contrast, by histopathological investigation all endometria revealed alterations, varying in quantity and quality. 92 % showed a periglandular fibrosis ("bovine endometrosis"), 77 % angiopathies, and 63 % an endometritis, mostly nonpurulent. 35 of 46 biopsies were usable for lightmicroscopy, 11 showed extensive artefacts due to the extraction. The findings in biopsies showed a far-ranging conformance to those in post-mortem examinations of the uterus. The results show that the majority of cows with subclinical fertility disturbances exhibit endometrial findings which are not apparent by conventional clinical examination but detectable by biopsy. A comparison of findings in the biopsies and the whole uteri revealed nearly identical diagnoses taking the quality and quantity into account. Therefore the endometrial biopsy is a potential tool for the diagnosis of subclinical endometrial alterations in infertile / subfertile cattle.

11.18 ANTIOXIDATIVE STATUS IN SOWS DURING THE PERIPARTURIENT PERIOD IN A FARM WITH AN HIGH INCIDENCE OF MMA

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Problem: The mastitis, metritis, agalactia complex (MMA) in sow is the most important puerperal disease in the industrial pig production. The typical clinical picture with mastitis, metritis and agalactia has changed in the last years. Nowadays we see different clinicals forms and courses which can vary from farm to farm. Because of the high economical relevance for the farmers the most important way is to search for practicable possibilities for prophylaxis of MMA. **Aim:** From studies in other animal species is known that the antioxidative system can be variably stressed during pregnancy and lactation. This can have a significant effect on the health of the animals. In this study the underlying stress of the antioxidative system, especially superoxide dismutase (SOD), glutathione peroxidase (GPX), trolox equivalent antioxidative capacity (TEAC) and Vitamin E, was investigated during the periparturient period in sows in a farm with a high incidence of MMA. Especially was tested if it is possible to get retrospective information about the susceptibility to disease.

Material and methods: Blood samples were taken four times during the periparturient period from 67 sows of a pig production farm in Thuringia. The parameters of the antioxidative status (SOD and GPX activity, TEAC and Vitamin E concentrations) were measured. The sows were divided in two groups, depending on whether they stayed healthy or fell ill for MMA.

Results: The parameters of the antioxidative status of the sows with later MMA was different from the healthy sows even before parturition. Such was the SOD activity in ill sows one week ante partum significantly higher than in healthy sows. In contrast the GPX activity and TEAC concentration were significantly lower than in healthy sows. After parturition the SOD activity in healthy sows increased until eight days post partum whereas it decreased in ill sows. The Vitamin E concentration was in both groups one week ante partum alike and decreased one day after parturition in both groups significantly. Altogether showed all parameters a great deviation in both groups.

Conclusions: The results allow the conclusion that the antioxidative status of sows with later MMA is more stressed before parturition an in healthy sows. An important point to prevent the development of MMA is therefore a good management and a suitable feeding already before and during parturition to reduce possible stress for the sows.

12 UDDER HEALTH, MILK QUALITY

12.1 VARIATION IN THE UNDERLYING MECHANISMS OF INNATE DEFENSE AND OUTCOME OF BOVINE MAMMARY E.COLI INFECTIONS

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Abstract

Mastitis is the most costly production disease in dairy herds worldwide. During the last years the proportion of Gram-positive mastitis has declined, while Gram-negative mastitis has risen. Progress was made in reducing subclinical contagious mastitis but these measures didn't have any impact on Gram-negative mastitis. Gram-negative pathogens commonly implicated are *E. coli* and *Klebsiella pneumoniae*. Neutrophils migrate from blood to the mammary gland in response to infection and constitute a major host defense mechanism. Many factors related to stage of lactation and parity may influence normal neutrophil function. The innate defense mechanisms become temporarily impaired during the periparturient period and the greatest effect of *E. coli* on the mammary gland is at that period. Of those cows with severe Gram-negative infections, nearly 25% will either die or culled. Endotoxin or lipopolysaccharide (LPS), the major constituent of the Gram-negative cell wall is released during bacterial growth and destruction, and is implicated in the pathogenesis. LPS provokes host cells to produce large amounts of pro-inflammatory mediators by activating transcription factors. One target is NF-kappa B, a key regulator of the inflammatory response. Cellular recognition of LPS is mediated by the transmembrane Toll receptor-4 (TLR-4). LPS also binds to CD14 and this is greatly enhanced by LBP. There is strong evidence for a protective role for CD14 and LBP in mediating the host response to Gram-negative infections. Although the detection of LPS is a critical event in the activation of the innate response to Gram-negative bacteria, excess of LPS signaling can lead to exaggerated host responses culminating in the development of septic shock. Although antimicrobial agents are continuously being developed, intramammary infection with *E. coli* in a compromised host remains a serious problem. No antibiotic can clear infections without a good functional immune system. Therefore, the development of novel interventions that modulate the inflammatory response elicited by LPS and/or contribute to the elimination of the pathogen, remains an important animal health priority.

Innate immunity in the bovine mammary gland

Innate immunity is the most universal and the most rapidly acting type of immunity. Invertebrates survive through innate immune mechanisms alone. Vertebrates developed alternative systems for pathogen recognition and elimination, called adaptive immunity. The innate immune system is an evolutionary old system that has been refined for a longer period of time than the adaptive immune system. It is more perfect in almost every way (Beutler, 2004). In the lactating bovine mammary gland it is the most important defense system. Vaccinating cows against mastitis generally is not very successful (Nickerson, 1985). The elimination of even one subset of innate immune effector cells (for example, neutrophils) may be sufficient to cause a profound immunodeficiency state (Jain et al, 1971).

Cells are not required for all innate immune reactions. Proteins and other molecules are sufficient to kill microbes that have not yet been engulfed by cells (Beutler, 2004). Complement, lysozyme, lactoferrin, and antimicrobial peptides are among the proteins most commonly cited in this regard (Grün, 1985 ; Malinowski, 2002 ; Schmitz et al., 2004). There is no doubt that several innate immune proteins provide a measure of protection in the mammary gland cisterns. However, in the bovine udder the protective role of some of them seem to be unimportant (e.g. lysozyme is not a significant defense protein in the bovine mammary gland). Furthermore some of them have actions that depend on the stage of the lactation cycle (e.g. lactoferrin, only effective during steady state involution in absence of citrate; Smith and Schanbacher 1977; Rejman et al. 1989). At the end of this review special attention will be drawn on the protective effect of CD14 and LPS binding protein (LBP).

Innate immunity is an enormously broad study object and one effector, the bovine neutrophil, has been extensively studied over the last 20 years (see reviews of Paape et al., 2000, 2002 & 2003). Three aspects of innate immunity are important in the defense against intramammary *E.coli* infections : 1) the afferent (sensing) arm that recognizes a diverse array of pathogens, 2) the efferent (effector) arm that kills these pathogens. Each arm is divided into cellular and humoral components. The molecules that sense microbes are not necessarily the same as those that kill them, and 3) sparing tissues of the host ; i.e. there must be self-tolerance (Beutler, 2004).

The innate immune system of the bovine mammary gland seems to have a battle plan against intramammary infections. The underlying mechanism seems to be dependent on the stage of lactation ; i.e. dry period, colostragenesis and established lactation (Burvenich et al., 2003 & 2004) and parity (Vangroenweghe et al., 2004a & 2004b).

Intramammary infections and early mammary involution

Susceptibility of the mammary gland to new intramammary infections is markedly increased during early involution (drying off ; active involution, first week or two, highest incidence of new infections) and during the periparturient period

(colostrogenesis) (Nickerson 1989; Oliver and Sordillo, 1988). These periods coincide with unique local and systemic physiological phenomena that interrupt or induce lactation. Considerable changes in mammary tissue remodeling and nutritional demands occur.

A number of local phenomena contribute to the high incidence of new intramammary infections during the early dry period. Milk, an excellent growth medium for bacteria, is no longer periodically removed from the gland and leakage from the teats occurs because of the increase in intramammary pressure during the first days. Leukocytes, mainly phagocytes, start entering the gland by day +6 after dry-off (McDonald and Anderson, 1981). The cell counts do not reach protective levels until day 8, about 1 million. One of us just finished an experiment where the cell count was increased to a million over the first 8 days by injecting an irritant into the gland and we got better protection than with antibiotics (Paape, 2005, USDA Patent Application S.N. 10/895, 797, November 12, 2004, Methods for Prevention and Treatment of Mastitis). An immunocompromised condition of these phagocytes is established through local factors. Indeed, after diapedesis, phagocytes start ingesting milk fat, casein and cell debris. Ingestion decreases the phagocytic function and induces apoptosis. The citrate : lactoferrin ratio is very high which makes any bacteriostatic action of lactoferrin ineffective. Citrate chelates iron and make it available to bacteria.

Intramammary infections and mammary steady state involution

In the cow, active involution is completed by 3 to 4 weeks after drying off and followed by a phase of steady state involution. This is the period of greatest resistance to intramammary infection ; especially to Gram-negatives. If an infection occurs, it usually is spontaneously eliminated. This mid-dry period shows the lowest incidence of new infections because teats have become sealed, the small fluid volume in the udder cisterns and the unfavorable composition of medium to bacterial growth. Very high concentrations of phagocytes are reached. The immunocompromised condition is suspended because there is practically no milk fat or casein that could eventually inhibit phagocyte function. Defensins are relatively small, cationic peptides with an amphiphilic charge distribution that enables them to interact with, and disrupt, bacterial cell membranes. Their antibacterial action appears to result from their ability to form pores in target membranes leading to cell lysis. Defensins are produced in neutrophils and macrophages (Kaiser and Diamond, 2000). Lactoferrin, the major protein found in mammary secretions during steady state involution reaches very high concentrations (Smith and Schanbacher 1977 ; Rejman et al. 1989). The mechanism of lactoferrin activity has not been clearly defined but appears to originate primarily through iron sequestration and/or through direct interaction of its cationic N-terminal region with bacterial components. Bacteria which have high iron requirements are susceptible to lactoferrin. Among mastitis pathogens, *E. coli* are the most susceptible, followed by *S. aureus*. Streptococci seem to be resistant (Rainard, 1986a). The citrate : lactoferrin ratio is lowered (because milk synthesis has stopped) and immunoglobulin concentrations are elevated. The bacteriostatic activity of lactoferrin can be enhanced

by antibodies specific to mastitis-causing bacteria, possibly by interfering with the bacterial iron-acquisition systems (Oliver and Bushe, 1987; Rainard, 1986b).

Intramammary infections and end of dry period

Transition cows are referred to as cows in their second half of the dry period through 2 to 4 weeks postpartum. Any physiological maladaptation from a pregnant, non-lactating to a non-pregnant, lactating status represents a challenging period in the production cycle of a modern dairy cow. Most metabolic diseases of dairy cows such as milk fever and ketosis, and abomasal displacement occur within the first 2 weeks of lactation.

From the second half of the transition period and especially during colostrogenesis, cows become more susceptible to new intramammary bacterial infections (Smith et al., 1985a & 1985b). The contribution of lactoferrin to protection of the mammary gland is compromised by its low concentration in milk and the presence of citrate. Milk citrate can effectively compete with lactoferrin for iron binding and the resulting iron-citrate complex can be utilized by bacteria (Schanbacher et al., 1993). The phagocyte numbers not only decrease but their phagocytic capacity is reduced again because of the appearance of fat and casein (immunocompromised condition induced by local factors). However, during colostrogenesis systemic factors also affect the immune condition of the cow (immunocompromised condition induced by systemic factors). In addition the majority of infectious diseases experienced by the dairy cow also become clinically apparent during early lactation. This is especially the case for mastitis, but also includes diseases such as Johne's disease and salmonellosis.

Severe septic mastitis during the periparturient period

A high proportion of intramammary coliform infections present at parturition, may develop disease characterized by severe inflammatory signs and sepsis during the first 60-70 days of lactation (Malinowski et al., 1983, Smith et al., 1985b, Smith et al., 1985a, Hogan et al., 1989). The clinical picture is reputed as toxic mastitis and is very well known by veterinarians. Of those cows with severe Gram-negative infections, nearly 25% will either die or be culled. There is remarkable variability in the clinical expression and complications of coliform mastitis around parturition, ranging from clinical severe to moderate and mild (Hill et al., 1979, Hill, 1981, Heyneman et al., 1990, Vandeputte-Van Messom et al., 1993, Burvenich et al., 1994, Shuster et al., 1996, Hirvonen et al., 1999, Burvenich et al., 2003). This is in sharp contrast with the more moderate clinical expression of coliform mastitis during established lactation (Burvenich et al., 1994, Shuster et al., 1996). Delays in the inflammatory response in cows with peracute coliform mastitis have been reported in certain cows shortly after calving (Hill et al., 1979, Hill, 1981, Heyneman et al., 1990, Vandeputte-Van Messom et al., 1993). The autotoxic character of the severe inflammatory response to infection falls in the category of sepsis research. The major point to be made about sepsis is that

the same mechanisms that contain a small infection can, if generalized, threaten the survival of the host. Nonetheless, evolution has calculated that it is best that these mechanisms be maintained (Beutler, 2004).

Cow parity and severity of clinical mastitis

There is a tendency to see more severe clinical coliform mastitis cases in multiparous cows (Van Werven et al., 1997, Mehrzad et al., 2001a & 2001b, Mehrzad et al., 2002, Burvenich et al., 2003, Vangroenweghe et al., 2004a). Blood neutrophil function was higher in younger animals than in cows after their 4th parturition. The drop in neutrophil ROS production around parturition is more pronounced in multiparous cows (Mehrzad et al., 2002). The pronounced reduction in neutrophil oxidative burst capacity and viability in milk neutrophils of multiparous cows may be involved in the underlying mechanisms that make these animals more susceptible to periparturient infectious diseases. Moreover, white blood cell viability and oxidative burst have been found to be significantly different between primiparous cows and multiparous cows during the periparturient period. Using relative high *E. coli* inoculum doses, all primiparous cows reacted as moderate responders based on their quarter milk production in the non-infected quarter on day +2 post-infection. Based on clinical severity scoring, all animals were scored as mild to moderate in their clinical response throughout the entire experimental challenge period (Vangroenweghe et al., 2004a & 2004b).

The efferent arm of innate defense

In vertebrates, innate immunity is largely dependent upon myeloid cells. Professional phagocytes engulf and destroy pathogens. The polymorphonuclear phagocytes (which include neutrophils, basophils, and eosinophils) are of key importance in the containment of infection. In particular neutrophils are specialized killers, endowed with a broad array of weapons with which to destroy their microbial prey (Beutler, 2004).

Detilleux et al. (2006) designed a mechanistic mathematical model of the acute inflammatory response to *E. coli*, for which estimated parameters were based on studies of the cellular kinetics during experimentally induced infection of the mammary gland. The model explained most of the variation observed in cell dynamics during the early response to intramammary *E. coli* infection.

Neutrophils migrate from blood to the mammary gland in response to infection and constitute a major host defense mechanism (first line defense; Paape et al., 2000). Protection is only effective if rapid influx of neutrophils and subsequent phagocytosis and killing of bacteria occurs (Paape et al., 2002 & 2003). Neutrophils have stand-alone capabilities: they are capable of killing bacteria *in vitro* in absence of serum factors (Paape et al., 2000, 2002 & 2003 ; Beutler, 2004). However, they have evolved to function optimally in conjunction with cells and proteins of the adaptive immune

system. Antibodies produced by lymphoid cells of the adaptive immune system opsonize bacteria for destruction by neutrophils. The process of opsonization promotes the uptake of bacteria. Immunological recognition is mainly accomplished by specific antibodies (IgG2 and IgM) which recognize the bacterium through Fab-regions and bind to neutrophils via Fc-receptors on the neutrophil plasma membrane (Burvenich et al., 1994).

Hydroxyl radicals, singlet oxygen, oxygen halides, hydrogen peroxide, and NO kill coliforms and are released during the process of phagocytosis into the phagolysosome and react with diverse molecular targets of the pathogen (lipids, proteins, and nucleic acids). In humans and in some other mammals, mutations affecting the generation of these reactive oxygen species (ROS; i.e. mutations of the components of NADPH oxidase, or myeloperoxidase) create severe immunodeficiencies, despite the presumed integrity of innate immune signaling adaptive immune responses (Beutler, 2004). Experimentally induced coliform mastitis in cows made neutropenic by an equine anti-bovine leukocyte serum resulted in fatal toxic mastitis cases, indicating the importance of the neutrophil in the defense against intramammary coliform infections (Jain et al., 1971). These facts bespeak the importance of innate immunity to host defense.

On the other hand, these reactive oxygen species may cause substantial injury to healthy tissues. In order to minimize mammary tissue damage caused by bacterial toxins and ROS released by neutrophils, elimination of invading bacteria should proceed quickly. Therefore, the inflammatory response needs to be regulated. The concept of a well balanced inflammatory response where pro- and anti-inflammatory local mediators, such as cytokines, regulate the outcome of the inflammatory process, is also applicable to systemic factors. Hormones, metabolites and acute phase proteins, influence the outcome of mastitis. This is especially the case around parturition.

The world-wide consistency of findings on periparturient immunosuppression has been remarkable. For example, production of ROS by neutrophils isolated from blood were evaluated in two longitudinal studies in dairy cows from 3 weeks before until 5 weeks after calving, in the United States and in Europe. In both studies, a significant decrease in oxidative burst activity of neutrophils was observed (Dosogne et al., 1999).

Interestingly, in the combined longitudinal U.S. and European study of Dosogne et al. (1999), ingestion of bacteria and ROS production were inversely correlated. While the phagocytic ingestion capacity increased during the two weeks before parturition, ROS levels were decreasing. Immature neutrophils cannot be held responsible for this inverse correlation, since they are not present in circulation prior to calving. Therefore, it might be hypothesized that the reduced ROS production of blood neutrophils around parturition underlies the selective depression of activation pathways involved in oxidative burst generation without affecting the ingestion capacity. Furthermore, the increased capacity for ingestion of pathogens by neutrophils may simply be a function of the fact that the cellular energy required to support the respiratory burst of oxidative metabolism is not used due to defects in that pathway, thus allowing neutrophils to deplete their energy stores by ingesting excessive numbers of bacteria.

The afferent arm of innate defense

To enable the host to orchestrate a balanced inflammatory reaction following an intramammary coliform infection, the pathogen has to be detected promptly. Here again innate immune receptors must detect pathogen molecules within the infectious nidus before microbes proliferate, disseminate, and overwhelm the host (Beutler, 2004). Over the years intraluminal E.coli sensing by 'milk macrophages' and subsequent communication with the epithelium of the cistern of the mammary gland has been accepted as dogma. Details of the underlying mechanisms remained unknown and research was mainly focused on the interaction between the pathogen and the phagocyte, as it occurs during receptor mediated and lectin phagocytosis (Burvenich et al., 1994).

Generally, mucosal pathogens trigger a local innate host response by activating epithelial cells and bacterial adherence signaling has been implicated as key events in this process. However, because E.coli doesn't seem to colonize the mammary gland as does S.aureus, the interaction between the E.coli and mammary epithelium only received little attention (Burvenich et al., 2003). Yet, since the detection of Toll receptors as very evolutionary conservative molecules and their role in innate defense, a new area of interest is now opened (Burvenich et al., 2000 ; Burvenich et al., 2004). The molecular machinery utilized by mammary innate immune cells as well as by the epithelial cells for the recognition of E. coli is now under investigation in several laboratories worldwide.

Sensing the presence of bacteria in the lumen of the mammary gland is an important step in innate immunity. In the bovine mammary gland it seems that the host response observed after E. coli infection is not initiated until bacterial concentrations reaches a certain threshold (Shuster et al., 1996). Because bacterial growth is accompanied by the release of metabolites and toxins, it seems logic that bacteria can be detected by the host through the release of these soluble molecules. Endotoxin detection by the host to detect infections with Gram-negatives, seems to be an evolutionary choice.

The mechanism of endotoxin sensing has immense practical importance. Endotoxins are elements of the pathogen associated molecular patterns (PAMP) and bind to pattern-recognition receptors (PRR) that are present on a variety of defense cells of the body. It is striking to see how a very conservative molecule, such as the endotoxin, is used by the host to recognize the Gram-negative pathogen and how at the same time it is used by the pathogen to affect the host (Burvenich et al., 2004).

Cellular recognition of LPS is mediated by the transmembrane Toll-like receptor TLR-4 (Poltorak et al., 1998). TLR-4 is a member of the larger family of Toll-like receptors involved in innate immunity (Poltorak et al., 1998). Bovine mammary tissue expresses mRNA for TLR2, 4 and 9 (Goldammer et al., 2004)

A total of five cytoplasmic adapter proteins are presently known to be available to carry signals from the TLRs into the cytoplasm. These adapters, known as MyD88, MAL (or Tirap), TRIF (or Ticam-1), MyD88-4 (sometimes called TIRP or TRAM), and MyD88-5 (Beutler, 2004).

Although the exact mechanism by which LPS is recognized by TLR-4 remains unclear, cell activation is dependent upon the cell surface assembly of a multi-protein recognition complex consisting of mCD14, MD-2, and TLR-4 (Akashi et al., 2000). Endotoxin has been implicated as a 'recognition molecule' for all Gram-negative bacteria and recognition of endotoxin by host cells requires an array of proteins. Endotoxin presentation to this multiprotein complex is enhanced by the acute phase protein, LPS-binding protein (LBP) (Tobias et al., 1999; Schumann and Latz, 2000). LBP and soluble CD14 (sCD14) are present in the serum and facilitate the transfer of LPS to membrane-bound CD14 (mCD14), a glycosylphosphatidylinositol-linked receptor on the surfaces of some host cells (Bannerman et al., 2003). It is thought that mCD14 subsequently interacts with TLR4, the signaling component of the LPS receptor.

Cells lacking mCD14, including epithelial and endothelial cells, are capable of recognizing and responding to LPS. In the absence of mCD14, LBP facilitates the transfer of circulating LPS to sCD14 and this complex is recognized by TLR-4 (Tobias et al., 1999). Therefore, a common denominator to host cell recognition of LPS is the presence of CD14 as either a membrane-bound (mCD14) or soluble molecule (sCD14) and LBP.

The mCD14 is a 55 kDa glycoprotein found on cells such as monocytes and macrophages, and to a lesser extent on neutrophils (Viriyakosol and Kirkland, 1995). sCD14 is derived from these cells by both direct exocytosis and proteolytic cleavage of cell surface mCD14. Although LPS directly binds CD14, this process is greatly enhanced by LBP (Hailman et al., 1994). LBP is a hepatocyte-derived acute phase protein, the expression of which is upregulated by IL-1 and IL-6 (Tobias et al., 1999; Schumann and Latz, 2000). LBP is a lipid transfer molecule that dissociates LPS aggregates into monomers, and catalyzes the transfer of these monomers to CD14. In addition, LBP has recently been shown to stabilize the association of LPS and CD14 by forming a tripartite complex (Thomas et al., 2002).

There is strong evidence for a protective role for CD14 and LBP in mediating the host response to Gram-negative infections; also in the mammary gland of the cow (Lee et al., 2003; Bannerman et al., 2003; Vangroenweghe et al., 2004b). Detection of LPS is a critical event in the activation of the innate response to Gram-negative bacteria, however excess of LPS signaling can lead to exaggerated host responses culminating in the development of septic shock.

NF- κ B, key regulator of inflammatory responses

The transcription factor NF-kappa B is the master regulator of all TLR-induced responses and its activation is the pivotal event in TLR-mediated activation of the innate immune response. Many of the key molecular events required for TLR-induced NF-kappaB (NF- κ B) activation have been elucidated (Carmody and Chen, 2007).

LPS provokes host cells to produce a large amount of pro-inflammatory mediators, including tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, by activating several types of transcription factors. One major LPS signaling target is NF- κ B a key regulator of immune and inflammatory responses. Activation of NF- κ B in turn results in the expression of a vast array of effector molecules that are critically involved in inflammatory responses such as the upregulation of adhesion molecules and the production of pro-inflammatory cytokines that contribute to host defense mechanisms. Recent advances in understanding the mechanisms of recognition of LPS by hosts and LPS-mediated signaling are offering promising approaches to block bacterial infection-induced overproduction of pro-inflammatory mediators and subsequent septic shock (Zhang and Ghosh, 2000; Ghosh et al., 1998; May and Ghosh, 1998).

Although the receptor for LPS, Toll-like receptor-4 (TLR-4) has been identified, the signaling pathways activated by LPS/TLR-4 have yet to be fully elucidated. Many molecular components involved in the LPS-induced NF- κ B activation have been recently uncovered. As expected, LPS signals activation of NF- κ B using an analogous molecular framework as IL-1 and IL-18 due to a highly conserved cytoplasmic signaling motif among their transmembrane receptor. MyD88, IRAK, TRAF6, TRAF6-interacting proteins and IKKs are all involved in three cases.

Under resting conditions, NF- κ B is sequestered in the cytosol as an inactive homodimer or heterodimer form, which non-covalently interacts with its inhibitory proteins. Upon activation of the TLR-4 receptor, these inhibitory proteins are phosphorylated by IKK through the MyD88/IRAK/TRAF6 pathway, and subsequently ubiquitinated and degraded, resulting in nuclear translocation and DNA binding of NF- κ B. Once in the nucleus, NF- κ B dimers are subject to a second level of regulation mainly through phosphorylation of the Rel proteins, which is required for full induction of NF- κ B target genes (Karin and Lin, 2002).

Genes that are targeted after LPS stimulation by NF- κ B, include those encoding cytokines (TNF-alpha, IL-1, IL-6, G-CSF, GM-CSF, M-CSF), chemokines (IL-8, MCP-1), adhesion molecules, acute phase proteins, transcription factors (p50, c-Rel, Egr-1, IRF-1) and also iNOS and cyclooxygenase 2 (COX-2) (Guha and Mackman, 2001). They collectively provide immediate protection for hosts and induce the development of adaptive immune responses as well (Zhang and Ghosh, 2000; Ghosh et al., 1998; May and Ghosh, 1998).

Much remains to be learned about the ability of TLRs to generate pathogen-specific responses using a limited number of transcription factors (Carmody and Chen, 2007). Recently, Lee et al. (2006) found that the expression of IFN-gamma was only increased in milk somatic cells isolated from *E. coli*, but not *S. aureus*, challenged mammary glands. Moreover, upregulated expression of cytokine genes had higher magnitudes and/or faster responses in glands challenged with *E. coli* in comparison with those challenged with *S. aureus*.

Endotoxin, TNF-alpha, NO connection and sepsis

Endotoxin or lipopolysaccharide (LPS), the major constituent of the Gram-negative bacterial cell wall, is released during bacterial growth and destruction, and is implicated in the pathogenesis of Gram-negative bacteria-induced mastitis. Endotoxin is a key molecule that contributes to both the localized and systemic inflammatory responses that accompany Gram-negative bacterial-induced mastitis. The bovine mammary gland is highly sensitive to low doses of endotoxin (Carroll et al., 1964; Dhondt et al., 1977; Mattila and Frost, 1989) and injection of endotoxin into the mammary glands of healthy cows induces mastitis (Paape et al., 1974). Endotoxin is detectable in the milk of cows with coliform mastitis (Anri, 1989) and absorption into blood following naturally occurring and experimental *E. coli* mastitis (Katholm and Andersen, 1992, Dosogne et al., 2002a) has been reported. Further, a significant portion of cows with acute coliform mastitis develop bacteraemia, thus, introducing LPS directly into the circulation (Wenz et al., 2001). The neutrophils are involved in the clearance of both the pathogen and its endotoxin from the udder cavities. A factor contributing to severity of coliform mastitis in postpartum cows is a reduction of acyloxyacyl hydrolase activity in circulating neutrophils (Dosogne et al., 1998). This enzyme is critical in detoxification of endotoxin (McDermott et al., 1991a & 1991b).

The deleterious outcome associated with Gram-negative infection is the result of an exaggerated inflammatory response and this response is elicited largely by endotoxin (Jackson and Bramley, 1983). Since the 70's, LPS has been largely studied for its capacity to mediate septic shock with severe impact on the intermediate metabolism (Lohuis et al., 1988a & 1988b).

Endotoxin provokes host cells to produce large amounts of pro-inflammatory mediators by activating transcription factors. One target is NF- κ B, a key regulator of immune and inflammatory responses. Induction of NF- κ B transcriptional activity by endotoxin was observed in cells isolated from infected quarters (Boulanger et al, 2003). LPS and cytokines stimulate the synthesis of a vasodilator called nitrogen oxide (NO). NO is a diatomic free radical. It is lipid-soluble and diffuses easily through cell membranes. It is short-lived and usually degrades or reacts within a few seconds. The natural form is a gas that reacts with a variety of molecules and mediates a large spectrum of biological effects. NO is synthesized from L-arginine. An inducible NO synthase (iNOS) is expressed by a variety of cells, especially monocytes, as a result of triggering with substances of microbial origin, such as LPS and TNF-alpha. Bovine

monocytes produce NO in response to LPS (Adler et al., 1995; Jungi et al., 1998; Stich et al., 1998). NO is produced by macrophages in response to invading pathogens and acts as an antimicrobial agent. It is also cytotoxic and can induce apoptosis if produced in excessive amounts (Anggard, 1994; Moncada et al., 1991).

Several studies on ruminants have shown a relationship between LPS, TNF-alpha and production of NO. The increase in TNF-alpha is followed by a delayed increase in NO_x (NO₂ + NO₃) (Blum et al., 2000; Bouchard et al., 1999). The NO_x production lasts longer in *E. coli* challenged quarters. A causal relationship between TNF-alpha and NO_x production was observed in studies in which *E. coli* LPS was injected intravenously (Kahl et al., 1997). It seems that severe cases of coliform mastitis are accompanied by the highest increase in blood plasma concentrations of both TNF-alpha (Hoeben et al., 2000) and NO_x (Blum et al., 2000; Hirvonen et al., 1999). Initially called endothelium derived factor (EDRF), NO causes vasodilation. It is released after the fever peak, which is at the moment of the delayed phase of vasodilation occurring during LPS-induced mastitis in lactating goats and cows (Dhondt et al., 1977). It is not impossible that NO is involved in this delayed phase of hyperemia.

Endotoxin: Friend or Foe?

A common denominator to all of Gram-negative bacteria is the presence of endotoxin or lipopolysaccharide (LPS), which is found in the outer membrane of all Gram-negative bacteria. LPS is a highly pro-inflammatory molecule that is shed from the bacterial surface during bacterial replication or death. The deleterious outcome associated with Gram-negative infection is the result of an exaggerated inflammatory response and this response is elicited largely by LPS (Jackson and Bramley, 1983; Erskine et al., 1991; Ziv, 1992). The bovine mammary gland is highly sensitive to low doses of LPS (Carroll et al., 1964; Dhont et al., 1977; Mattila and Frost, 1989). Injection of LPS into the mammary glands of healthy cows induces mastitis (Jain et al., 1969; Paape et al., 1974; Dhont et al., 1977, Bannerman et al., 2003) and LPS is detectable in the milk of cows with coliform mastitis (Anri, 1989; Hakogi et al., 1989). Absorption of LPS into blood following LPS injection into healthy mammary glands (Ziv et al., 1976) or during naturally occurring *E. coli* mastitis (Katholm and Andersen, 1992, Dosogne et al., 2002) has been reported. Further, a significant portion of cows with acute coliform mastitis develop bacteremia, thus, introducing LPS directly into the circulation (Wenz et al., 2001). It is well established that the systemic inflammatory response that accompanies peracute coliform mastitis is mediated, in part, by LPS (Carroll et al., 1964; Schalm et al., 1964; Jackson and Bramley, 1983; Ziv, 1992). Thus, LPS is a key pro-inflammatory molecule that contributes to both the localized and systemic inflammatory responses that accompany Gram-negative bacterial-induced mastitis.

A factor contributing to severity of coliform mastitis in postpartum cows could be a reduction of acyloxyacyl hydrolase activity in circulating neutrophils. This enzyme is

critical in detoxification of endotoxin (Dosogne et al., 1998; Mehrzad et al., 2007). In this context it is important to mention again the variability of the inflammatory response during coliform mastitis around parturition, ranging from severe to moderate to mild, and that this variability is unpredictable (Burvenich et al., 2003).

Since the 70's, LPS has been largely studied for its capacity to mediate septic shock with severe impact on the intermediate metabolism (Lohuis et al., 1988a,b). Since then, we have come to understand that LPS is not only 'toxic' but that it has a vast array of characteristics ranging from immunodepressive to immunostimulatory, depending on the circumstances during which it is released and the released amount. More recently, endotoxin has been implicated as 'recognition molecule' for all Gram-negative bacteria. They are elements of the pathogen associated molecular patterns (PAMP) and bind to pattern-recognition receptors (PRR) that are present on a variety of defense cells of the body. It is striking to see how a very conservative molecule, such as LPS of Gram-negative bacteria, is used by the host to recognize the pathogen and how at the same time is used by the pathogen to affect the host (Burvenich et al., 2004).

Homeostasis and homeorhesis in the mammary gland

The mammary gland is a complex and open self-regulatory system with a continuous flow of matter, energy and information. From both biochemical and evolutionary viewpoints it is interesting to learn that in the mammary gland, two signaling pathways, NF- κ B and Jak/Stat, play central roles in inflammation (innate defense) as well as in lactation (Vorbach et al., 2006).

Metabolically, the mammary gland has absolute priority over most other tissues except the brain. Self-regulation with change over time is characterized by a dynamic equilibrium between two mechanisms: homeostatic and homeorhetic (Bauman and Currie, 1980). Homeostasis is a short-term control representing the mechanisms that enable the animal to function under a range of environmental conditions (Greek : homeo, similar; stasis, remain). It refers to ways the body acts to maintain a stable internal environment in spite of environmental variations and disturbances. It is one of the most remarkable and most typical properties of highly complex open systems. The 'concept' of homeostasis was first formulated by Claude Bernard in the XIXth century; the term itself was coined by Walter B. Cannon (1932) and popularized in his book *The Wisdom of the Body*. Homeorhesis (Greek : homeo, similar; rhesis, flow) is defined as a long-term control that expresses the genetic make-up of the animal and/or the animal's potential. It describes the tendency of developing or changing organisms to continue development or change towards a given state, even if disturbed in development. This term was first coined by the embryologist Conrad H. Waddington (Slack, 2002) around 1957. Homeorhesis can be defined as the co-ordinated changes of body tissues to support a physiological state (Piaget, 1974) and indicates a situation of stable flow. It is the counterpoint of homeostasis. Homeostasis is a spatial metaphor, whereas homeorhesis is a developmental process. Scientists have integrated both

homeostatic and homeorhetic controls of protein and body lipid turnover into mathematical models allowing for mechanistic representations of nutrient partitioning.

Similar mechanisms keep also 'milk composition' constant and complex. Details of how various milk constituents are secreted by different intracellular routes (transcellular and paracellular pathways) have been studied over the years. However, milk secretion mechanisms change according to the lactation cycle. According to Shennan and Peaker (2000) it is time for investigations of the coordinated regulation to extend to those changes in the composition of the secretion that occur with physiological state; e.g. the formation of colostrum, the shift to milk production at around the time of parturition and drying off. Long-term regulation, through changes in galactopoietic hormone receptors, provides an efficient mechanism for integrating acute intramammary regulation of lactation with strategic endocrine control of mammary tissue development (Wilde et al., 1998). Many positive feedback mechanisms will induce the mammary gland to grow and to produce milk. Mammogenesis, colostrogenesis and maintenance of milk secretion are controlled by mainly homeorhetic mechanisms (Bauman and Currie, 1980).

The same concept is true for mammary defense. The defense against invaders by innate immunity and auto-repair of the damaged tissues are covered by many homeostatic mechanisms. However, immunity also has to function and develop in time, depending on the lactation cycle, and the development in such a dynamical system seems to be a challenge that has sometimes to compete with the primary goal of lactation and mammary development during specific periods of the lactation cycle. During the lactation cycle the flow of energy in the organism is permanently redirected. Lactation comes with high metabolic costs, which are manifested in parent-offspring conflict, and special physiological adaptations have evolved which match milk supply to demand by the young (Peaker, 2002). In such a complex dynamic situation it is not surprising that physiology is not far away from pathology.

The cows udder is continuously challenged with bacteria from the environment and both have a high evolutionary degree of adaptation. Whereas *E. coli* mastitis can be a severe problem at the beginning of lactation, it is completely self-curing after peak lactation (Burvenich et al., 2004). Several systemic factors related to the stage of lactation and parity may contribute to function of the defense system in an abnormal or incomplete manner. For example, the activities of neutrophils in combating infection are complex and involve expenditure of cellular energy. The average cow has ~3,500 neutrophils per ml of blood, this translates into $\sim 1.4 \times 10^{11}$ neutrophils in an 800 kg Holstein cow. The circulating half-life of neutrophils is about 6 hours, so a cow is replacing half of her neutrophils every 6 hours from bone marrow stores. Clearly, a component of dietary energy and protein consumption for maintenance is spent on replenishment of neutrophils by the bone marrow. Next to this, it is also known that there is considerable genetic control over the immune system capacity to function, especially around parturition (Dettloux et al., 1994, Dettloux, 2002, Paape et al., 2002). Therefore, a high milk yield will not likely dictate that a cow will experience a greater impact on its innate defense than a lower producing cow.

Conclusive evidence that the demands of lactation contribute to postpartum immune suppression derives from recent studies at the National Animal Disease Center, USDA-ARS. In studies with mastectomized cows it was found that they recover from periparturient immunosuppression within one week after calving, whereas intact lactating cows can be immunosuppressed for 2-3 weeks postpartum (Kimura et al., 1999, Kimura et al., 2002, Nonnecke et al., 2003). Negative energy and protein balances that exist during early lactation contribute to impaired immune function and, thus, account for a portion of the periparturient immunosuppression observed.

Enhanced disease resistance

A potential application includes enhanced disease resistance. At the moment, many mastitis research groups are searching to enhance resistance of the udder by enabling mammary epithelium to secrete immune or antibacterial self and non-self (foreign) proteins. Proof of concept has already been obtained with transgenic mice and cow (Wall et al, 2005). Interest is focused on e.g. lysostaphin (a prokaryotic protein that has potent anti-staphylococcal activity), lysozyme, lactoferrin and soluble CD14. Inserting the gene for CD14 into the mammary gland will provide the CD14 necessary for recruitment of neutrophils and elimination of invading coliform mastitis causing pathogens. Lysostaphin and human lysozyme are ineffective against *E. coli* and *S. uberis* isolates obtained from mastitis milk. Bovine beta-defensin related peptide has been expressed in the lactating mammary gland of transgenic mice (Yarus et al., 1996)

There are at least two potential benefits of transgenic application of immune or antibacterial proteins : 1) unveiling candidate genes whose promoter elements will enable temporal expression patterns. Inflammation inducible expression constructs may be superior to constitutive expression (delivering antibacterial proteins only when needed) and 2) the sparing in agricultural use of antibiotics currently used in human medicine.

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12.2 IMMUNE REACTION OF THE BOVINE MAMMARY GLAND IN RESPONSE TO BACTERIAL TOXIN STIMULATION

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Abstract

An infection of the mammary gland occurs if pathogens invade the mammary gland. They induce an inflammatory reaction, which is regulated by cytokines and causes an increase of somatic cells in milk and activation of bacteriostatic enzymes and proteins in milk. This defense mechanism is induced if specific pathogen associated molecular patterns (PAMP) are recognized by the immune system of the host. The more rapidly they are recognized the more effective is the defense against them. To study the mammary immune defense mechanisms PAMP of common mastitis pathogens like lipopolysaccharide (LPS) from gram-negative and lipoteichoic acid (LTA) from gram-positive bacteria were used in *in vivo* and *in vitro* studies.

In vivo LPS-challenged mammary gland quarters increased their somatic cell count (SCC) and the mRNA expression of tumor necrosis factor α (TNF- α), interleukin (IL)-1 β and cyclooxygenase-2 expression to highest values within hours after LPS-challenge. Expression of lactoferrin, lysozyme, inducible nitric oxide synthase, I-selectin, NF κ B and of the apoptotic factors caspase-3, caspase-7 and Fas (FS-7 associated surface antigen) was elevated. No significant increase in expression of platelet-activating factor, acetylhydrolase, 5-lipoxygenase, β -integrin and insulin-like growth factor 1 was found. Gene expression of major milk proteins did not change significantly in response to the LPS challenge (α S1-casein (CN), α S2-CN, β -CN and β -lactoglobulin) except for α -lactalbumin which decreased in LPS-treated and control quarters and for κ -CN which decreased in the LPS-treated quarters. LTA challenged quarters showed also increased SCC and IL-8 and lactoferrin but not TNF- α mRNA expression. Comparable immune responses to LPS and LTA and cytokine regulation have been shown *in vitro* in cell culture models.

In conclusion, *in vitro* or *in vivo* the use of PAMP lead to far-reaching knowledge of immune regulation in the mammary gland during mastitis. The response to PAMP of different pathogens could be studied under defined experimental conditions and excluded greatest possible environmental influences.

Introduction

An infection of the bovine mammary gland is mostly induced by pathogenic microorganisms if they overcome the anatomical-histological barrier in the teat canal, the main entrance pathway of pathogens. To combat the infection firstly the innate unspecific cellular immune response is stimulated, which is considered the crucial defense against pathogens.

In order to allow an immediate immune reaction and to inhibit microbial growth in the mammary gland, a fast and efficient reaction of the defense systems is necessary. This includes a fast and effective recognition of potential pathogens. In response to the detection of pathogens antimicrobial enzymes and proteins have to be synthesized and activated, and, most importantly, an efficient transfer of leukocytes from the blood into the mammary tissue and milk must be elicited as soon as possible after pathogen invasion.

Pathogens are recognized by their pathogen-associated molecular patterns (PAMP) that are specific components commonly found in cell walls of pathogens and that are not present in the host organism. These PAMP are the endotoxins of the bacteria that are released when they degrade. PAMP bind to specific receptors on the surfaces of host cells, the toll-like receptors (TLR). Upon the binding of the PAMP to the TLR a signalling pathway is initiated that stimulates the host defense.

Common PAMP are the cell wall components lipopolysaccharide (LPS) from gram-negative and lipoteichoic acid (LTA) from gram-positive bacteria. To study the immune response PAMP from different mastitis pathogens were used in several *in vivo* and *in vitro* studies to model a mammary gland infection. The advantage of using PAMP for these experiments is the possibility that defined concentrations can be applied which are totally independent of the growth of bacteria, which can hardly be predicted and which vary due to pathogen, environmental, and cow factors. Thus, for *in vivo* experiments the inflammation is exactly controllable and the immune response is limited in time if PAMP are used.

The present review focuses on *in vivo* and *in vitro* studies to investigate the mammary immune response by the stimulation with different PAMP.

Escherichia coli lipopolysaccharid stimulation

E. coli is a common mastitis pathogen that induces mostly acute and severe infections of the mammary gland. The pathogenic component of this gram-negative bacterium is LPS from the cell wall. It is responsible for many of the clinical signs, i.e. fever, pain, increasing somatic cell counts (SCC) and loss of milk character in coliform mastitis (Jain et al., 1978; Hill, 1981; Guidry et al., 1983). Therefore, it is used in concentrations up to 500 µg to induce mastitis experimentally to study the inflammatory response in the

bovine udder after the release of endotoxin from the infecting pathogens (Hoeben et al., 2000; Mehrzad et al., 2001; Van Oostveldt et al., 2002; Schmitz et al., 2004).

In our studies (Schmitz et al., 2004; Werner-Misof et al., 2007a; 2007b) lactating dairy cows were intramammarily injected in one quarter with *E. coli* LPS (Serotype O26:B6; Sigma Chem. Co., St. Louis, USA), the contralateral quarter served as control. Mammary biopsy samples of both quarters were taken and mRNA expression of different immunomodulators measured by qRT-PCR. The LPS induction of mammary immune response was shown to be dose dependent (Werner-Misof et al., 2007a; 2007b).

Cytokines and other immunomodulators

The entire immune response in the mammary gland is regulated by cytokines produced by leukocytes and epithelial cells. Cytokines that function as chemokines, i.e. attract leukocytes, are responsible for augmented leukocyte influx from blood into milk. This recruitment of immune cells is crucial to eliminate invaded bacteria by phagocytosis. An intramammary LPS challenge increases the SCC within some hours (Schmitz et al., 2004; Werner-Misof et al., 2007a).

Tumor necrosis factor- α (TNF- α) is a cytokine, which serves as a rapidly responding central mediator of inflammatory functions (Watanabe et al., 2000). It is known to play an important role in mastitis and is responsible for the endotoxin shock in an acute phase of coliform mastitis (Sordillo and Streicher, 2002). The gene expression of TNF- α increased transiently in LPS-challenged quarters to highest values at 3 h after LPS challenge (Schmitz et al., 2004). Highest levels were about 47-fold above baseline. Thereafter, TNF- α mRNA showed a steady decrease. TNF- α in the mammary gland is produced by leukocytes (Pfaffl et al., 2003; Alluwaimi et al., 2003) and also by the mammary epithelial cells in response to LPS stimulation (Wellnitz and Kerr, 2004).

A similar pattern as for TNF- α in LPS stimulated mammary glands was observed for interleukin (IL)-1 β which showed highest expression levels at 3 h after LPS treatment and decreased thereafter (Schmitz et al., 2004). Both, TNF- α and IL-1 β expression showed also a slight increase in the control quarters, likely in response to the biopsy treatment thus indicating the highly sensitive reaction of these factors in response to minimal stimuli. IL-1 β , like TNF- α , plays a key role in eliciting the acute phase response following bacterial invasion of the udder, including accumulation of leukocytes at the site of infection (Riollet et al., 2000a). Recombinant IL-1 β increases the rate of mammary gland involution and concomitantly the lactoferrin concentrations in milk (Wedlock et al., 2004). In addition, both, TNF- α and IL-1 β have been demonstrated to be involved in inducible nitric oxide synthase (iNOS) up-regulation that is important for microbistatic and microbicidal activity, in response to LPS challenge (Kleinert et al., 2003).

IL-8 is an additional cytokine known to be involved in the immune response of the mammary gland. It is a potent chemokine for neutrophils produced by leukocytes and was also shown to be produced during an immune response by bovine mammary epithelial cells (Wellnitz and Kerr, 2004). In freshly removed milk incubated at 37°C the mRNA expression of IL-8 decreased from 3 to 6h of incubation and this effect was lower after 3h if the milk was enriched with 0.5 µg LPS/ml (Baumert et al., 2007). This may show that cells once present in milk cannot markedly be stimulated by PAMP. Therefore, for the mammary defense mechanism a recruitment of fresh immune cells from the blood in response to PAMP stimulation is more effective than the immune response of cells that are already in the cistern and milk ducts.

Mammary gland explants (2 x 2 x 2 mm tissue isolated directly after slaughter from lactating cows) have the advantage to cell culture models that the tissue formation is still intact and milk proteins are still produced. They show increased IL-1β, IL-6, and IL-8 mRNA expression in response to LPS stimulation (10 µg LPS/ml culture medium)

(Rabot et al., 2007). Besides cytokines, also lipid derived mediators such as prostaglandins and leukotrienes are involved in the immune response of the mammary gland (Wittmann et al., 2002).

In response to LPS *in vivo*, the gene expression of the key enzyme of prostaglandin biosynthesis, cyclooxygenase-2, was transiently increased and peaked at 3 h after challenge (Schmitz et al., 2004). In contrast, there was no significant change of key enzymes related to platelet activating factor (platelet activating factor-acetylhydrolase) or leukotriene (5-lipoxygenase) metabolism within the 12 h of our experiment. Possibly some of these factors which do not belong to the class of the pro-inflammatory mediators, would react after this period in a true infection scenario. Insulin-like growth factor-I (IGF-I) mRNA expression did not change significantly in response to LPS challenge (Schmitz et al., 2004). This finding is surprising because in a previous investigation, IGF-I levels increased about 3-fold within 6 h in the milk of LPS-treated quarters (Bruckmaier et al., 1993). However, in this earlier study the LPS dosage was about 10-fold higher than in the recent study by Schmitz et al. (2004). Possibly, the elevated IGF-I concentration in response to LPS challenge was due to an increased paracellular transfer of IGF-I from blood into milk via leaky tight junctions, i.e. on a similar basis as the concentrations of sodium and chloride ions (Bruckmaier et al., 2004) or serum albumin in milk are regulated. The assumption of leaking of IGF-I from blood toward milk along a concentration gradient is also supported by a study of Lacasse et al. (1996a).

Adhesion molecules expressed on the surface of endothelial cells of blood vessels play a crucial role in the recruitment of PMNs. Fast flowing PMNs get in contact with L-selectin to initiate contact with a variety of ligands present on the cell surface. As soon as contact is made the PMNs begin to roll along the endothelial cells surveying the blood vessel for inflammation (Burton and Erskine, 2003). Close to the site of inflammation the expression of β-integrin initiates the paracellular migration through endothelial cells to the inflammatory site. In our study (Schmitz et al., 2004), LPS challenge caused a 5-fold increase of mRNA for L-selectin in the biopsy samples

within 3 h, and this elevated level remained unchanged until the final sample at 12 h. Surprisingly, the expression of β -integrin remained unchanged in LPS and control quarters, respectively, throughout the experiment. Possibly, some of the crucial factors involved in PMN recruitment, do not undergo a short-term regulation of their expression and may be activated without changing their gene expression.

Antibacterial proteins and enzymes

Lactoferrin is an iron binding glycoprotein with non-specific antimicrobial activity that is expressed by numerous cell types. Besides synthesis by the secretory epithelium (Persson et al., 1992; Pfaffl et al., 2003; Schmitz et al., 2004), lactoferrin is also released by PMNs during inflammation (Harmon and Newbould, 1980). It is known to increase in bovine milk during clinical mastitis (Harmon et al., 1976; Kawai et al., 1999). Increased concentration of lactoferrin in the mammary secretion during mastitis or involution indicates that the regulation of lactoferrin in the mammary gland is contrary to that of other milk proteins (Schanbacher et al., 1993). These changes were paralleled by changes in lactoferrin expression (Neville and Zhang, 2000). In response to the intramammary LPS injection (Schmitz et al., 2004) the gene expression of lactoferrin increased significantly within 3 h and peaked at 6 h after challenge. Lactoferrin mRNA expression and protein secretion is also dramatically increased in LPS challenged mammary epithelial cells *in vitro* (Wellnitz and Kerr, 2004). In freshly removed milk kept at 37°C the lactoferrin mRNA expression in the somatic cells decreased within 3 to 6 h but not if 0.5 μ g LPS/ml was added to the milk (Baumert et al., 2007).

Lysozyme is of relevance to the natural defense system of the mammary gland due to its bacteriostatic and even bactericidal effects on udder pathogens (Lunau, 1989). In several studies, lysozyme concentration was low in normal bovine milk but increased during mastitis (Carlsson et al., 1989; Persson et al., 1992). A significant correlation between concentrations of lysozyme and SCC was also observed (Götze et al., 1977; Persson et al., 1992). Persson et al. (1992) and Steinhoff et al. (1994) concluded that leukocytes are the most likely source of lysozyme during inflammation. Intramammary LPS injection (Schmitz et al., 2004) caused an increased lysozyme mRNA expression. Therefore, an important contribution of the mammary epithelial cells in lysozyme synthesis is most likely.

NO is synthesized by activated macrophages as an important component of immune response. NO may play a role in mediating microbistatic or microbicidal activity (Jungi, 2000) or in increasing mammary blood flow which helps to transport more leukocytes to the site of infection (Lacasse et al., 1996b). Intramammary infusion of LPS caused an enhanced intramammary production of nitric oxide (NO) (Bouchard et al., 1999; Blum et al., 2000). NO synthesis is catalyzed by iNOS which is known to be induced by LPS. TNF- α is supposedly responsible for iNOS up-regulation and hence increased NO production (Blum et al., 2000; Kleinert et al., 2003). This assumption is supported by augmented iNOS expression in response to intramammary LPS challenge

(Schmitz et al., 2004) showing a peak of mRNA for TNF- α and the transcription factor NF- κ B (Bruckmaier and Meyer, 2005) clearly before the peak of iNOS expression. NF- κ B expression increased about 8-fold within 3 h in response to LPS challenge and gradually decreased thereafter in our study. NF- κ B plays a crucial role, possibly together with other transcription factors such as STAT-1 α , in the synthesis of iNOS because it is a central target of activators or inhibitors of iNOS expression such as LPS, TNF- α , and IL-1 β (Kleinert et al., 2003).

Apoptosis factors

Bacterial endotoxins may induce apoptosis in both milk leukocytes and in mammary tissue. Apoptosis is characterized by a defined cascade of morphological and biochemical events finally leading to the controlled death and removal of the respective cells without any signs of inflammation. TNF α , as released in response to LPS challenge, may be one of the factors to induce apoptosis (Maianski et al., 2003). A major event during the course of apoptosis is the activation of caspases that contribute to cleavage of nuclear proteins and DNA fragmentation (Robertson et al., 2000). Caspase-3 and caspase-7 belong to the group of effector caspases. Caspase-3 participates in bone marrow derived neutrophil apoptosis (Woo et al., 1998). In response to intramammary LPS injection (Schmitz et al., 2004), caspase-3 and caspase-7 expression increased dramatically until 6 h from the start of infusion and thereafter decreased again. In addition, Fas (FS-7 cell associated surface antigen, Nagata, 1994) expression increased with a peak at 3 h from infusion of LPS (Didier and Bruckmaier, 2004). Although caspases are stored as pro-enzymes and activated by cleavage of the pro-domain in response to a “death signal” (Zhvivotovsky et al., 1999) there is obviously also a short-term up-regulation of the mRNA of these factors. LPS induces apoptosis in neutrophils (Van Oostveldt et al., 2002) but also in mammary secretory tissue cells (Didier and Bruckmaier, 2004). Possibly, the activation of apoptotic enzymes during inflammation (Didier and Bruckmaier, 2004) comes along with a down-regulation of milk proteins and simultaneous up-regulation of lactoferrin (Schmitz et al., 2004). In freshly removed milk kept at 37°C the number of somatic cells decreased faster if stimulated with 0.5 μ g LPS/ml compared to control samples (Baumert et al., 2007) most likely due to apoptosis.

Receptors involved in the immune response

PAMP induce their effects through binding to TLR on the cell surface. This binding induces a signal cascade within the cell that induces finally an activation of NF- κ B and the transcription of diverse immunomodulators. The activation of mammary gland epithelial cells by LPS is mediated via TLR-4. They were shown to express TLR-2 and TLR-4 mRNA (Strandberg et al., 2005).

A co-receptor of TLR-4 is CD14. It is a specific LPS receptor expressed on the surface of circulating monocytes and macrophages, and also present in a soluble form (Wang et al., 2002). Soluble CD14 results from the shedding of membrane CD14 and prevents LPS

from binding to the cell membrane. It, thus, prevents the excess production of inflammatory factors such as TNF- α (Paape et al., 2002). It is tempting to speculate that mammary epithelial cells undergoing programmed cell death in response to bacterial toxins are changing their protein expression pattern during early stages of apoptosis. This could cause the observed decline of synthesis of milk proteins like α -lactalbumin and κ -casein while concomitantly the synthesis of antibacterial proteins and enzymes like lactoferrin and lysozyme is dramatically increased (Schmitz et al., 2004). On the other hand it seems possible that during the inflammation state after LPS challenge the massive influx of PMN is partially responsible for the increased expression of apoptosis factors in the biopsy samples. PMNs are short living cells and will rapidly undergo apoptosis after migration into milk. In contrast, it is also reported that LPS increases viability of PMN in vitro (Boulanger et al., 2002).

Immunoglobulins are also part of the soluble defense that elicit effective protective responses to invading pathogens. If the mammary tight junctions are damaged immunoglobulins from the blood appear in the milk. However, immunoglobulin receptors are present in the mammary gland. In mammary gland explants the mRNA expression of the neonatal Fc receptor (FcRn) and the polymeric immunoglobulin receptor (pIGR) was stimulated after 3h if 10 μ g LPS was added to the medium then recovered to the level of the controls level at 6h (Rabot et al., 2007). These results show that epithelial cells could have the capacity to enhance their production of Ig receptor in order to concentrate Ig at the place of infection to thwart the bacterial invasion.

Acute phase proteins

Acute phase proteins are important regulators of the immune system and have stimulatory effects in the early stage of immune response. Serum amyloid A (SAA) and haptoglobin were shown to be increased in blood and milk during acute *Staphylococcus aureus* mastitis while only SAA was also increased during chronic mastitis (Grönlund et al., 2003). Changes in the expression of haptoglobin, representing acute phase proteins, were investigated in response to LPS challenge (Hiss et al., 2004). A clear increase of expression in LPS treated quarters was found. SAA may play an important role in leukocyte recruitment in the mammary gland as it has chemotactic effects on leukocytes (Badolato et al., 1994). Its expression is dramatically increased in mammary epithelial cells in response to *E. coli* LPS treatment or *S. aureus* infection (Wellnitz and Kerr, 2004) or heat killed *E. coli* or *S. aureus* (Wellnitz et al., 2006).

Milk constituents

The milk proteins α S1-Casein (CN)-, α S2-CN-, β -CN- and β -lactoglobulin expression did not change significantly in response to intramammary LPS infusion albeit their values were numerically lower at 9 h after LPS administration as compared to 0 h. A significant decrease in mRNA expression at 9 h post inoculation relative to time 0 was

observed for α -lactalbumin in both, LPS and control quarter, and for κ -CN in the LPS quarter (Schmitz et al., 2004). This effect may be caused by early apoptotic reactions of the mammary epithelial cells (Didier and Bruckmaier, 2004), i.e. proteins which determine milk yield or casein micelle formation, respectively, are down-regulated while simultaneously proteins involved in the immune response like lactoferrin and lysozyme are up-regulated.

The disaccharide lactose is a milk component that is responsible for the milk yield due to its osmotic function. Lactose concentrations in milk decrease within the first 12 hours after an intramammary LPS stimulation in a dose dependent way (Werner-Mishof et al., 2007a) due to damaged tight-junctions (Stelwagen et al., 1997; Nguyen and Neville, 1998) that allows the lactose to leave the milk into the blood in the direction of the concentration gradient.

The damaged tight junctions are also responsible for an increase of electrical conductivity due to an influx of electrolytes from the blood. This is a typical sign of mammary gland infection and is seen after LPS challenge (Werner-Misof et al. 2007a)

Other bacterial toxins

Lipoteichoic acid (LTA) is, comparable with LPS in gram-negative bacteria, the most important pathogenic component of gram-positive bacteria like the common mastitis pathogens *S. aureus* and *Streptococcus uberis*. LTA induces the immune response of cells via the TLR-2 (Aderem and Ulevitch, 2000) and has been described to stimulate mammary epithelial cell immune response by increased mRNA expression of cytokines *in vitro* (Strandberg et al., 2005; Wellnitz et al., 2006). Compared with the immune response of epithelial cells to LPS the response to LTA was much smaller in regard to TNF- α , IL-1 β , IL-8 and β -defensin expression (Strandberg et al., 2005). However, in these experiments a smaller amount of LTA than LPS was used.

In vivo LTA induced a rapid increase of SCC if applied into the mammary gland through the teat canal (20 μ g/quarter) and, in addition, it increased the mRNA expression of IL-8 and lactoferrin in the tissue after 6 and 12 h, respectively. However, it did not stimulate the TNF- α mRNA expression (Stenger et al., 2007).

Whereas in gram-negative bacteria as the major pathogenic component only the cell wall component LPS is described, in gram-positive bacteria, like *S. aureus*, two different pathogenic components: LTA and peptidoglycans (PGN) are described. They both bind to the TLR-2 (Aderem and Ulevitch, 2000). Commercially available *S. aureus* PGN was shown to induce the mRNA expression of immunomodulators in mammary gland epithelial cells *in vitro* (Wellnitz et al., 2006). However, Travassos et al., (2004) discussed that the TLR-2 recognition of PGN is the result of LTA contamination.

Comparison of LPS and LTA

E. coli and *S. aureus* induce normally a different course of mastitis: *E. coli* predominantly acute and severe, *S. aureus* often subclinical and chronic forms. These pathogens seem to induce the mammary immune response differently if used in the same concentration, because a smaller increase of cytokines and chemokines was shown in *E. coli* treatment of mammary gland tissue *in vivo* (Riollet et al., 2000b; Bannerman et al., 2004) and of mammary epithelial cells *in vitro* (Wellnitz et al., 2006). Same findings were made using the pathogenic components LPS and LTA *in vitro* on mammary epithelial cells (Strandberg et al., 2005). However, own investigations indicate that these responses depend on the origin of LTA/LPS and are dose dependent. Heat inactivated *Streptococcus uberis* as induced did not induce an immune response like *S. aureus* in the same concentration although both bacteria are LTA containing gram-positive pathogens (Wellnitz et al., 2006). In addition, LPS extracted from a bovine mastitis pathogen induced a comparable immune response (increase of SCC) in mammary glands if used in a dose of 1/1000 as a commercial available LPS extracted from a different than mastitis case (unpublished). In addition, with LTA extracted from a *S. aureus* strain that was isolated from a mastitis case, comparable immune responses of the mammary gland were found (Stenger et al., 2007) as detected previously in the studies with LPS challenge (Schmitz et al., 2004). An exception was found in TNF- α mRNA expression that was lower in LTA treatment and the IL-8 mRNA expression was pronounced. Yet, this matches with results in human blood cells which show that LTA is a much weaker inducer of TNF- α release but a stronger inducer of IL-8 expression (von Aulock et al., 2006).

Conclusion

In conclusion, the use of PAMP of different pathogens to study mammary gland immune response either *in vitro* or *in vivo* is a convenient model that provides defined experimental conditions and excludes greatest possible environmental influences. An enormous knowledge has been received in the mechanisms of immune defense of the mammary gland, specifically in the regulation of immunomodulators, in response to stimulation with the different endotoxins of mastitis pathogens.

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12.3 THE VOLUNTARY UDDER HEALTH SANITATION PROGRAM IN BAVARIA

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The task of the Udder Health Service (EGD) in Bavaria acts as a control and consulting centre between dairy farms, dairy and veterinary practitioners. The EGD is the relevant organization in case of excessive SCC based on national law. The decision of a farmer to consult the EGD is voluntary. In 1961 a program was developed, intending to improve and stabilize the udder health significantly. A continuous control facilitates an immediate and targeted assistance for the respective dairy herds. Since 2006 the program will be continued by a EU permitted project named: Udder Health and Protection of Udder Health in Bavarian Dairy Farms. The Project will run by three modules: technology, health as well as technology and health. Within the three modules three aims could be selected protection, improvement or rehabilitation. This project to assure good milk quality and food safety is supported by the dairies in Bavaria. Dairy farmers cover the fees proportionally. The MAF and the Animal Health Insurance support the investigations by proportionate funding. Herds with an increased mastitis frequency will become noticeable by the SCC data of the monthly taken samples through the Dairy Board (MPR) and the milk yield data of the Association for Animal Recording (LKV). The MPR warns the farmer if bulk tank milk SCC exceeds the limit of 300,000 for the first time. He is requested to take measures to solve the problems and to consult the EGD e.g.. A 1992 jointly developed 'Early Warning System' of LKV and EGD is designed to make out individual cows and herds at risk. It underlines the readiness and acceptance for self control in the farms. If more than 30 % of all cows under milk control exceed the SCC > 200,000/ml, the monthly milk report will be supplemented with a 'comment'. After exceeding the limit repeatedly the respective farmer is offered to consult the EGD. The project is offered countrywide. Tests and investigations cover housing, general and specific milking hygiene, milking equipment and its technical condition including cytological status of all cows in milk. Investigations and consulting is performed by technical and veterinary personal. Quarter milk samples are taken from all lactating cows and evaluated in own laboratories. The detailed written findings are sent to both the farmer and the respective veterinarian. The milking unit report, the laboratory findings and the veterinary recommendation provide reliable support to start necessary treatment and adequate hygiene measures and to clear specific technical problems. In the last 18 years the EGD has looked every year after some 10% of all dairy farms in Bavaria. Up to 168,428 farm visits were carried out as well as 2,391,455 cows and 10,906,606 quarter milk samples were investigated.

12.4 INFLUENCE OF HOUSING SYSTEM FOR OUTBREAKS OF MASTITIS IN WALLOON DAIRY HERDS

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Nowadays, mastitis can still have profound economic and health impacts on dairy farms. The negative effects of this pathology on farm profitability are universally recognized and the multifactorial nature of this disease has been confirmed by numerous studies. However, these studies mostly focused on the description of the frequency, nature of bacteria and pathogenicity through individual approaches. The results gained by these previous studies are therefore difficult to interpret due to the limitation of experimental frameworks. Through an ecopathological study, our project aims at accurately quantify the mammary health status on dairy farms in Wallonia (Belgium). In particular, this project focuses on the identification of the risk factors (milking conditions, environment and management) involved in mastitis frequency through detailed analysis of individual farm audits.

Material and methods: We selected 400 farms for the audit on the basis of six criteria applied on a 4 months period (from August to November 2005) :1.Number of animals (>20) 2.Cellular score 3.Percent of animals with a Somatic Cell Count (SCC)> 400 000 cell/ml during the present lactation4.Percent of animals with a SCC> 400 000 cell/ml during the present and last lactation 5.Frequency of bulk milk SCC > 400 000 cell/ml on the observed period 6.Geographical repartition.In each farm, the audit consisted in a one shot visit with observations on milking conditions, environment and herd management. Overall, 430 potential risk factors were observed for each visit.To evaluate the risk's infection, an Infection's Risk Scale or IRS, based on criteria 2 to 4 has been proposed. It extends from 0 (low risk) to 6 (high risk). Separate analysis of the influence of each factor on the IRS has been carried out.Results and discussionThe observations showed a large diversity of practices in Wallonia related to milking systems, milking conditions and herd management. Among the numerous factors susceptible to influence the infection's risk, we chose to focus on housing system.The free-stalls are more widespread (52%) than the straw yards (30%) and the tie-stalls (18%). IRS differed with the housing conditions and was greater with straw yards than with free-stalls or tie-stall. However, no significant difference was observed between tie-stall and free-stall. Yet, the cleanness of the cows was better with free-stall than with the tie-stall housing system.

12.5 ASSOCIATION OF THE PITUITARY-SPECIFIC TRANSCRIPTION FACTOR-1 AND LEPTIN GENES WITH TRAITS RELATED TO THE TEAT DEVELOPMENT IN PIGS

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The development of the mammary gland is well recognized, but still not fully understood. Different genes are known to play a role for the teat development through hormone- receptor reaction. The development and lactation of mammary gland is a complex process regulated by various growth factors including the Leptin (LEP) gene. LEP is known to influence the development and lactation of mammary gland. The Pituitary-Specific Transcription Factor-1 (PIT-1) is responsible for pituitary development and hormone expression in mammals and is a candidate gene for growth and muscle development in pigs. Further it is a cell-specific activator of gene transcription of Growth hormone or Prolactin, genes which are involved in the development of the mammary gland. The aim of this study was to investigate the association of PIT-1 and LEP genes with characteristics of the mammary gland in pigs, especially the inverted teat and the number of teats. Due to the later application as markers for selection the study was carried out in two pig populations. Genotyping was performed in pigs arising from an experimental population generated by reciprocal crossing of Berlin Miniature Pig and Duroc and animals of the dam breeds German Landrace (DL) and German Large White (DE). The PIT-1 polymorphism was detected and confirmed by PCR-RFLP analyses with *RsaI* endonuclease. The restriction enzyme *HinfI* was used for genotyping the polymorphism in LEP using PCR-RFLP. The statistical analysis was carried out using the Family-based association analysis (FBAT) program. The analysis of PIT-1 showed highly significant association with the affection status for inverted teats ($p \leq 0.001$), the left, right and total number of teats ($p \leq 0.001$). Significant association could also be shown with the number of inverted teats on the left, the right side and in total ($p \leq 0.05$). LEP polymorphisms were also highly significant associated with the left, right and total number of teats ($p \leq 0.01$). Significant association could also be shown with the affection status with inverted teats ($p \leq 0.05$) but not with the number of inverted teats. In the analysis of the commercial population no significance was found. This result indicates that PIT-1 gene and LEP gene affect the mammary gland development in pigs. To be a useful molecular marker for the selection in breeding programs for improved teat performance further studies are necessary as in this study no significance could be detected in the dam lines.

12.6 THE DIAGNOSTIC PROPERTIES OF A QPCR BASED ASSAY TO DETECT STAPHYLOCOCCUS AUREUS IN MILK AND COMPARISON WITH STANDARD BACTERIOLOGY

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1. Setting and aim of the study. Bacteriological culture as a diagnostic tool for *Staphylococcus aureus* (*S. aureus*) is not completely satisfying. The cyclic shedding pattern of *S. aureus* with intervals of low excretion is considered to be the main reason for this. A novel assay for *S. aureus* in milk, based on real-time quantitative PCR (QPCR), was developed by Graber et al. (see proceeding abstract of H.U. Graber). In order to analyze the diagnostic properties of this test, its diagnostic sensitivity and specificity was evaluated by a longitudinal study under field conditions. In addition, the shedding pattern was analyzed. 2.

Material and methods: Eleven quarters of Swiss SIXRH cows, naturally infected with *S. aureus* and not treated, were selected. Quarters were considered *S. aureus*-positive if 3 consecutive samples had revealed a positive bacteriological result. Eleven control quarters of cows in the same herds were selected. They were determined free of *S. aureus* if bacteriology was 3 times negative. Milk was collected during 14 consecutive days before evening milking. For bacteriology, 100µl were spread on sheep blood agar and on Baird-Parker. Growing bacteria were evaluated according to the guidelines of NMC. For the novel assay, preparation of bacteria from raw milk, DNA extraction and QPCR (2 primers and a fluorescent probe) were done according Graber et al.3.

Results: The overall diagnostic sensitivity of the QPCR-assay was 99.4%, meaning that an intramammary infection with *S. aureus* will be correctly detected with a probability of 0.994. On quarter level, the diagnostic sensitivity ranged between 92.9% and 100%. The overall diagnostic specificity was 99.3% (quarter level: 92.9% to 100%). Considering routine bacteriology using 10 µl aliquots, the overall diagnostic sensitivity was 79.9% with a range between 21.4% and 100% on quarter level. In the case of the quarter with very low shedding frequency, QPCR always gave a positive result. The diagnostic specificity of bacteriology was 100% (overall and quarter level). Using log₁₀ transformed QPCR data and plotting them across sampling time, 6 quarters showed a cyclic shedding pattern, 2 a log linear increasing pattern whereas 3 had no definite pattern. Considering the cyclic pattern, a sine-forming dependency with a half-period of 6.5 days was observed. 4.

Conclusion: The novel test has a very high diagnostic sensitivity and specificity so that quarters infected with *S. aureus* are reliably detected, independent from their actual shedding quantity. Reliable identification of infected quarters can be done now by single sampling. This is in contrast to bacteriology which requires consecutive samples. Bacterial shedding frequently shows a cyclic, sine-forming pattern.

12.7 DEVELOPMENT OF A HIGHLY SENSITIVE AND SPECIFIC ASSAY TO DETECT STAPHYLOCOCCUS AUREUS IN BOVINE MASTITIC MILK

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1. Setting and aim of the study Bacteriological analysis of milk on agar plates is still the gold standard for the microbiological diagnosis of *Staphylococcus aureus* (*S. aureus*) intramammary infections. However, it often provides unsatisfactory results. Using aliquots of 100µl per sample, the overall bacteriological sensitivity is 75% but may vary between 41% and 100% for individual cows. Under routine conditions with smaller aliquots (10µl), the results are even worse. Only when 3 consecutive milk samples are taken, the sensitivity improves to 98%. This procedure, however, is expensive and is not satisfactory for clinical purposes. To improve the diagnostics for *S. aureus* in mastitic milk, we developed a test that allows the highly specific detection of *S. aureus* in bovine milk samples at very low concentrations.2.

Material and methods: An assay was developed which is based on a novel, fast and patented procedure to prepare *S. aureus* from milk processing 400µl of milk. This step is followed by DNA extraction and real-time quantitative PCR (QPCR) with a *S. aureus*-specific gene as a target. QPCR was performed with 2 primers and a fluorescent probe. An internal control DNA was included so that negative results of target gene amplification could be interpreted. The QPCR method was compared to classical bacteriology using 77 clinical milk samples. Bacteriological analysis of each sample was done according to the guidelines of the NMC using 100µl-aliquots.3.

Results: For the clinical milk samples, the analytical sensitivity of the novel assay was 50.7 times and 507 times higher than conventional bacteriology with plating volumes of 100µl and 10µl (calculated), respectively. The diagnostic specificity was 100% and was not influenced by contaminating DNA extracted from various *Staphylococcus* spp. The test is further characterized by a low intra- and inter-assay variability as well as by a very good recovery (94%). Furthermore, a high correlation ($r = 0.925$) between the agar plate counts and the QPCR methodology over the whole range was found. In addition, our test revealed considerably more positive results than bacteriology ($p < 0.001$). Finally, the novel assay is fast as a definite result is obtained within 5 hours including bacteria preparation, DNA extraction and QPCR.4.

Conclusion: We developed a novel QPCR-based assay to detect and quantify *S. aureus* in milk. It is characterized by excellent diagnostic properties such as a very high analytical sensitivity and specificity. Due to its distinctive features, the assay is expected to become an important diagnostic tool in the context of bovine mastitis caused by *S. aureus*.

12.8 A PATHOGEN-SPECIFIC ANALYSIS OF INTRA-MAMMARY INFECTIONS IN DAIRY COWS: ENVIRONMENTAL AND GENETIC ASPECTS

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Subclinical mastitis most often is the cause of disappointing cell counts in dairy herds. Infected animals are at high risk for subsequently exhibiting mastitis as well as other diseases. The present project was carried out by the State Research Institute of Agriculture in Thuringia in cooperation with the Animal Breeding Group of the IANS in Halle. Data originated from 55 dairy farms during 1999 to 2001 and comprised a total of 22,462 cows with 93,293 foremilk samples. Aim of the study was to assess the udder health status, i.e. the prevalence of intra-mammary infections (IMI), based on bacteriological analyses of milk samples, and its relationship with somatic cell score. The results showed a IMI prevalence of 33.1 % (positive samples). Most important pathogens (% of positive samples) were Coagulase negative staphylococci (CNS, 40.8 %) and *S. aureus* (28.57 %). The study included the estimation of environmental effects like parity or housing systems as well as genetic effects. Heritabilities for binary traits describing the status of healthy vs. infected for infections in general and for infections caused by specific pathogens were estimated as around 5 %. Bivariate genetic analyses were used to examine the relationship between infections and somatic cell score. The relevance of an analysis according to pathogen is underlined by the fact that the two most important pathogens in this study, e.g. *S. aureus* and CNS, are clearly contrasting with respect to the incidence within and across parities.

12.9 ELECTRICAL CONDUCTIVITY AND N-ACETYL- β -D-GLUCOSAMINIDASE ACTIVITY OF DIFFERENT MILK FRACTIONS IN RELATION TO UDDER HEALTH IN DAIRY COWS

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Present study sought to determine how the electrical conductivity (EC) and N-acetyl-beta-D-glucosaminidase (NAGase) activity were affected over different milk fractions considering udder health, and which milk fraction was most effective in regard to mastitis diagnosis. A total of 24 lactating dairy cows were involved. Three different milk fractions viz., quarter strict foremilk, foremilk and strippings were collected and analyzed for microbiology, EC and NAGase activity. To rule out any possible effect due to management, animal physiology and analytical procedures, the collection and processing of milk samples from each cow was done thrice at weekly intervals, and the means of three-day values were used.

Results revealed 10% and 5% of the quarters suffering from specific and non-specific mastitis (IDF criteria). The latent infections accounted for 21 % of quarters. Both, udder health and milk fractions showed significant effects on the parameters studied. Investigation of interdependence of udder quarters indicated significant differences for EC and NAGase between healthy and diseased quarters of identical udders. Moreover, these parameters for healthy quarters from healthy udders differed significantly from healthy quarters of udders having one or more diseased quarters, thus putting a question mark on the independence of quarters. Over the milking process, EC decreased while NAGase increased towards end of milking (strippings). But, the magnitude of change differed for healthy and mastitis quarters e. g. fall in EC was more pronounced in healthy than in diseased quarters and the reverse was true for NAGase. Overall, strippings milk fraction could differentiate in a better way between the healthy and mastitis quarters.

12.10 FERTILITY INDICES OF MASTITIC DAIRY COWS TREATED INTRAMAMMARILY WITH ANTIBIOTICS IN CONNECTION WITH PARENTERAL INJECTION OF ANTIOXIDANTS, IMMUNOMODULATOR OR NSAID

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Mastitis is the most frequent disease of dairy cows that decreases a reproduction efficiency apart of milk yield and quality. The purpose of the study was to establish the effect of an additional medicine injection to intramammary treated mastitic cows on results of both mastitis treatment and fertility indices of these animals. Field trials were conducted in 3 farms on 161 cows with acute form of mastitis (221 inflamed quarters) that occurred before a 100 d post calving vs. 60 healthy control cows (group I). After clinical examination of cows, udders, and milk sampling for laboratory examinations, mastitic animals were allocated to four groups. Some cows were intramammary treated with antibiotic products only according to recommendations of manufacturers (50 animals; II group) or with the same antibiotics connected with one i.m. injection of antioxidants: vitamin C (0.01 mg/kg), beta-caroten (0.4 mg/kg) and vitamin E (1 mg/kg) connected with 0.01 mg/kg of Se (46 cows; III group), or with the lysozyme dimer in dose of 0.02 mg/kg b.w. (30 cows; IV group) or with Flunixin meglumine in dose of 2.2 mg/kg b.w. (35 cows; V group). The recovery rates were 52.0 % (II group), 54.3 % (V group), 65.2 % (III) and 67.0 % (IV group) of cows. The best fertility indices: time from calving to first insemination (CI = 80 d), conception rate (CR = 72%), artificial insemination index (AI = 1.38) and calving pregnancy period (CP = 91d) were in healthy control animals (I group). Among mastitic cows, fertility indices were better in groups treated with additional medicine (V, III, IV, successive) than in the group II (CI = 120d; CR = 25%; AI = 2.2; CP = 150 d, respectively), independently on recovery rates. However, it was found that cows recovered from mastitis had better fertility indices than animals refractory to the first therapy, except of IV group (no differences).

In conclusion, mastitis negatively affects the reproductive efficiency of cows. One i.m. injection of lysozyme dimer or antioxidants increases the efficacy of local antibiotic treatment of acute udder inflammations caused by CNS, *E. coli*, *Streptococcus* sp., *Cor. bovis*, and even *Staph. aureus*. The injection of additional medicine: flunixin meglumine, antioxidants or lysozyme dimer can improve the reproductive performance of cows with acute mastitis, traditionally treated with antibiotics.

12.11 THE PHARMACOKINETICS AND MILK RESIDUAL BEHAVIOUR OF TYLOSIN IN LACTATING NAJDI EWES

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Problem and Aim: The residual depletion in milk and plasma kinetics of tylosin were evaluated in Najdi lactating ewes (n=5) injected intramuscularly with 10 mg kg⁻¹ b.wt. **Maternal and Methods:** Blood and milk samples were collected before and at different time intervals after treatment. Tylosin concentrations in plasma and milk were determined by a microbiological agar plate assay using *Bacillus subtilis* ATCC 6633 as the test organism. **Results:** The plasma levels of tylosin against time were adequately described by the one compartment open model with a fairly low area under the concentration time curve (AUC) (2.99 µg.h/ml) and maximum plasma concentration (C_{max}) (0.626 µg/ml) at t_{max} (1.33 h). The plasma elimination half-life (t_{1/2el}) was 2.302 h and the mean residual time (MRT) was 3.863 h. A different pattern was shown for milk, in which measurable residual levels are found in all animals up to 72 hours after treatment. The mean value of milk AUC was 88.124 µg.h/ml and the t_{1/2el} was 3.29 h. In vitro mean plasma and milk proteins binding of tylosin were 19.30 % and 30.23 %, respectively.

Conclusion: The milk withdrawal period of tylosin in lactating Najdi ewes should be at least 72 h to avoid risks for human consumption.

12.12 ASSESSMENT OF MAMMARIAN BLOOD FLOW IN DAIRY COWS

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It is well known that milk synthesis of the mammary gland depends upon an adequate supply of nutrients from arterial blood, but up to now there are no clinical studies of blood supply of the mammary gland in lactating cows. The aims of this study were to establish transrectal colour Doppler sonography as a tool for the non-invasive measurement of blood flow to the mammary gland and to compare it with milk yield of cows. Mammary blood flow was quantified by measuring the blood flow volume (BFV) in the right and the left pudendoepigastric trunks. Examinations were carried out in 44 multiparous Holstein-Friesian cows (lactation number: 2.67) in weeks 21, 25, 29, 33, 36 and 39 of pregnancy as well as on days 1, 7, 14, 28, 56 and 84 post partum (p.p.). As the time of drying off varied between weeks 28 and 34 of pregnancy, BFV-values were standardized in relation to this time point (weeks -12 to 12). Relating to the median of milk yield (30.1 L/day) during early lactation (days 7 to 84 p.p.), cows were divided into two groups: low (<30 L/day, mean: 26.6 L/day) and high (>30 L/day, mean: 34.7 L/day) yielding cows. In all cows changes in BFV followed a characteristic pattern, but there was a high individual variability (coefficient of variation range from 19.6% to 45.0%). Between weeks -12 and -1 mean BFV decreased ($P < 0.05$) slightly from 9,774 ml/min to 7,123 ml/min. After drying off, BFV-values reached ($P < 0.05$) basic levels (mean of weeks 1 to 12: 2,145 ml/min). On day 1 p.p. BFV had steeply inclined ($P < 0.05$) to 13,857 ml/min. Afterwards it decreased ($P < 0.05$) to 10,533 ml/min on day 7 p.p., followed by a slight increase ($P < 0.05$) between days 14 (10,015 ml/min) and 84 p.p. (11,399 ml/min). High yielding cows showed higher ($P < 0.05$) BFV-values only between weeks -9 and -4 and on days 14 and 28 p.p. than low yielding cows. During the lactating period, milk yield was weakly to moderately related to BFV ($r = 0.36$ to 0.54 ; $P < 0.05$).

In conclusion, Doppler sonography is a useful tool for measuring blood flow volume in the pudendoepigastric trunks. There is a high variability in mammary blood supply between cows. Most distinct changes in mammary blood flow occur around times of drying off and parturition, but there are only slight alterations in blood flow during early and late lactation. Mammary blood supply is higher in high yielding dairy cows, but there are no good correlations between mammary blood flow and milk yield.

12.13 EFFECT OF SOME FACTORS UPON THE VARIOUS TYPES OF MASTITIS IN COWS IN BULGARIA

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The aim of the present study was to determine the effect of the farm with different traits and practices, the number of lactation, phase of lactation and the season upon the incidence of subclinical mastitis, nonspecific mastitis and latent infections. A total of 1564 quarter milk samples from 391 cows reared at 5 farms were examined. The cows were from the Bulgarian Black-and-white breed at their 1st, 2nd and 3rd lactation with an average milk productivity between 4000 and 6900 kg. Each sampling corresponded to 30% of the overall farm capacity (80-150 cows). Milk samples were obtained throughout the four seasons (spring, summer, autumn and winter) in various lactation phases (1-2; 3-4; 5-6; 7-8 lactation month). According to the bacteriological examination, the milk somatic cell counts (SCC) and the clinical inspection of the udder, the type of mastitis was determined. The subclinical mastitis was defined at $SCC \geq 250\ 000/ml$ and presence of mastitis pathogens at $\geq 500\ cfu/ml$ milk; nonspecific mastitis - at the same SCC and lack of mastitis pathogens and latent infection - at $SCC < 250\ 000/ml$ and mastitis pathogens at $\geq 500\ cfu/ml$ milk. The effect of each farm was determined taking into consideration the rearing technology, mastitis control, the sanitation, milking system and management practices. The effect of the number of lactation, the phase of lactation and the season (spring, summer, autumn, winter) was also estimated. The determination of the effect of all those factors was done by the least squares means (LSM) analysis. The analysis of variance of controlled factors upon the total morbidity rate revealed a highly statistically significant effect only of the farm. Positive LS estimation had farms with bad management and irregular mastitis control. A statistically significant effect on latent infections ($P < 0.001$) had only season. Winter and spring were seasons with positive LS value. The season ($P < 0.001$) and farm ($P < 0.01$) effects were most important for the manifestation of nonspecific mastitis. For this type, the farms with bad management and poor mastitis control had a positive LS estimation value. The autumn and the winter were the seasons favourizing its appearance and LS ranged between +0.2 and +0.1, respectively. The appearance of subclinical mastitis, compared to the other mastitis types was influenced by the highest number of factors. The strongest effect ($P < 0.001$) was that of the farm according the farm practices, the number of lactation ($P < 0.01$) and the season ($P < 0.05$).

12.14 CYTOKINE MRNA EXPRESSION PROFILES IN MAMMARY GLAND EPITHELIAL CELLS INDUCED BY STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

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Epithelial cells of the mammary gland are the final barrier that prevent invading pathogens from entering circulation. Their role in the immune defence of the mammary gland is not only building a mechanical border but also initiating and transmitting essential immunological signals which is a crucial mechanism for eliminating microorganisms and, thus, for the course of infection. Epithelial cells isolated from mammary glands of twentyeight primiparous German Holstein Friesian dairy cows were cultured and treated in the third passage with the same concentration (10 MOI) of heat-inactivated *Staphylococcus aureus* (*S. aureus*) representing pathogens causing chronic mastitis or *Escherichia coli* (*E. coli*) often involved in acute mastitis. After 1, 6 and 24h total RNA was harvested and mRNA expression of tumour necrosis factor alpha (TNFalpha), interleukin (IL)-1beta, IL-6, IL-8 and RANTES was measured by quantitative real-time RT-PCR using mastercycler realplex (Eppendorf). TNFalpha and IL-1beta mRNA expression showed a significant ($P < 0.05$) increase in both *S. aureus* and *E. coli* treated cells after 6h. RANTES and IL-6 mRNA expressions were up-regulated significantly after 24h in *S. aureus* exposed samples and already after 6h in *E. coli* treated cells. Moreover, RANTES showed a further significant increase after 24h in the *E. coli* group. IL-8 showed a significant up-regulation after 6h only in *E. coli* treated samples. At all time points *E. coli* exposed cells are characterized by a significantly higher expression of TNF alpha and IL-1beta mRNA than *S. aureus* affected epithelial cells. Furthermore expression levels of IL-6 and IL-8 in *E. coli* treated cells are significantly higher after 1 and 6h, but no difference between the pathogens could be detected after 24h. However, RANTES showed a significantly higher expression in *E. coli* affected cells than in *S. aureus* treated samples after 6 and 24h. None of the measured factors changed in control cells within time in both treatments.

In conclusion, this work, distinguished from other experiments especially by its large number of primary cell cultures from different animals, provides a reliable explanation for deviating cytokine patterns during acute and chronic mastitis. These deviations are characterized by differences in time dependent up-regulation and also by different levels of up-regulation of proinflammatory cytokines in mammary gland epithelial cells treated with the same concentration of *S. aureus* and *E. coli*.

12.15 SUBCLINICAL MASTITIS IN DAIRY COWS HELD IN ORGANIC AND CONVENTIONAL FARMS

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Subclinical mastitis (SM) accounts for high economic losses. There is non-consistent evidence that SM and high somatic cell counts (SCC) are a greater problem in organic (OP) than in conventional production (CP) farms. We have studied the prevalence of SM and of udder pathogens in 60 Swiss OP and 60 CP farms, risk factors for SM and antibiotic resistance of udder pathogens isolated from cows with SM. Cows ($n = 970$) were studied at median 31 and 102 d postpartum. Cows with a 1+ positive California Mastitis Test (CMT) in at least one quarter were considered to have SM. At 31 and 102 d cow-level prevalences of SM (39 and 40% in OP, 34% and 35% in CP) were similar, but quarter-level prevalences of SM were higher in OP than CP (15% and 18% in OP, 12% and 15% in CP). Median SCC at 31 d were higher in OP than CP cows (43,000 and 28,000 cells/mL), but were similar at 102 d in OP and CP cows (45,000 and 38,000 cells/mL). In milks from quarters with a CMT reaction $\geq 2+$ prevalences of coagulase negative staphylococci were lower at 102 d, whereas of non-agalactiae streptococci were higher in OP than in CP cows at 31 and 102 d. Differences in the percentage of antibiotic resistance were mainly species-dependent, but there were no differences between isolates from cows held on OP or CP farms. The percentage of resistance to ceftiofur, erythromycin, clindamycin, enrofloxacin, chloramphenicol, penicillin, oxacillin, gentamicin, tetracycline, and quinupristin-dalfopristin was different, but no strain was resistant to amoxicillin-clavulanic acid and vancomycin.

In conclusion, under Swiss conditions, udder health was in general good in both OP and CP farms, but SM was a greater problem in OP than in CP cows, although differences were not marked. Differences of risk factors for SM between OP and CP cows were partially related to system-specific management, such as antibiotic dry cow therapy, nutrition and milking routine. Monitoring of antibiotic resistance of mastitis pathogens to avoid the emergence of multi-drug resistant bacteria is necessary also in OP farms, in which they are not expected because of restricted use.

12.16 PLASMA FIBRINOGEN CONCENTRATION IN BOVINE MASTITIS

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The objective of the present study was to determine plasma fibrinogen concentration in dairy cows with clinical mastitis in order to evaluate as one diagnostic test. The study was carried out in several farms in La Laguna, Torreon, Mexico. Seventy-four Holstein dairy cows were included in the study. To be included in the testing group of this study, cows had to meet all of the following inclusion criteria: 1) clinical mastitis in only one quarter, 2) macroscopically abnormal milk without or with systemic signs, 3) no current disease, 4) no teat lesions and 5) no clinical mastitis or anti-infectious or anti-inflammatory treatment within the last 14 d. The animals were meeting in groups by clinical examination including general condition, rectal temperature, heart rate, udder edema and appearance of milk. Testing group (n=39), cows with clinical mastitis, which were classified as chronic mastitis (CM), moderately acute mastitis (MAM), severely acute mastitis (SAM) and healthy group (n=35), cows clinically healthy. The results for testing group were 12.8%, 51.2% and 35.8% to CM, MAM and SAM, respectively. Blood samples from this group showed an average of total plasma proteins (TPP) and plasma fibrinogen (PF) concentration of 95.4 ± 10.7 g/L and 8.38 ± 5.09 g/L, respectively. According to the mastitis degree, the values obtained for TPP were 104 ± 8.5 , 92.7 ± 11.5 and 96.2 ± 8.8 for CM, MAM and SAM, respectively. For PF, 8.20 ± 5.76 , 7.45 ± 4.68 and 9.79 ± 5.48 g/L for CM, MAM and SAM, respectively. For healthy group, the average of TPP was 91.0 ± 6.0 g/L and PF of 6.86 ± 4.5 g/L.

The results did not show a significant difference between both groups, however, the plasma fibrinogen concentration could be used as an important and complementary indicator of inflammation in the diagnosis of mastitis.

12.17 INFLUENCE OF DRY COW THERAPY IN DAIRY CATTLE DURING TRANSITION PERIOD AND EARLY LACTATION

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The dry cow therapy in dairy farms with antimicrobials have been used in the last years as an important part of mastitis control programmes. The aim of the present study was to follow clinical mastitis incidences during transition period and up to 3th lactation month after parturition in dairy cows with or without dry cow therapy. Our survey was carried out in three commercial dairy farms (A, B and C) with Holstain-Fresian and Bulgarian Brown cattle breed. The milk samples for bacteriologic examination and somatic cell count (SCC) were received two week before draining. From animals in each farm were setted up 3 groups (48 quaters in group) according to microbial culture. First group included animals with obligated microorganisms, second group with mastitis pathogens and third group with associated microbial culture. The cows from first two groups were treated with Orbenin syringie intramammariae (PfaiserTM) at draining day and animals from third group (control) were untreated. The clinical mastitis incidences during transition period were recorded. Milk samples on day 5, 15, 30 and 60 after parturition were received and bacteriologic examination and SCC were performed. The mastitis incidences establishment up to 3th lactation month was done. During the transition period clinical mastitis incidences in first group for all three farms, second groups in farm A and farm B had not been registrated. In second group in farm C and third group from all farms clinical mastitis incidences were registrated. Significant higher values ($p < 0.05$) of somatic cells were found in animals without dry cow therapy comparing to animals with this one. The our results showed that dry cow therapy reduced in 80% mastitis incidences during transition period and early lactation and could be effective part of mastitis control programmes.

Key words: dairy cows, dry cow therapy, mastitis

12.18 ASSOCIATION BETWEEN THE QUARTER-LEVEL SOMATIC CELL COUNT AND INFECTION STATUS IN DAIRY HEIFERS IN EARLY LACTATION

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Problem: Although dairy heifers represent the future production of a dairy herd, many freshen with an intramammary infection (IMI). Knowledge about the dynamics of those IMI (transient versus persisting) and their impact on the quarter-milk somatic cell count (qSCC) in early lactation are, however, scarce.

Aim: The main objective of this study was to picture the prevalence and distribution of mastitis pathogens isolated from dairy heifers in Flanders between 1-4 days in milk (DIM) and between 5-8 DIM, and to explore the relation with qSCC in early lactation.

Materials and methods: A total of 146 dairy heifers from 20 dairy herds were included. Quarter-milk samples were collected between 1-4 DIM, and between 5-8 DIM after calving for bacteriological culture and determination of qSCC. Quarters were subdivided into four different groups according to their IMI-status. A quarter was defined as (1) culture-negative when the two subsequent samples were culture-negative, (2) single NAS-positive if the first sample yielded non-aureus staphylococci (NAS) and if the second sample was culture-negative, (3) double NAS-positive if NAS-species were cultured from the two consecutive samples and (4) major pathogen-positive if *Staphylococcus aureus* or esculin-positive cocci were isolated from the first sample, regardless of the result of the second sample. The association between the natural logarithmic transformed qSCC (LnqSCC) and the IMI-status was analyzed using analysis of variance (ANOVA) at first and second sampling, respectively.

Results: The majority of IMI in dairy heifers in early lactation were caused by NAS. Remarkably, half of the NAS-species isolated at day 1-4 could not be cultured again from the subsequent sample at day 5-8. The overall average LnqSCC decreased from 5.78 at 1-4 DIM to 4.66 at 5-8 DIM. At 1-4 DIM, the average LnqSCC was significantly lower in culture-negative quarters (5.45) than in quarters infected with a major pathogen (6.79). At 5-8 DIM, the average LnqSCC was significantly lower in culture-negative quarters (4.18) compared to single NAS-positive quarters (4.74), double NAS-positive quarters (5.14), and quarters infected with major pathogens (6.13). Single NAS-positive quarters had a significantly lower LnqSCC than quarters infected with major pathogens. No significant difference in LnqSCC between single NAS-positive and double NAS-positive quarters was present at second sampling.

Conclusions: A bacterial-specific impact (minor versus major pathogen; transient versus persisting IMI) on the qSCC of dairy heifers in early lactation is present.

12.19 STABILITY OF MILK, FRACTIONS OF PROTEINS AND IONIZED CALCIUM

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The stability of milk to ethanol is a very ancient test used for the control of public health and it is still carried out in many countries. The test is positive when milk coagulation happens after to add alcohol, it indicates acid or unstable milk. Some dairy farms have an increased frequency of milk tests positives in some seasonal periods, thus their bulk milk is rejected by the industry. This circumstance is a challenge for milk producers because they have no evident solutions. The instability of milk has been related to changes in composition and high ionized calcium. The aim is to explore the relation among ethanol instability of milk, fractions of proteins and amount of ionized calcium.

Material and methods: Bulk and individual cow milk were analyzed of 20 commercial herds. Studies were performed immediately after milking for Ca⁺⁺, pH and alcohol test. Ionized calcium was analyzed with a specific electrode (ISE/Orion). Stability of milk samples were tested on mixing an equal volume of ethanol 70° and milk. Skim milk was analyzed to determinate: total protein by BCA, protein fractions by electrophoresis with PAGE-SDS gels (12%) and quantified by software (Gel-Pro).

Results: The values of total protein in positive milk to alcohol test differed from negative one (respectively: 3.54±0.67% and 3.23±0.40%) (P< 0.01). The amount of total casein was significant lower (P< 0.01) in milk positive (72.98±5.80%) than in negative (75.02±6.58%). Some fractions of proteins showed variations when its values were compared between positive and negative milk: the concentrations of kappa-casein and beta-lactoglobulin were lower in positives than in negatives. Their values were: kappa-casein: 4.18±1.3% and 4.59±1.6% (P< 0.03) and beta-lactoglobulin: 6.09±1.3% and 6.21±2.9% (p < 0.01), respectively. The alcohol positive milk contains higher Ca⁺⁺ (0.118±0.030 g/l) than the negative (0.103±0.026 g/l) (P< 0.01), measured either in bulk milk or individual cows. Values of pH were not different. Ionized calcium has a dominant role in controlling ethanol stability. The depletion of these two fractions is related to the stability of the electrostatic complex Casein-Ca and also to the size of the micelles. Precipitation of the milk protein induced by Ca⁺⁺ might be possible because of the interaction of a larger micelles action in liquid phase associated with a low formation of the complex beta-lactoglobulin/kappa-casein in presence of a high level of Ca⁺⁺.

Conclusions: Studies of milk stability in Uruguay concluded that high amounts of ionized calcium had a significant relationship with the positive alcohol test. Analysis of protein composition revealed decreased total casein and lower percentage values of kappa-casein and beta-lactoglobulin in unstable milk.

12.20 LIPOTEICHOIC ACID INDUCED IMMUNE RESPONSE IN THE BOVINE MAMMARY GLAND

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The gram-positive *Staphylococcus aureus* is one of the major mastitis pathogens. Lipoteichoic acid (LTA) is a gram-positive bacterial cell wall component and one of the pathogen associated molecular patterns (PAMPs) of *S. aureus*. The aim of the present study was to characterize the LTA induced immune response by *in vivo* stimulation of the bovine mammary gland. 15 dairy cows were treated in one quarter with LTA (20 µg LTA diluted in 10 ml 0.9 % NaCl) and one quarter served as control (NaCl). The LTA was isolated from a *S. aureus* strain that has induced mastitis in cows. Pre-treatment somatic cell count (SCC) of the quarters was $69 \pm 14 \times 10^3$ cells/ml. During the experiment either milk samples were collected each h from 0 to 12 h for SCC measurement (7 cows) or mammary gland biopsies were taken at 0, 6, and 12 h after LTA treatment (8 cows) for RNA extraction from mammary tissue and measurement of mRNA expression by qRT-PCR of tumour necrosis factor alpha (TNF), lactoferrin (LF), and interleukin 8 (IL8) and GAPDH as a housekeeping gene. Rectal temperature was measured hourly during the experiment. In the LTA-treated quarter SCC increased within 1h ($P < 0.05$) and increased further up to a maximum ($2604 \pm 916 \times 10^3$ cells/ml) at 8 h after LTA treatment and started to decrease thereafter. Also in the control quarters an increase of SCC was observed with a maximum at 2 h after NaCl infusion ($391 \pm 196 \times 10^3$ cells/ml). The mRNA expression of TNF did not differ between groups or during the course of the experiment in both groups. In contrast, the mRNA expression of LF was increased ($P < 0.05$) in the LTA-treated quarters at 12 h but not in the control quarter. The mRNA expression of IL8 was increased at 6 h in the LTA-treated quarters and at 12 h in the controls. No significant changes of rectal temperature were detectable during the experiments.

In conclusion the stimulation with LTA increases SCC dramatically and causes a significant increase in mRNA expression of LF and IL8. TNF seems to be not affected by LTA stimulation. The results show that the LTA of *S. aureus* stimulates the mammary gland immune system and provokes a somatic cell influx into the mammary gland.

12.21 VIABILITY AND IMMUNE COMPETENCE OF CELLS IN MILK EX VIVO

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The somatic cell count (SCC) is used as a criterion to measure the hygienic status of milk. At the same time milk cells are the most important players in mammary immune response. The goal of this experiment was to test the viability of the different cell population and the immune competence of cells in removed milk with time and in response to lipopolysaccharide (LPS) stimulation. Proportional whole milk samples (machine milking) were taken from 9 dairy cows in their 2nd to 6th lactation, with a SCC of $60 \pm 10 \times 1000/\text{ml}$. 250 ml milk was incubated with or without 125 ug LPS (from *E. coli*; O111:B4; SIGMA) at 37°C. After 0, 1, 3, and 6h, resp., the cells were washed, isolated (centrifugation), microscopically counted, the viability tested (Trypan blue stain), and microscopically differentiated (Pappenheim stain). In addition, cells of 4 of these cows were tested for the mRNA-expression of important immune modulators: Tumor necrosis factor alpha (TNF), interleukin-8 (IL8), lactoferrin (Lf) with quantitative RT-PCR and their expression was normalized by GAPDH.

Results are described as changes if $P < 0.05$. Cell concentrations decreased within 1h in LPS treated cells and decreased further until 6h, whereas in controls only after 6h a decrease in cell concentration was detected (35 ± 6 , 31 ± 6 , 46 ± 6 % reduction in treated, and 17 ± 7 , 15 ± 8 , 35 ± 7 % reduction, in control cells at 1, 3, and 6h, resp.). The viability of the cells decreased in treated and control cells within 6h of incubation with the greatest reduction from 3 to 6h (from 78 ± 3 to 75 ± 3 , 73 ± 3 , 62 ± 3 % in treated, and 77 ± 4 , 76 ± 4 , 63 ± 2 % in control cells at 1, 3, and 6h, resp.). PMN fraction decreased within 1h in treated and control cells and was 74 ± 5 , 63 ± 6 , 73 ± 5 , 75 ± 7 % in treated, and 74 ± 5 , 67 ± 6 , 74 ± 6 , 69 ± 12 % in control cells, at 0, 1, 3, and 6h, resp., whereas the fraction of macrophages increased after 1h of LPS treatment (19 ± 4 , 28 ± 6 , 23 ± 7 , 23 ± 6 % in treated, and 19 ± 4 , 24 ± 5 , 21 ± 6 , 28 ± 13 % in control cells at 0, 1, 3, and 6h, resp.). No changes in lymphocyte fraction were detectable in all groups. mRNA expression of TNF did not differ in all samples. However, the expression of IL8 was lower in LPS treated cells than in control cells after 3h and decreased from 3 to 6h in both treatments. Lf expression was reduced in control cells from 3 to 6h but not changed in LPS treatments.

In conclusion, in milk kept at 37°C the cell count is reduced with time and PMN fraction already decreases within one hour. The immune response of the cells decreases even faster with LPS compared to controls. The results show that immune cells once present in milk lose viability and, in addition, the capability to respond to pathogenic components.

12.22 EFFECTS OF PRECULTURE FREEZING AND INCUBATION ON RECOVERY OF STAPHYLOCOCCI FROM SUBCLINICAL BOVINE MASTITIS

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Bacteriological culture of quarter milk samples is the most common way for isolation of mastitis pathogens. However, culture of quarter milk samples of cows with a sub-clinical mastitis often yields no pathogens. There are several methods to reduce the number of false negative culture results of mastitis milk samples.

The objective of this study was to determine the effect of preculture freezing and incubation on the results of bacteriologic culture of milk samples collected from sub-clinical mastitis. A total of 163 milk samples from cows with subclinical mastitis were taken from problem herds in Urmia in Iran. Milk samples were cultured using a conventional culture technique (0.01 ml of fresh milk streaked on a blood agar plate). The remaining was split into two equal sub-samples. One was frozen at -20°C over night and another one incubated at 37°C for 24 hours. Fifty-nine out of 163 samples were positive for coagulase positive staphylococci; nine out of 163 were positive for coagulase negative staphylococci; 3 out of 163 were positive for coliforms; 76 out of 163 were negative, and 16 out of 163 were contaminated. The results showed that the isolation percentage of coagulase positive and coagulase negative *Staphylococcus* was 36.2% and 5.52% respectively, for conventional culture technique. The results for freezing and incubation were as the same as conventional culture technique, except for the of isolation rate of coagulase positive *Staphylococcus* which was 38.04% for incubation method. Freezing method did not have any effect on the positive culture rate of coagulase positive and coagulase negative staphylococci. Our results are consistent with the results of Schukken & et al. (1989). They did not find any effect of freezing. These results may be due to short period of pre-culture freezing of samples. However, Villanueva & et al. (1991) found significant increase for freezing method at the rate of 1.48 times greater than conventional method in isolating *Staphylococcus aureus*. On the other hand, in our study freezing resulted in increased colony count of the majority of coagulase positive staphylococci cultures.

Our results indicated that incubation method was the most effective one among these three methods in respective of increasing isolation rate of *Staphylococcus* species in mastitis milk samples.

12.23 SEASONAL VARIATION OF THE LACTOFERRIN CONCENTRATION, SOMATIC CELL COUNT AND SUBCLINICAL MASTITIS OCCURRENCE IN COWS

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Problem: The udder inflammation is one of the most widespread diseases in dairy cows in Latvia. In total, 6% of cows are affected by clinical mastitis, but up to 30% and more of all dairy cows - by subclinical mastitis. The most important bacteria causing mastitis, isolated from milk samples in the National Diagnostic Center, Latvia, were *Staphylococcus aureus* in 54% of samples, *Streptococcus uberis* - 14%, *Escherichia coli* - 10%. In recent years, considerable research has been focused on enhancing the natural defence mechanisms of the mammary gland during periods of increased susceptibility to diseases. Lactoferrin (Lf) is one of the most important natural antibacterial factors in mammary gland. **AIM.** The aim of this study was to evaluate and compare lactoferrin and somatic cell count (SCC) in milk during the housing and grazing periods.

Materials and Methods: Basing on an analogue by lactation stage principle a group of 16 cows was completed from a dairy herd consisting of 75 Latvian Brown and Holstein cows kept in a cold loose housing system. Indices of the udder health and changes of the lactoferrin concentration, and SCC in milk were investigated (164 milk samples in total) during the housing period and grazing period. The SCC was measured by the Somacount analyzer, and the lactoferrin level detected by the immunoferritin assay. Milk from the cows with a positive California Mastitis Test reaction were examined bacteriologically with a direct culture on the blood agar.

Results: The investigation showed that in 82.5% of cows the udder health indices were good (SCC less than 250 000 per ml). In milk samples with higher SCC, *Staphylococcus aureus* 32.10%, coagulase-negative staphylococci 48.60%, *Streptococcus uberis* 18.90 % bacteriologically were found. In cases of bacterial infection compared to non-infectious cases the somatic cell count and Lf concentration were significantly increased by 55.55% (P less than 0.001) and by 40.33% (P less than 0.05), respectively. During the grazing period the mean SCC in milk was significantly higher (P less than 0.05), but mean Lf concentration was significantly lower (P less than 0.05) than that in the housing one. **CONCLUSIONS.** 1) Lactoferrin is a strong antibacterial factor, however, not increased during grazing period simultaneously with somatic cell count. 2) The main reason of the increase of somatic cell count in the grazing period, is a non-infectious udder irritation.

12.24 THE STUDY OF IMPORTANT FACTORS ON REPRODUCTION OF AZERBAIJAN BUFFALO (BUBALUS BUBALIS)

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Buffalos are one of cattle, which kept in different part of world, but the most population of this animal is in Asia. Buffalo`s population is 400000 in Iran and 112393 in Azerbaijan particularly. Azerbaijan buffaloes produce 4355 tons milk and 58771 tons meat in one year. This survey was performed on 833 Azerbaijan buffalo in during ten years. Considered important factor were age of first parturition, period of between of two parturition, length of pregnancy period, length of milking period, length of dry period, mean of milk production in one year, mean of daily milk production, mean of fat percentage and economic age. For this survey many populations of Azerbaijan buffalo were selected in different regions of East Azerbaijan randomly and studies were performed on them. Studied buffalos had Indian husbandry. Obtained results of this survey were appeared that the age of first parturition is 1260 days after birth, period of between of two parturition is 480-540days, length of pregnancy period is 305-315 days, length of milking period is 206 days, length of dry period is 90-120days, mean of milk production in one year is 1244 liters, mean of daily milk production is 6/04 liters, mean of fat percentage is 7/56% and economic age is ten years.

Key words: Azerbaijan, Buffalo, Physiology, Reproduction

12.25 STUDY INTO THE OCCURRENCE OF EXTERNALLY VISIBLE SIGNS OF APPROACHING PARTURITION IN SUCKLER COWS AND HEIFERS: CHANGES TO THE UDDER

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The objective of this study was to examine to what extent changes to the mammary gland are suitable for determining the precise time of parturition in suckler cows. The following parameters were examined in a total of 105 animals every eight hours during the last seven days ante partum: udder development, udder edema and fore udder edema. Development of the udder and the number of animals with udder and fore udder edema increased with approaching parturition ($p < 0.001$). However, only 5.7 % of the animals had a completely developed udder with shiny teats filled with milk eight hours before parturition, whereas the teats of 32.4 % of them were not yet filled with milk at this time. 86.7% of the animals had udder edema and 12.4 % of the animals had edema of the fore udder, this characteristic being observed in 50 % of the heifers and in 10.3 % of the cows. This difference is statistically highly significant ($p < 0.001$).

No influence of the breed was detected on any of the three parameters. Changes to the mammary gland in cows are therefore not suitable for fixing the time of parturition more precisely within the last seven days ante partum.

13 CLAW HEALTH

13.1 METABOLIC DISORDERS AND LAMINITIS IN CATTLE – A REVIEW

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Abstract

Pathophysiology of laminitis is as complex as the multifactorial etiology of the laminitis syndrome and still far away from being completely understood. However, the acquisition of new information is accelerating with the ongoing broad research activities focusing on metabolic and environmental aspects of the pathogenesis of laminitis. This paper reviews the knowledge of the pathophysiological events in the different tissue components of the bovine claw during laminitis. It focuses on tissue responses to challenges originating from metabolic disorders, their implications on function of the claw. The functional Anatomy, Physiology and Pathophysiology of the digital cushion, the suspensory system of the pedal bone, the dermal vascular system, the dermo-epidermal junction and the horn producing living epidermis are explored. Possible links between metabolic problems and local tissue damage are described and discussed. Included are new hypotheses explaining potential links. Finally the paper provides some conclusions from the recent scientific findings and a summary of their practical implications.

Key words: laminitis, cattle, bovine, lameness, metabolic disorder, claw

Introduction

The performance of the bovine claw is genetically determined and its capacity of adaptation to metabolic and environmental challenges is limited. The interaction between the tissue elements of the claw and the metabolism, or more precisely the bio-active molecules and by-products originating from metabolic disorders, and the environment results in a cascade of pathophysiological events leading to adaptation changes, alterations or damage in the tissues. In high-performing dairy cows housed under conditions of intensive-management the claw is continuously challenged by environmental and metabolic factors. Any weakening of the structural integrity of the claw has immediate functional implications followed by all of its biological, economical and welfare consequences (Mülling and Greenough, 2006). Lameness in dairy cattle is a major cause of suffering and economic loss with approximately one quarter of cows experiencing lameness annually. Lameness is an unacceptable condition which causes severe pain, decreased milk yield, reduced reproductive performance, high culling rates and increased cost of veterinary intervention. Laminitis

is regarded as a major predisposing factor in lameness caused by claw diseases, particularly white line lesions, solear haemorrhages and sole ulcers. Laminitis is a multi-factorial problem resulting from a multitude of factors inherent to dairy farming (Bosman et al., 1991; Ebeid, 1993; Greenough & Vermont, 1991; Lischer & Ossent, 1994). Predisposing factors and causes include management, housing, genetics, breeding, nutrition and physiological state (pregnancy, parturition and transition to lactation).

The marked increase in milk production on dairy cows has negative effects on fertility, the mammary gland and the claws of the animal (Ingvarsen, 2006; Fleischer et al., 2001). Most of the diseases in these system occur around parturition or in early lactation. High annual culling rates are the consequence causing substantial economic losses of 300 € and more for a single case of sole ulcer (Kossaibaiti and Esslemont, 1997). There is increasing evidence that the high milk yield and associated metabolic demand impact on the function of the udder, reproductive system and the claws. A growing number of researchers postulate that the increase of NEFA during lipid mobilisation is linked to a progressive insulin resistance and the same time an increase in production of cytokines.

Research of the past 10 years has become more and more interdisciplinary employing modern methods of epidemiology, cellular biology and molecular biology (Bergsten & Mülling, 2004). Research has focused in particular on a condition or syndrome called subclinical laminitis (SCL). Laminitis is term that is a scientifically inaccurate description of this disorder as is the recently suggested term 'claw horn disruption' (CHD) (Hoblet & Weiss, 2001). SCL weakens the integrity of claw tissues predisposing them to secondary lesions such as white line disease and sole ulcers. It is now accepted that SCL has a multi-factorial etiology and a complex physiopathology resulting from a multitude of 'risk factors' inherent to dairy farming (Bosman et al., 1991; Ebeid, 1993; Greenough, 1985; Greenough & Vermont, 1991; Lischer & Ossent, 1994; Mülling & Lischer, 2002). Nocek (1997) postulates that nutritional management and improved cow comfort are major risk factors to be considered in the attempt to reduce lameness in dairy cattle. Inappropriate policies for genetic selection, accelerated breeding protocols, poor peri-parturient management and even farmer knowledge and skills have been implicated as risk factors.

Basing on the results of the ongoing research new hypotheses have been developed to demonstrate possible links between metabolic problems and local tissue damage causing the variety of clinical lesions in the claw.

New models for studying pathophysiological mechanisms in claw tissue

To establish and understand the links between metabolic disorders and local alterations in the claw tissues studies into these local mechanisms and their initiation by bioactive molecules deriving from central metabolic problems will be of central importance. Many attempts have been made to establish a valid animal model for studying bovine

laminitis in living cattle. The most recent attempt demonstrated the early acute events and tissue changes occurring in acute laminitis (Thoefner et al., 2004; Thoefner et al., 2005). However, animal models are expensive use and are invariably associated with concerns about animal welfare. In recent years in vitro models have gained more importance in studying the physiopathology of claw diseases. Tissue explant studies have already provided important insights into regulation of differentiation in healthy and diseased claw tissue (Hendry et al., 1997, 1999, 2001, 2003). Cell lines and simple three-dimensional cell cultures are already available (Nebel et al., 2002, 2003, 2004). The development and experimental application of novel in vitro models was a major task of the EU framework 5 project Lamecow (<http://www.abdn.ac.uk/lamecow>). Parts of the outcome of this project are different cell culture systems including three dimensional organotypic culture systems and tissue explants systems. In addition a novel ex vivo model, the isolated haemoperfused distal cow limb model (Wüstenberg, 2004), has been developed and used for experiments. These models enable in vitro studies under standardised conditions in cell clines, complex organotypic cultures and finally testing in the isolated limb perfusion model under conditions as close as possible in a model to the in vivo situation.

Dietary and metabolic problems and laminitis

The composition of the diet, the preparation of the diet and the way it is fed, but also the feeding behaviour are risk factors for laminitis (Bergsten, 1994) because these factors interfere with ruminal fermentation and the animals metabolism. Ruminal acidosis, often the result of improper feeding management, has frequently been associated with laminitis and is thought to be a major cause for laminitis in cattle. There are, however, studies where no correlation between a low ruminal pH (<5.8) and claw lesions was detected (Chant et al., 1998). The same study showed an significant increase in sole lesions 8 to 12 weeks after calving when the diet was changed suddenly from high to low fibre compared to feeding the same diet all the time. Logue et al. (2000) compared wet and dry forage feeding to heifers before calving under identical conditions. Wet diets (grass and silage with 19% DM) were related to significant higher incidence of lameness and claw lesions compared to dry diets (straw and concentrates with 86% DM). These and other studies have in common that they identify the transition period as a problematic time with a high risk of developing laminitis.

Many physiological changes occur in the transition period, i.e. in late gestation and early lactation which affect nutrient uptake and flow. Despite the quantity of research conducted on nutrition and physiology of transition cows, this period remains a problem on many dairy farms; metabolic disorders and laminitis with all it's associated claw lesions continue to occur in this period and cause the already mentioned economical losses (Burhans et al., 2003). Many cows in early lactation suffer claw abnormalities (Green et al., 2002) which may be partly due to the result of nutritional deficiencies or hormonal changes occurring during the periparturient period. An interesting area of developing research relates to the hormonal control of horn protein

production and how changes at parturition may create a predisposition for future lameness. One of the primary physiological adaptations of transition cows is the need to synthesize and direct glucose to the mammary gland. The cow accomplishes this by concurrently increasing hepatic gluconeogenesis (Reynolds et al., 2003) and decreasing oxidation of glucose by peripheral tissues. Already Vermunt and Greenough (1994) suggested that overfeeding during the dry period, which gives rise to hyperinsulinemia and hyperglycemia in early lactation, appeared to predispose cows to laminitis. Green et al. (2002) reported that incidents of first lameness were highest three months after calving, suggesting that factors affecting horn growth during the dry period and in early lactation result in production of inferior horn and subsequent lameness in early lactation. Epidermal growth factor (EGF) was reported to have potent mitogenic and anti-differentiating effects in epithelial tissues other than the claw (alimentary and uterine tracts). Hendry et al. (1999) reported that EGF may impact keratin formation and result in formation of inferior horn production. A hormone of particular interest during the periparturient period is the major lactogenic hormone prolactin. Stimulation of protein synthesis by EGF in hoof explants was partly antagonized by prolactin. Although prolactin itself did not influence hoof protein synthesis, its ability to decrease EGF-stimulated protein synthesis in hoof tissue cultures may be another factor in reducing keratin synthesis during lactation (Hendry et al., 1999). Goff and Horst (1997) described that periparturient dairy cows are often subjected to stress with a subsequent increase in cortisol levels. Hendry et al. (1999) found that hydrocortisone inhibited keratin protein synthesis in bovine hoof tissue explants. A causative relationship between systemic glucocorticoid concentration and laminitis in dairy cows still awaits the scientific proof. However, it is notable that highly productive herds, which have a greater incidence of laminitis (Nocek, 1997) also have higher glucocorticoid levels (Johnson & Vanjonack, 1976). Stress and subsequent elevation of cortisol during the periparturient period and during lactation (Goff & Horst, 1997) may predispose dairy cows to claw disorders resulting from production of inferior claw horn.

Initial changes in the dermal vascular system of the claw

The dermal vascular system of the claw is the structural and functional link between factors originating from metabolic disorders and any local events occurring in the claw causing structural damage and subsequent functional loss. All factors have to travel via the vascular system to cause an effect in the claw. The same time the vascular lining, i.e. the endothelial cells, will interact with the arriving challenging factors and release factors activating local regulatory or inflammatory cascades.

The dermal vascular system of the claw is unique in its three-dimensional arrangement and complexity. It responds to a variety of vaso-active factors and by-products present at increased levels in association with metabolic disorders. It is susceptible to structural damage and disturbances to the perfusion of blood (Hirschberg et al., 1998, 2001). Structural peculiarities in the vascular system and arterio-venous anastomoses (AVAs) in particular have been described as having a central role in development of laminitis.

AVA's are shunts between the arterioles bringing the blood into the capillary bed and the venule draining the blood from the capillary bed. They are supplied with nerves which innervate smooth muscle in vessel walls which can close blocking perfusion of the capillary bed of the dermis. More recent micro-corrosion cast studies by Hirschberg and others (1999, 2001) have demonstrated that there are almost no AVAs in the vascular system of the digits of healthy claws.

The overall microvascular system in the dermal papillae and lamellae is quite extensive, but not all branches of this microvascular bed are perfused at all times. A primary pathway supplies the dermal and the adjacent epidermal cells with their basic needs. This is different from the functional pathway which responds to the functional demands required from the dermis and epidermis, e.g. region-specific rate of proliferation and horn production. The actual perfusion pattern is adapted permanently to the tissue needs by precapillary sphincters. The primary pathways ('thoroughfare channels') are the main routes within the capillary bed where the central arteriole and venule are connected inside a papilla. The functional pathway required for proper supply of the adjacent epidermal layers is provided by the extensive capillary network of the papilla. Arteriovenous anastomoses are located outside the microvascular bed and regulate extreme demands for perfusion. Therefore, they occur at the base of the lamellae and papillae and in the deeper layers of the connective tissue of the claw, not within the lamellae and papillae. These arteriovenous anastomoses are also subject to function related remodelling and are formed on demand that is, mostly in response to changes in the environmental temperature (Hirschberg, 2001; Hirschberg & Plendl, 2005).

The dermal microvascularisation and the perfusion patterns are highly adaptable to metabolic and functional requirements. Mechanical forces during weight-bearing deform the papillae and open or close pathways within the vascular system of the papillae. Thus, the perfusion pattern of the microvascular bed is regulated by demand and by mechanical forces. Structural adaptation of the angioarchitecture is facilitated by active remodelling processes of the capillaries by sprouting and intussusception (Hirschberg & Plendl, 2005). Pressure in the vessels and tissues increases during laminitis which could be explained by compromised function of AVA's but a much more likely explanation would be coagulopathy. The pressure increases in the capillaries together with transvascular movement of fluid in tissues may be caused by an increased post-capillary resistance. This resistance is believed to be the result of a reduction in the diameter of the venules in the periphery draining blood from the capillary bed (Christmann et al. 2002). The linkage between metabolic problems and local changes in the claw tissue and the causes of vascular failure still awaits clear explanations. A number of candidate factors and mediators are listed and discussed in the literature. Ranking high on the list are endotoxins, histamine and lactate. The initial local reaction to laminitis that takes place in claw tissues are alterations in the vascular endothelial lining (roughening) and in the microcirculation (changes in the rate of perfusion). These events are followed by the activation of a variety of interacting and cross linked inflammatory and regulatory cascades including MMP activation. It also may be that there is direct initial damage to the epidermis by mechanical overload. The damage

will cause the release of interleukin 1 (IL1) which is a potent pro-inflammatory cytokine produced and stored by the epidermal cells. IL1 will then diffuse into the dermis, binding to fibroblasts and triggering the release of keratinocyte growth factor (KGF). KGF is a potent mitogen activating basal proliferation in the epidermis. Parallel to this, IL 1 also activates MMPs. In addition there is positive and negative feed-back between these auto and paracrine regulators (Mülling et al., 2004). These observations strongly support the idea of complex rather than a simple physiopathology. The results of Christmann and others (2002) provide initial evidence that alterations in microcirculation related to early laminitic-like events are different in the bovine claw compared to the equine hoof. They demonstrated in grain overloaded steers an increase in capillary pressure and post-capillary resistance. This facilitates transvascular movement and an increase in tissue pressure. Digital venous constriction is thought to be the initial step in these events. However, horses showed no significant changes in pre-capillary resistance and digital blood flow was found to be normal. The differences in the haemodynamic changes observed between the species may contribute to the differences in clinical presentation of laminitis (Christmann et al., 2002). Nilsson described, in 1963, the formation of 'Neocapillaries' in the dermis of claws of animals suffering from subacute laminitis. Recently Hirschberg and Plendl (2005) investigated the formation of new blood vessels in diseased claws. Based on their studies of the morphology of microvascularisation and angioadaptation of the claw they postulate a central role of pododermal angiogenesis in the pathogenesis of laminitis. It well may be that studies on the angiogenesis, i.e. the de novo formation of blood vessels will significantly contribute to the understanding of laminitis and open new doors for the prevention of laminitis.

Structural and functional alterations in the suspending system of the pedal bone

The suspensory apparatus of the pedal bone consists of collagen fibres that run upwards from their insertion in the bone to the basement membrane of the dermal lamellae where they are anchored and thus connected to the lamellar epidermal of the claw capsule (Westerfeld et al., 2000, 2004). Collagen fibres of the connective tissue are the crucial structural and functional components of the suspensory apparatus of the digit. The system of fibres suspending the pedal bone is responsible for transferring the load (weight of the animal) from the pedal bone to the claw capsule (Westerfeld et al., 2000, 2004). The quality of these fibres is of critical importance if the pedal bone is to be held in a stable position inside the claw capsule (Lischer et al., 2002; Maierl et al., 2002; Tarlton and Webster, 2002; Westerfeld & Mülling, 2000). For whatever reason a loosening or increase in length of this connective tissue occurs it will lead to displacement (sinking, rotation, tilting) of the pedal bone within the horn capsule and subsequent increase in pressure onto the soft tissue. An elongation, loosening or an increasing elasticity in the collagen system suspending the pedal bone inside the claw capsule are central to hypotheses explaining the pathogenesis of subclinical laminitis (Mülling & Lischer, 2002; Mülling et al., 2004). During the periparturition period and throughout the onset of lactation the properties of the connective tissue of the suspensory apparatus (more precisely the extracellular matrix in the connective tissue,

the collagen fibres) undergo changes leading to decreased stability of the dermis (Holah et al., 2002; Mülling et al., 2004). As a result, there is increased mobility of the pedal bone inside the claw capsule (Lischer et al., 2002; Mülling & Lischer, 2002).

Recent experiments designed to explore the importance of housing, feeding and parturition/lactation indicate that the structural integrity of connective tissue was most severely compromised by housing in cubicles. Parturition and lactation amplified this effect whereas feeding had no significant influence (Webster, 2001, 2003; Webster et al., 2005). Within this context it must be re-emphasized that the dermis is exposed to high local mechanical pressure (Hinterhofer et al., 2006; van der Tol, 2002), particularly when cows stand for excessively long period throughout the day. Cubicle housing in comparison to straw yards leads to elevated level of pro MMP2 and active MMP 2 in the connective tissue of the claw (Tarlton et al., 2000; Webster et al., 2005).

A group of proteolytic enzymes resident in connective tissue, the Matrix Metalloproteinases (MMPs) play a central role in the degradation of collagen. Basing on recent studies on the effects of MMP-2 and MMP-9 on dermo-epidermal explants (Hendry et al., 2003) and a proteolytic enzyme, "hoofase", (Tarlton & Webster, 2000) two major hypothesis have been developed. One hypothesis favours the central role of MMPs and their activation by proteases (Tarlton & Webster, 2000) or other known activators of MMPs such as cytokines and inflammatory factors (Mülling et al., 2004). The other hypothesis is based on the direct effects of hormones on the connective tissue leading to instability or a loosening of the collagen fibre system. Relaxin present in the peripartal period (Jönsson & Person, 1969) is discussed as the major candidate. Activation of MMPs can be caused by factors derived from biological mediators circulating in the blood such as endotoxin, lactate, cytokines like TNF alpha or Interleukin-1. This activation is mediated by cytokines released from local tissues. Under conditions of physiological homeostasis MMPs are controlled by their tissue inhibitors (TIMPs) which normally prevent excessive MMP activity. In disease, elevated levels of active MMP result from decreased TIMP activity and increased conversion of inactive pro MMP into active MMP. When MMP expression is elevated and TIMP activity decreased bioactive molecules activate MMP's which start degrading collagen fibres. Pathological activity of MMPs leads to increased collagen degradation and loosening and elongation of collagen fibres. The subsequent increased mobility of the pedal bone within the capsule causes displacement (sinking and/or rotation or tilting or a combination of these movements) of the pedal bone to a degree depending on the localization and severity of the collagen degradation. If housing and exposure to concrete are the major hazards to claw tissue integrity then mechanical irritation and or overload are the cause of MMP activation and collagen degradation.

Recent findings from in vitro studies (Mülling et al., 2004) provide initial insights into the collagen alterations causing increased instability of the suspensory apparatus of the pedal bone. The structural alterations demonstrated are comparable to the collagen removal process described in human and equine tendons (Birch et al., 1998; Riley et al., 2002). Exposure of dermal collagen to activated MMP-2 and MMP-9 leads to a removal of collagen from the dermal network and subsequent disintegration of the

system as revealed by electron microscopy. Degradation of a certain number of collagen microfibrils within the collagen fibres can prepare the pathway for mechanical induced microruptures which has been described for stressed tendons. Damage of a limited number of collagen fibres leads to a microrupture and a slight increase in length of a small fibre bundle. The overall elongation and instability will depend on the number of fibres affected. If the damaged matrix is loaded, elongation is the consequence leading to displacement of the pedal bone in the claw capsule without actual separation of the connective tissue.

Structural and functional alterations in the supporting digital cushions of the claw

The digital cushion extends forward beneath the pedal bone and is made up of three cylindrical parallel oriented bodies each with a capsule of connective tissue filled with soft fat (Räber et al., 2004).

During normal gait the heel bulbs make the first contact with the ground and the weight will be distributed equally between the outer and inner claw. While the resilient bulbs reduce the initial shock on the posterior part of the claws the weight of the animal is smoothly transferred to the wall and adjacent sole by slight splaying of the claws. The dermis of the sole and heel and the underlying fat cushions in the subcutis function as 'shock absorbers' bearing a considerable proportion of the impact of the first phase of each step the animal makes when walking.

There is a marked change in the composition of the digital cushions as the animal gets older. In heifers the fat cushions are not completely developed and functional. They develop to full shock absorbing capacity during the first 2 lactations. The fat content is significantly higher in cows (38%) than in heifers (27%) (Räber, 2000; Räber et al., 2004). The cushions in the heifers are composed predominantly of loose connective tissue with abundant amorphous ground substance. In cows however, there was a marked increase of adipose tissue with progressing age (Räber et al., 2004). A comparison of the digital cushions between sound claws and claws with sole ulcers revealed that the phalanx of ulcerated claws had sunken and the solear dermis and subcutis were thinner than in the controls. The cushions contained significantly less adipose tissue than the controls but had been replaced by collagenous connective tissue (Lischer et al., 2002). The fatty acid composition and the size of the fat cushions change under the influence of metabolic disorders, in particular in cows with lipid mobilisation syndrome (LMS, ketosis). The fat in the digital cushion has a high content of monounsaturated fatty acids (MUFA). MUFA are mainly produced endogenously and the greater the quantity of these fatty acids in the fat tissue, the softer it is; the structural fat in the digital cushion possesses its own 'fat softener' (Räber et al., 2002). Heifers have significantly less fat in the cushions and slightly more saturated fatty acids (SFA) than the cows. This indicates that the change from SFA to MUFA and the proliferation of fat occurs at first parturition and during the following lactation. It is possible that these changes in the heifer's digital cushions make them less resistant to pressure load. Epidemiological studies have shown that there is a higher tendency for

sole lesions to occur at the beginning of the first lactation (Boosman et al., 1991; Enevoldsen et al., 1991; Greenough & Vermunt 1991; Smilie et al., 1999).

Research into structural and biochemical changes in the fat cushions in relation to the metabolic status and profile of the animal seems to be a very promising area to establish links between metabolic disorders and laminitic change in the claw and further improve our understanding of this syndrome.

Changes in the dermo-epidermal junction zone

The dermo-epidermal interface is a highly developed and specialized region at the border between dermis (connective tissue) and epidermis (epithelium) (Mülling & Budras, 2002). The living epidermal cells located on the interface proliferate and show high metabolic activity. All nutrients, substances and factors required for the epidermal activities have to pass from the dermis into the epidermis and vice versa. During proliferation and synthesis the mitotic cells have to withstand a high mechanical load while transferring all the mechanical forces between the underlying bone and the outer horn capsule and the environment. With its complex functions the dermo-epidermal interface is a structure of crucial importance for the integrity and normal function of the claw. It establishes the attachment of the living epidermis to the underlying dermis. Signals between dermal and epidermal cells also run through this interface.

Epidermal-dermal interactions play an important role in regulating the proliferation and differentiation of keratinocytes which play an important role in repairing surgical and traumatic injuries of the claw capsule. Early in the pathogenesis of laminitis alterations in the dermo-epidermal region have been reported such as initial molecular and structural changes followed by functional disturbances. In addition, on the dermal side, activation of MMPs (Tarlton et al., 2000) leading to degradation of collagen as well as activation of growth and necrosis factors, molecular and structural alterations in the basement membrane (Hendry et al., 2003) and alterations of capillary walls.

Changes on epidermal side have to be considered as secondary changes due to disturbed nutrient and oxygen supply. A double paracrine regulation of keratinocyte growth and differentiation has recently been postulated and described *in vitro* (Mülling et al., 2004). Interleukin-1 (Il-1) and keratinocyte growth factor (KGF/FGF-7) and their receptors are major mediators in this epidermal-dermal signalling. Il-1 is synthesised and stored in the Keratinocytes of claw epidermis. It is released upon physical or chemical injury of the cells but also by the effects of cytokines arriving in the dermis via the vascular system. Il-1 migrates into the dermis where it binds to receptors on dermal fibrocytes. There it causes release of Keratinocyte Growth factor (KGF). KGF is an important stimulator of keratinocyte proliferation as demonstrated in co-culture systems of claw fibroblasts and keratinocytes. There is preliminary evidence for a reciprocal regulatory mechanism present in the bovine claw involving Il-1 produced in the epidermis and KGF originating from fibroblasts. It is reasonable to assume that increased cytokine levels associated with metabolic disorders trigger Il-1 release and

thus activate the local paracrine regulation. Increased rate of proliferation and horn formation would be the consequence. In addition MMPs would become activated by the released Interleukin 1. The collagen of the innermost layer of the basement membrane (*lamina fibroreticularis*) is a substrate for activated MMP's which degrade this collagen disrupting the integrity and regulatory/communicative functions of the basement membrane (Hendry et al., 2003).

Disturbed or disrupted horn production on the living layers of the claw epidermis

A significant weakening of the horn capsule is a central result of subclinical laminitis. The consequence is an increased susceptibility of the claw to damage and lesions secondary to laminitis. There is growing evidence from morphological and *in vitro* studies that disruption of the differentiation of keratinocytes in the differentiating hoof epidermis is the major reactive event during pathogenesis of laminitis. This disruption occurs following dermal alterations resulting in disruption of appropriate supplies to the epidermis (Hendry et al., 1999, 2001). This was the theory that has been explored also by Ekfalck (1991) and Wattle (2001). Most of the findings observed in the claw capsule in subacute or chronic laminitis is related to or a result of the reactive changes in the epidermis.

Formation of claw horn is the result of a dynamic process of proliferation, cellular differentiation (i.e. keratinisation) and programmed cell death called cornification all occurring in the living layers of the epidermis (Mülling & Budras, 1998; Tomlinson et al, 2004). This process is controlled by a variety of bioactive molecules including growth factors and neuropeptides provided by the dermal cells and/or the vascular system. Already in the basal layer they start to produce keratin proteins with increasing intensity in the superimposed layers. At the end of the differentiation during cornification, the keratins are cross-linked by formation of disulphide-bonds which consist of a stable protein-complex which provides mechanical and chemical stability to the horn. The second product of keratinising epidermal cells is the intercellular cementing substance consisting of glycoprotein and complex lipids such as phospholipids glycolipids and acylglycosylceramides. Its major function is to establish cell to cell adhesion providing mechanical stability to the horn. The lipids of the cementing substance establish a permeability barrier in the intercellular space. This barrier prevents the passage of aqueous solutions through the horn and thus protects horn cells from excessive loss of water as well as from extreme hydration (Mülling & Budras, 1998).

The highly active horn producing epidermal cells depend on a sufficient and balanced supply of nutrients and oxygen, minerals vitamins and trace elements. Bioactive molecules derived from metabolic activity or systemic disease will impact on vascular walls and perfusion. These factors have the potential to change the diameter of the dermal vessels or to damage the endothelial wall. Of particular relevance is metabolic stress related to parturition, lactation or dietary problems resulting in metabolic disorders like ketosis or acidosis. Some factors such as histamine, lactate, endotoxin

can directly damage the endothelial lining of the vessels and increase transvascular movement. Vasoactive factors such as serotonin or bradykinine will cause constriction of vascular walls with the result of reduced perfusion or reduced drainage from the capillary bed. The latter will result in increased transvascular movement and increased pressure inside the claw capsule (Christmann et al., 2002). Both reduced perfusion and alterations in the vessel themselves will impair horn production and finally provoke horn of inferior quality.

Conclusions and practical implications

As metabolic disorders, infertility and mastitis, laminitis with secondary claw lesions is frequently a herd problem. As with the other production diseases, laminitis is multifactorial and management decisions are critical to reduce most laminitis risk factors. Thus, the possibility to prevent laminitis increases if farmers or managers understand and are aware of the problem and its consequences. Optimized nutrition and good environmental conditions (cow comfort) are important for high yielding cows, the most critical period in this aspect is the time around calving.

There are anatomical differences in the digital cushion between heifers and cows. It is therefore important to give the heifers enough time to adapt to the new housing conditions of dairy cows.

Although we do not completely understand the link between metabolic disease and claw lesions, adequate nutrition and optimal feeding management of dairy cows are of outstanding importance.

Sole ulcers develop as a consequence of a partial failure of the suspensory apparatus. Whenever possible the ulcerated claw should be unloaded with a claw block on the sound claw. Looking at the biomechanical components of laminitis it is clear that professional functional hoof trimming helps to reduce the impact of laminitic alterations on the structure and function of the claw.

The linkage between metabolic problems and local changes in the claw tissue and the causes of vascular failure still awaits clear explanations. A number of candidate factors and mediators are listed and discussed in the literature. Ranking high on the list are endotoxins, histamine and lactate. The initial local reaction to metabolic disorders that takes place in claw tissues are alterations in the vascular endothelial lining and in the microcirculation (changes in the rate of perfusion). These events are followed by the activation of a variety of interacting and cross linked inflammatory and regulatory cascades. MMP activation is only one part of the jigsaw. It also may be that there is direct initial damage to the epidermis by mechanical overload. The damage will cause the release of interleukin 1 (IL1) which is a potent pro-inflammatory cytokine produced and stored by the epidermal cells. These observations strongly support the idea of complex rather than a simple physiopathology.

The concept of a multi-factorial etiology of claw diseases is as valid as it ever was. Preventive measures still must be geared to managing the stress level that precipitates the complex physiopathology that is intrinsic to the foot. Routine claw trimming helps to balance the load borne by the claw. Eliminating overcrowding improves freedom of movement and the perfusion of blood through the foot. Cow Comfort must be interpreted as part of the interaction between the foot of a dairy cow and its internal and external challenges. This is the background for the claw diseases occurring under modern intensive housing conditions. Multi-factorial means that practically every aspect of dairy cow management has to be scrutinized for its potential impact on the claw. The disorder can not be managed simply from a nutritional perspective. In addition to the measures applied to the animals and their environment it is imperative to develop the herd managers/farmers awareness of the problem, to improve education, dissemination of knowledge and to promote sustainable management strategies for reduction of foot problems.

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13.2 AGE-RELATED CHANGES IN THE SUSPENSORY APPARATUS OF THE CLAWS IN DAIRY COWS: 3D-TOPOGRAPHY OF THE DISTAL PHALANX IN RELATION TO THE CLAW CAPSULE MEASURED FROM CT-DATASETS

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On the one hand the coffin bone is suspended by the dermal lamellae of the wall segment, on the other hand it is supported by the elastic cushions of the bulb in the palmar/plantar region. Recent publications report about age-related changes in this suspension in dairy cattle resulting in an unphysiologically high pressure within the dermis below the flexor tubercle.

It was therefore the objective of this study to measure the distance distal phalanx - claw horn under various loading conditions in order to give evidence of the altered mechanical behaviour of the bulb tissue. Only heifers and cows ($n = 40$) with a minimum age of 18 months were chosen for this investigation. Animals that had been slaughtered for reasons primarily unrelated to claw health were divided into four groups depending on the number of pregnancies (nulliparous, primiparous, multiparous with either 2-4 or >4 pregnancies). The investigation included one fore- and hindfoot of each animal chosen randomly. After functional claw trimming feet were scanned in a computertomograph (Veterinary Surgical Clinics, Munich) in an unloaded and loaded state. CT-datasets were reconstructed three dimensionally and the distance between the claw horn and the distal phalanx was determined. In the dorsal, abaxial and axial surfaces almost no differences could be observed in the measured distances between the unloaded and the loaded digits. The distance values in these surfaces amounted to 4-6 mm respectively. In the solear surface, however, a distinct sinking of the coffin bone could be observed during loading (approx. 7-8 mm unloaded, approx. 3 mm loaded). In older animals the distance in the area of the flexor tubercle amounted to only 3-5 mm in unloaded digits. The compression during loading only yielded an average change of distance of maximally 1,5 mm.

Results suggest that bulb cushions lose volume with an increasing number of lactations. This results in a reduced damping capacity of these cushions which leads to peak loading of the dermis below the flexor tubercle. Thus this area is predisposed to sole ulcers which is even worsened with tissue composition of the bulb being altered with an increasing number of lactations.

13.3 TESTING THREE TYPES OF FOOT BATHS TO PREVENT DIGITAL DERMATITIS AND HEEL HORN EROSION IN DAIRY COWS

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As part of good dairy cattle management, disinfectants are used in foot baths to control infectious claw diseases. A wide range of new substances have been introduced in recent years. The aim of the present study was to test the effectiveness of three different substances and two application methods in an experimental dairy herd of Swedish Holstein and Swedish Red cows with severe problems of digital dermatitis (DD) and heel horn erosion (HE). DD and HE were scored from 0 (no lesion) to 2 (severe) before and after exposure to the disinfectants. In trial 1, 95 cows were walked twice daily for 56-113 days through a passage with a layer of foam containing peracetic acid and hydroxygen peroxide (Kovex, Ecolab, Denmark). Fresh foam was added while the cows were passing. Sixty-three cows were used as untreated controls. In trial 2, 105 cows were walked twice daily for 53-113 days through a foot bath with two longitudinal compartments equipped with Hoofmat (Sweetmans Ltd, New Zealand). The left compartment (left feet) was filled with 7% copper sulphate solution and the right compartment (right feet) with water as a control. In trial 3, 244 cows were walked twice daily through the same longitudinally split foot bath for 14-116 days, but the right compartment being filled with a 5% solution of acetic acid, glutar aldehyde, copper sulphate and N-dialkyl-N,N-dimethyle ammonium chloride (Footbath solution 500, DeLaval, Sweden) and the left with water. The foot bath was cleaned and new solution was added after passage of the whole group in trial 2 and half the group in trial 3. After reducing lesion scores to three binary outcomes (presence or not of HE, DD and the two lesions combined), statistical analysis was performed at the foot level using generalized linear mixed modelling, controlling for cow identity (random effect), breed, parity, days in milk, milk production, presence of other diseases and rear/front foot. Almost all cows were affected by HE and 15% by DD at the start of the study. No effect of peracetic acid and hydrogen peroxide foam was found. The foot bath of 7% copper sulphate decreased 4-fold the odds of having either HE, or HE and DD combined, and decreased 10-fold the odds of having DD. The combination solution in trial 3 reduced by half the odds of having HE but had no effect on DD. We conclude that a walk-through foot bath with copper sulphate reduces the risk of HE and DD, and that the combination of acetic acid and copper sulphate reduces the risk of HE. Further research is needed to evaluate the effect of other foot-bath substances. Financial support was provided from the Swedish Farmers' Foundation for Research and the EC framework 5 project 'LAMECOW' (No. QLK5-CT-2002-00969).

13.4 AGE-RELATED CHANGES IN THE SUSPENSORY APPARATUS OF THE CLAWS IN DAIRY COWS: 3D DISTRIBUTION OF MAXIMUM TENSILE STRESS

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According to recent publications an age-related slackening of the dermal lamellar suspension of the distal phalanx can be observed in dairy cattle with a higher number of lactations.

The objective of this study was to describe the loading situation of the suspensory apparatus of the distal phalanx within the claw capsule by tensile testing. Only heifers and cows ($n = 40$) with a minimum age of 18 months were chosen for this investigation. Animals that had been slaughtered for reasons primarily unrelated to claw health were divided into four groups depending on the number of pregnancies (nulliparous, primiparous, multiparous with either 2-4 or >4 pregnancies). Tests included one fore- and hindfoot of each animal chosen randomly. The tensile strength and peak loading of the suspensory apparatus of the third phalanx were tested at a total of 64 sampling sites per animal with a material testing machine. A representative number of sampling sites was examined histologically in order to determine the location of mechanical failure. Macroscopic lesions of the claws were rarely observed in maiden heifers but increased with the number of pregnancies. The tensile strength of the suspensory apparatus proved to be higher in heifers (2,6-3,9 MPa) and primiparous cows (2,4-3,6 MPa) compared to older multiparous cows which had the lowest values of tensile stability (1,9-2,6 MPa).

Histological findings revealed clear differences as to the site of failure within the suspensory apparatus. In the group of maiden heifers supporting structures were torn apart at the corio-epidermal junction and within the corium at equal parts. In older, multiparous cows, however, the tissue failed within the slackened corium in most cases. In addition to these differences a laceration at the level of the coffin bone occurred relatively often in the group of the heifers.

Findings strongly suggest that the suspensory apparatus of the third phalanx is losing tensile strength with an increasing number of pregnancies. The subsequent effect is a slackening of the phalangeal suspension with an altered position of the coffin bone within the claw. This is the reason - amongst others - for the formation of claw ulcers.

13.5 EFFECT OF SUPPORTING SHOES AFTER HOOF TRIMMING ON HEALING DURATION OF HOOF ULCERS IN DAIRY COWS

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Effect of supporting shoes after hoof trimming on healing duration of hoof ulcers was studied in 31 dairy cows with lameness. Fifteen cows were treated with hoof trimming and treated hoof was supported by rubberized shoe glued to the unaffected claw, and other sixteen cows were treated only with hoof trimming. The treatment results were recorded once a week for 6 consecutive weeks following treatment. The treatment scores were graded into 1 to 5 as follows: score 1, completely healed ulcer without pain on hoof tester; score 2, completely healed ulcer with remaining pain on hoof tester; score 3, ulcer diameter smaller than 1 cm; score 4, ulcer diameter between 1 and 3 cm; score 5, ulcer diameter greater than 3 cm. Average lesion scores after hoof trimming in cows with supporting shoes and cows with only hoof trimming were 4.0 and 3.93, respectively. At 6 wk after treatment, average lesion scores were 2.0 and 2.62 for cows with supporting shoes and cows with only hoof trimming, respectively.

Results revealed that average scores in cows with supporting shoes were lower following 6 weeks of treatment than cows with only hoof trimming.

In conclusion, treatment hoof ulcers in dairy cows by hoof trimming together with supporting shoes would help shorter healing duration of the ulcers than treatment by hoof trimming alone.

14 RESPIRATORY HEALTH

14.1 IMPACT OF CLINICALLY INAPPARENT INFECTIONS WITH CHLAMYDIAE ON ANIMAL HEALTH IN CATTLE

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Introduction

Chlamydiae are a group of obligatory intracellular bacteria that may cause a broad spectrum of diseases in humans and animals [Longbottom & Coulter, 2003]. Zoonotic chlamydial infections are known to generate acute illness in humans, and even life-threatening situations have been reported in patients who became infected with either *Chlamydophila* (*Cp.*) *psittaci* originating from birds or *Cp.* *abortus* from small ruminants [Walder et al. 2003, 2005, Haas et al. 2006, Janssen et al. 2006, Pandeli & Ernest 2006]. In traditional veterinary literature, the presence of chlamydial infections was mostly reported in connection with acute illness leading to severe, but rare, disease manifestations such as pneumonia, enteritis, polyarthritis, encephalomyelitis, abortion and fertility disorders [Shewen 1980, Reggiardo et al. 1989, Storz & Kaltenboeck 1993, Wittenbrink et al. 1993]. Recent investigations using modern sensitive ELISA and PCR techniques, however, show a different picture of chlamydial infections in livestock. High seroprevalence rates, approaching 100%, and the presence of chlamydial genomic DNA with a prevalence as high as 50-70% in many livestock species lead to the assumption that chlamydial infections can be regarded to be virtually ubiquitous in farm animals [Cavirani et al. 2001, Wang et al. 2001, DeGraves et al. 2003, Borel et al. 2004, Jee et al. 2004, Kaltenboeck et al. 2005, Reinhold et al. 2005].

At first glance, an inconsistency seems to exist between an obviously high prevalence of Chlamydiaceae in bovine herds and relatively few reports about acute clinical illness caused by these bacteria. This leads to the question whether chlamydial infections in clinically inconspicuous animals do have any impact on animal health. Consequently, there is an ongoing discussion among veterinarians whether these observations indicate a role of chlamydiae as by-standers, co-pathogens, or perpetrators of just latent infections. Subclinical chlamydial infection has rarely been addressed in veterinary literature, and the question arises whether the detection of chlamydial organisms without apparent clinical diseases has any functional consequences on the organ systems infected and whether subclinical infections have a tangible impact on animal health.

Impact of asymptomatic chlamydial infections on animal health

Dairy cows

Chlamydiae & subclinical mastitis

Economically, mastitis is one of the most important diseases in animal agriculture, affecting both milk quantity and quality. Infections with *Cp. abortus* and *Cp. pecorum* are ubiquitous in cattle, and have been experimentally and clinically associated with bovine mastitis, and even subclinical mastitis is of major interest to “production medicine” because of the large impact on profit margins of dairy farms.

In dairy cows, the presence of chlamydiae was found to be significantly associated with subclinical inflammation of the mammary gland Biesenkamp-Uhe *et al.* [2007]. In a prospective cohort study in a herd of 140 Holstein dairy cows, all cows had serum antibodies against chlamydiae, and 49% of the cows were positive for *Cp. abortus* on day 0 of the experiment in at least one PCR test of a conjunctival or vaginal swab. As shown in Fig. 1, subclinical inflammation of the bovine mammary gland was confirmed by the somatic cell count (SCC) in milk (SCC is known to be a sensitive quantitative indicator of subclinical mastitis, and 10^5 somatic cells per ml milk are considered the upper limit for a healthy bovine mammary gland).

An intervention approach by perturbation of the immune response to *Cp. abortus* and/or *Cp. pecorum* was used to further examine induction, and immune-mediated reduction, of mastitis caused by chlamydial infection. All dairy cows had established immunity to chlamydiae (as demonstrated by anti-chlamydial serum antibodies), and many had PCR-demonstrated chlamydial infection. They received two doses of an inactivated vaccine of *Cp. abortus*/*Cp. pecorum* elementary bodies (therapeutic vaccination) or a mock vaccine on days 0 and 35 of the investigation. This vaccination increased anti-chlamydial antibody levels (Fig. 2A), and highly significantly reduced milk SCC (Fig. 2B), thus reduced bovine mastitis, but did not reduce shedding of chlamydiae. Chlamydia vaccination also resulted in improved relative body condition of dairy cows after 10 weeks. The disease-protective effect was maximal 10 weeks after vaccination, and lasted for additional 4 weeks.

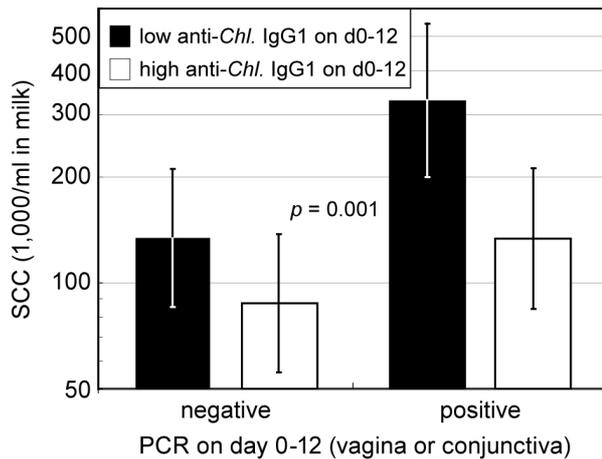


Figure 1: Effect of the interaction between *Chlamydomphila* PCR and anti-*Chlamydomphila* serum IgG1 on milk SCC on days 0 and 12 [Biesenkamp-Uhe et al. 2007].

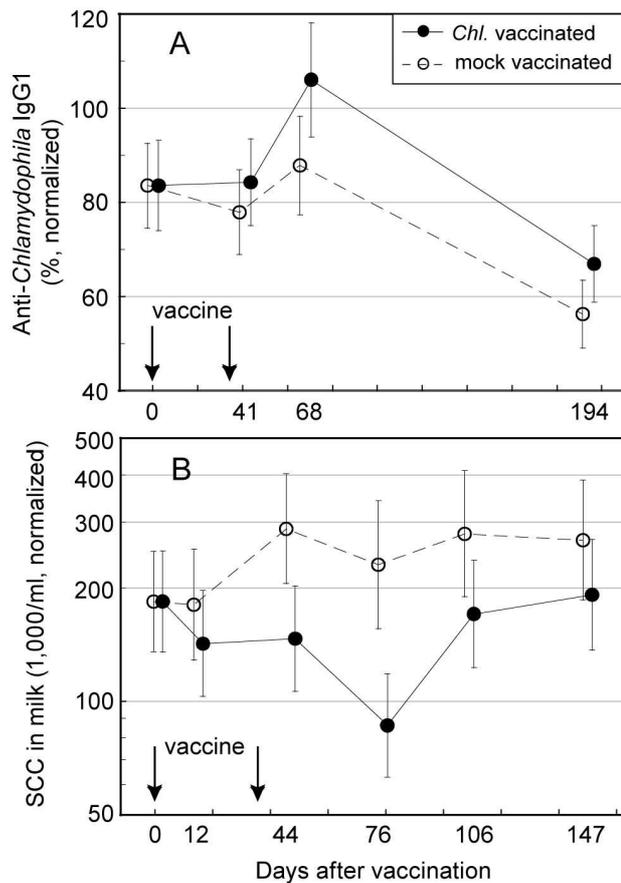


Figure 2: Effect of *Chlamydomphila* vaccination on anti-*Chlamydomphila* serum antibodies and milk somatic cell counts [Biesenkamp-Uhe et al. 2007].

A. *Chlamydomphila*-vaccinated cows have significantly higher anti-*Chlamydomphila* serum IgG1 levels than mock-vaccinated cows ($P = 0.018$; combined time points after day 0 in repeated measures ANOVA, Tukey HSD). Levels of anti-*Chlamydomphila* serum IgG1 antibodies are shown as percent optical density in comparison to a low-positive control serum. All cows had positive pre-vaccination antibody levels.

B. *Chlamydomphila*-vaccinated cows have significantly lower milk SCC than mock-vaccinated cows ($P = 0.007$ for all combined time points after day 0, repeated measures ANOVA, Tukey HSD). Data are shown as antilog of mean log SCC \pm 95% confidence interval and were normalized for identical day 0 means of *Chlamydomphila*- and mock-vaccinated animals (vaccine on days 0 and 35).

Chlamydomphila PCR-positive cows with low Chlamydomphila antibody levels before vaccination have significantly higher somatic cell counts on days 0 and 12 than cows that are Chlamydomphila PCR-negative and have high anti-Chlamydomphila antibody levels ($P = 0.001$; combined day 0 and 12 data in repeated measures ANOVA, Tukey HSD). Data are shown as antilog of mean log SCC \pm 95% confidence interval.

This study demonstrated an etiological involvement of the ubiquitous chlamydial infections in bovine mastitis, a herd disease of critical importance for the dairy industry. Furthermore, it shows the potential for transient improvement of chlamydial disease by therapeutic vaccination.

Chlamydiae & bovine fertility

DeGraves *et al.* [2004] investigated the effects of controlled re-infection on the fertility of cattle naturally pre-exposed to *Cp. abortus*. Twenty virgin heifers were estrus synchronized with prostaglandin F₂, artificially inseminated 2-3 days later, and challenged immediately by intra-uterine administration of 0, 10⁴, 10⁵, 10⁶, or 10⁸ inclusion forming units (IFU) of *Cp. abortus*. Ten heifers were estrus-synchronized, inseminated, and uterine-challenged 2 weeks later. These animals were also indirectly exposed to *Cp. abortus* infection (cohort challenged) by contact with their previously challenged cohorts. Pregnancy was determined by rectal palpation 42 days after insemination. All animals had prior serum antibodies against *Cp. abortus*, but showed no signs of clinical disease. One hundred percent, 83%, 50%, 66%, and 0% of heifers were pregnant after uterine challenge with 0, 10⁴, 10⁵, 10⁶, or 10⁸ IFU of *Cp. abortus*, respectively. Fifty percent and 65% of heifers were pregnant with or without cohort challenge, respectively. Uterine inoculum dose and cohort challenge, or alternatively a negative pregnancy outcome (infertility), correlated highly significantly with a rise in post-challenge over pre-challenge anti-*Cp. abortus* IgM. Logistic regression significantly modeled the uterine *Cp. abortus* inoculum causing infertility in combination with cohort exposure (Fig 3A) or in combination with anti-chlamydial IgM (Fig. 3B). These logistic regression models of fertility of heifers with established immunity against *Cp. abortus* indicate that with cohort challenge a uterine infection of 10^{4.38} IFU of *Cp. abortus* is necessary to reduce fertility of heifers from 100% to 50%, as compared to an 8.5-fold higher dose required for the same reduction without cohort challenge (Fig 3A); and at low pre-challenge anti-*C. abortus* IgM levels 10^{5.01} intrauterine IFU of *Cp. abortus* reduce fertility of heifers from 100% to 50%, as compared to a 17-fold higher dose required for the same reduction at high pre-challenge anti-*Cp. abortus* IgM levels (Fig. 3B). This investigation demonstrated that an asymptomatic, circulating, non-sexually transmitted herd infection by *Cp. abortus* has a profound influence on the fertility of cattle bred at this time.

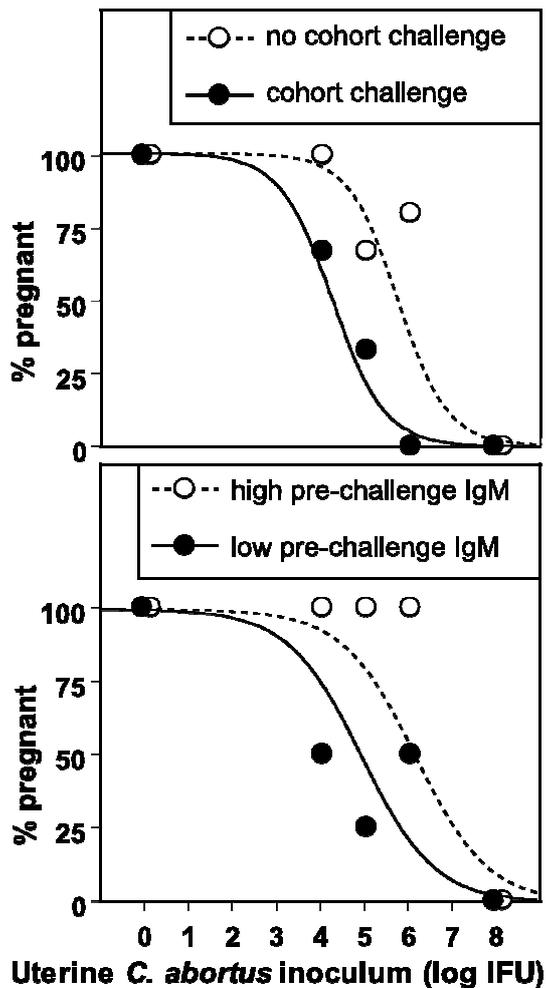


Figure 3: High uterine inoculum and cohort challenge with *Cp. abortus* and low pre-challenge anti-*Cp. abortus* serum IgM levels are associated with reduced fertility in heifers previously exposed to *Cp. abortus*.

Pregnancy as an indicator of fertility was evaluated 6 weeks after uterine challenge with *Cp. abortus* [DeGraves *et al.* 2004]. Fertility of challenged heifers (percent animals pregnant) is significantly predicted in logistic regression models by uterine *Cp. abortus* inoculum dose and cohort challenge by *Cp. abortus* (A), or by uterine inoculum dose and concentration of preformed IgM against *Cp. abortus* (B).

A — Overall pregnancy data and fertility modeled by logistic regression of Holstein heifers (n = 30) inoculated in the uterus with 0, 10⁴, 10⁵, 10⁶, or 10⁸ IFU, with or without cohort challenge.

B — Pregnancy data and logistic regression model for the same group of 30 heifers, with high (above-median) or low (below-median) pre-challenge anti-*Cp. abortus* IgM levels.

Collectively, these data suggest that reduction of bovine fertility by re-infection with *Cp. abortus* depends on the epidemiological parameters of herd infection with *Cp. abortus*, specifically on total challenge, via direct uterine inoculation and via cohort exposure, and the immune response to previous infection.

Calves

It is conceivable that chlamydial infections in young calves originating from cows with chlamydia-associated health problems as mentioned above may have an impact on health and development of young bovines. It is further known that the adult cows are the most likely source of infection for calves born in chlamydia-positive dairy herds. In such herds, calves are born free of

chlamydiae, but start to acquire both *Cp. pecorum* and *Cp. abortus* within 2 weeks post natum [Jee et al. 2004]. Both prevalence and intensity of animal chlamydial infections increased highly significantly with population density (crowding effect).

Chlamydial DNA can be detected in asymptomatic calves by PCR in nasal, rectal, and conjunctival swabs over months in an irregular and intermittent pattern that reveals persistent or frequently recurring infections [Reinhold et al. 2007]. Interestingly, *Cp. pecorum* is mainly detectable in gastro-intestinal samples, whereas *Cp. abortus* is predominant in samples of the respiratory tract and ocular secretions. In serology, antibody reactions are usually non-uniform in their pattern or even lacking (which is in contrast to the pattern of "synchronized" increase of humoral antibody production at the onset of host immune response).

Long term effects on general health and development in growing calves

Despite the absence of any clinical illness, naturally occurring persistent or recurring infections with *Chlamydomphila* spp. may have long lasting effects on animal health in calves. As shown recently, calves with clinically inapparent chlamydial infections had significantly higher body temperatures (subfebrile) and significantly lower body weights from two to seven months of age as compared to calves without chlamydial infections (Fig. 4).

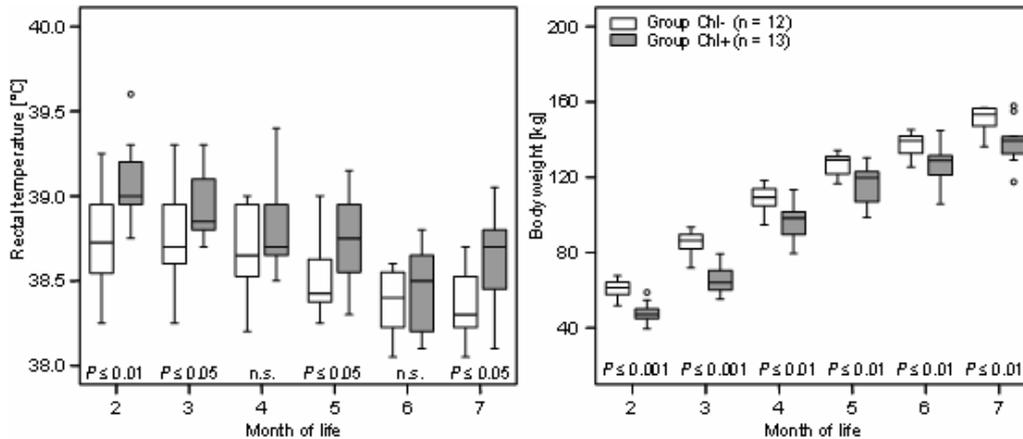


Figure 4: Rectal temperatures and body weights in calves derived from herds without (Group Chl-) or with known chlamydial infections (Group Chl+) aged two to seven months [Reinhold et al. 2007].

Legend: Box-and-Whisker Plot representing median value, 25%- and 75% percentiles (box), range, and outlier values (o). *P*-values indicate significant differences between groups at given time points (*W* test).

Furthermore, chlamydial infections were associated with reduced serum iron concentrations, and lower total hemoglobin and hematocrit values. Cell counts and flow cytometric differentiation of peripheral white blood cells revealed an overall decrease in leukocytes mainly caused by significantly reduced absolute numbers of lymphocytes in the peripheral blood of calves carrying chlamydiae. These findings suggest chronic effects on animal health at a subclinical level in calves naturally infected with *Chlamydophila* species although animals were kept under optimized and standardized conditions [Reinhold *et al.* 2007].

Chlamydiae & respiratory disorders

In traditional veterinary literature, respiratory chlamydial infections in bovines were mostly subsumed under the generic term of 'pneumonia' characterized by fever and depression, nasal secretions, cough, and dyspnoea [Storz & Kaltenboeck 1993]. Recently an outbreak of acute upper respiratory tract disease was reported in calves aged less than 6 months [Twomey *et al.* 2006]. Furthermore, chlamydial infections have been found to be associated with kerato-conjunctivitis in calves [Otter *et al.* 2003].

In calves persistently infected with *Cp. abortus* and/or *Cp. pecorum*, respiratory chlamydial infection was found to be associated with chronic inflammation of lung and airways [Jaeger *et al.* 2007]. Non-invasive lung function tests revealed significantly increased respiratory rates (Fig. 5A) and elevated peripheral airway resistances (Fig. 5B), indicating peripheral airway obstruction. Despite changes noted on a sub-clinical level (no respiratory distress was seen), pulmonary dysfunctions were already detectable at the age of 2 months and persisted over at least 6 months (Fig. 5).

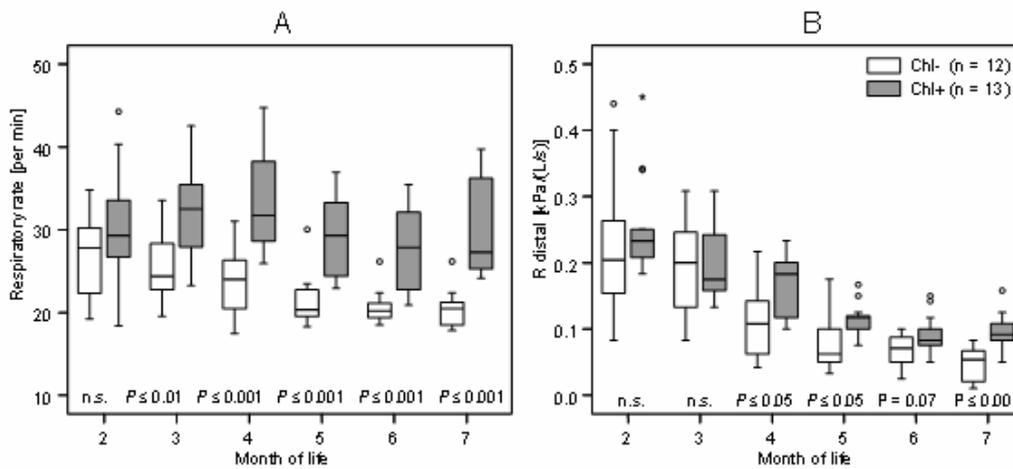


Figure 5: Respiratory rate and resistance of distal airways in calves derived from herds without (Group Chl-) or with known chlamydial infections (Group Chl+) aged two to seven months [Jaeger et al. 2007].

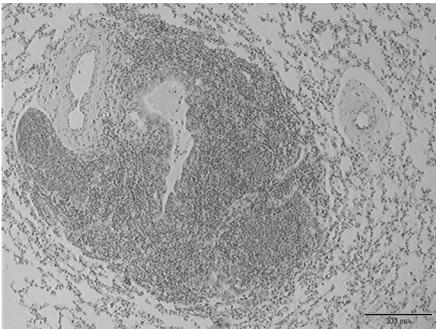
Legend: Box-and-Whisker Plot representing median value, 25%- and 75% percentiles (box), range, and outlier values (o). *P*-values indicate significant differences between groups at given time points (*W* test).

Pulmonary disorders were confirmed histologically by a markedly activated bronchus-associated lymphoid tissue (BALT) in the apical pulmonary lobes causing partial obstruction of bronchiolar lumina (Fig. 6). Furthermore, significantly elevated concentrations of total protein and 8-iso-prostane (8-IP), as well as increased activities of matrix metalloprotease 2 (MMP-2) were measured in broncho-alveolar lavage fluids (BALF) of calves with respiratory chlamydial infections indicating pulmonary inflammation (Table 1).



Figure 6: Comparison of pulmonary morphology in calves without (Chl-) or with known chlamydial infections (Chl+), Paraffin sections, H&E stain = 200µm [Jaeger et al. 2007]

Chl-: Two small lymphoid follicles close to bronchioles and an arteriole in a calf without chlamydial infection. Note the markedly folded mucosa of the bronchioles.



Chl+: Large activated lymphoid follicles with germinal centres completely surrounding a bronchiole ("cuffing") in a calf with chlamydial infection. There is loss of mucosal folding. The bronchiolar lumen is partially obstructed by protruding lymphoid follicles. The bronchiolar epithelium is hyperplastic. Neutrophils and exudates are present in the lumen.

Table 1: Markers of inflammation measured in broncho-alveolar lavage fluids (BALF) of 25 clinically normal calves aged 7 months without (Chl-) and with (Chl+) chlamydial infections [Jaeger et al. 2007].

	Unit	Chl-				Chl+				Wtest
		n	Median	Min.	Max.	n	Median	Min	Max.	
Protein	µg/mL	12	340.0	148.0	705.0	13	610.0	391.0	2205.0	P≤0.01
8-IP	pg/mL	12	104.2	26.1	647.0	13	340.0	64.7	908.0	P≤0.05
MMP-2	AU	12	403.0	177.0	954.0	12*	836.5	453.0	1591.0	P≤0.001

Legend: 8-IP: 8-iso-prostane; MMP-2: matrix metalloproteases 2; AU: arbitrary units

*one BALF sample was not possible to be analyzed

The detection of long lasting lung function disorders and pulmonary inflammation has quantitatively demonstrated the persisting effects of subclinical naturally acquired chlamydial infections on the pulmonary system. It should be taken into account that these pathophysiological features might interfere with postnatal lung development. In the bovine species, the period of postnatal lung growth and development is determined by further morphological differentiation and functional maturation of the lung, and functional maturity of the respiratory system is not reached before one year of age or a body weight of 300 kg [Lekeux *et al.* 1984, Lekeux 1993]. During this period, respiratory infections are likely involved in growth retardation that may cause impaired lung function for a long time and or even life long.

Pathogenesis of chlamydial infections

Being intracellular pathogens, chlamydiae are entirely dependent on the host for their replication within cells, where they form characteristic vacuole-like inclusions and replicate within an endosome. All chlamydial species have the potential to cause both acute and chronic infection, and the remarkable variety of clinical manifestations of chlamydial diseases should, at least in part, be a consequence of the distinctive features of the causative agents, particularly their unique biphasic developmental cycle. In the course of a replication cycle, infectious, but metabolically inactive elementary bodies (EBs) evolve into non-infectious, but metabolically active reticulate bodies (RBs). The latter reside in a vacuole-like inclusion of the host cell and undergo binary fission before transforming back into elementary bodies to start a fresh cycle. This life cycle enables the pathogen to pursue distinctive survival strategies in the host, which are giving rise to the evasion of host defence, as well as chronic and persistent courses of infection.

Persistence

There is increasing evidence that chlamydiae are capable of persistence and therefore may reactivate a present infection and so causing recurrent infections [Goellner *et al.* 2006, Hogan *et al.* 2004, Stephens 2003]. Consequently, the presence of chronic persistent infections with various clinical outcomes can be assumed in different hosts, and the hypothesis that chlamydiae may promote inflammatory processes in chronic diseases is based on the theoretical ability of this pathogen to survive in its host cells in a persistent state. This hypothesis, however, has yet to be proven *in vivo*.

In human medicine, an association between the intracellular persistence of *Cp. pneumoniae* and the manifestation of chronic diseases, like coronary artery disease, myocarditis, cancer or even neurological diseases is under discussion [Johnston & Martin 2005; Brandén *et al.* 2005]. Research on the interaction of *Cp. pneumoniae* with its host cells has demonstrated that the response of infected cells may contribute to the initiation and maintenance of inflammation as well as to tissue remodeling and fibrosis.

In respiratory medicine, *Cp. pneumoniae* infections have been reported to be involved in exacerbation of chronic obstructive pulmonary disease (COPD) and Asthma bronchiale, and more exacerbation results in faster progression of airway obstruction. Even after complete resolution of *Cp. pneumoniae* infection, hyperreactivity of the airways persists for months in otherwise healthy persons. It has been assumed that these pathogens may cause long lasting neurogenic inflammation, explaining these observations.

Altogether, establishment of chlamydial persistence is accompanied by diverse molecular mechanisms which are not yet fully understood. Nevertheless, there is little doubt that the persistence stage plays an important role in the chlamydial development and may be considered as the third phase of the developmental cycle

Inhibition of Host Cell Apoptosis

Chlamydiae are capable to either induce or inhibit host cell apoptosis, which is an important feature for their reproduction within the host cell. As persistent chlamydiae establish a long-term relationship with the host cell and persistent aberrant chlamydial bodies are not released at the end of the normal acute developmental cycle, enduring inhibition of apoptosis during the persistent state is conceivable.

Inhibition of host cell apoptosis may facilitate colonization of the organism and seems to play a role in long-term survival of persistent chlamydiae within the host cell. Indeed, there is evidence from *in-vitro* data that chlamydiae are able to inhibit apoptosis of infected cells [Haecker *et al.* 2006]. The resistance of infected cells against apoptotic stimuli may be essential for chlamydiae to complete their growth cycle but it may also promote intracellular persistence. In cultures of HEp-2 epithelial cells persistently infected with *Cp. pneumoniae* IL-6 production is continuously up-

regulated suggesting that a persistent infection may cause chronic inflammation [Kutlin *et al.* 2002].

Tissue Remodeling

Furthermore, chlamydiae were found to stimulate the expression of connective tissue growth factor (CTGF) and the production of basic fibroblast growth factor (bFGF) in different cells [Roedel *et al.* 2000, Peters *et al.* 2005]. Moreover, *Cp. pneumoniae* infection of smooth muscle cells increases the production of matrix metalloproteinases 1 (MMP-1) and -3, and infected macrophages secrete MMP-1 and -9 [Roedel *et al.* 2003, Kim *et al.* 2005]. All these mechanisms might be involved in irreversible structural changes of tissues called 'tissue remodeling'.

Conclusion

Recent data from epidemiological surveys indicate that chlamydial infections are distributed worldwide in bovine farms. Due to various pathogenetic peculiarities, these infections do not necessarily lead to clinical illness. Only if epidemiological risk factors coincide, such as a high-density host population, do these infections build up to become clinically manifest. Nevertheless, they may have a serious impact on livestock productivity and farm income. Causing chronic inflammatory reactions and dysfunctions on different organ levels, clinically inapparent chlamydial infections are probably economically more important than rare outbreaks of severe chlamydial disease. Precise data on the zoonotic impact of chlamydioses in bovines is not available.

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14.2 IOS AS A SUITABLE METHOD TO EVALUATE LUNG FUNCTION DISTURBANCES IN RESPIRATORY MECHANICS CAUSED BY CLINICALLY LATENT RESPIRATORY INFECTIONS WITH CHLAMYDIACEAE IN CALVES

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Aim: In symptom-free calves, Chlamydiaceae can be found quite regularly in samples obtained from the respiratory system. Whether this finding is related to functional and/or pathological changes within the respiratory tract has yet to be defined. This study evaluated the influence of chlamydial infections on pulmonary functions in conventionally raised calves. **Animals and Methods:** Twenty five calves aged 20 ± 5 days (mean \pm SD) were included. Group I ($n = 12$) was without any history of chlamydial infections, while Group II ($n = 13$) resulted from farms with known Chlamydia-associated health problems. Multiple bacteriological, serological and PCR analyses confirmed that both groups differed significantly with respect to Chlamydiaceae, but not for other confounding infections. All animals were examined between 2nd – 7th month of life clinically. In each animal, lung function was evaluated using the impulse oscillometry system (IOS). Twice per month, variables of ventilation (respiratory rate, tidal volume, minute volume) and respiratory mechanics (airway resistance, respiratory reactance) were measured. At the end of the study lungs were examined histologically. Statistical analyses were conducted to clarify whether the presence of Chlamydiaceae had any significant influence on lung function.

Results: *Chlamydophila abortus* and *Chlamydophila pecorum* were the most predominant chlamydial species found in nasal, faecal, and conjunctival swabs of Group II. Although there was no clinical illness in any group, mean rectal temperature was higher and average body weight was lower in calves of Group II compared to calves of Group I. In addition, pulmonary dysfunctions were observed in calves of Group II that were characterized by a significantly higher respiratory resistance in the frequency range 1 to 10 Hz (indicating peripheral airway obstruction) and significantly higher respiratory rates. Histologically, markedly activated BALT that caused partial obstruction of bronchiolar lumina was found in the apical pulmonary lobes of calves of Group II.

In conclusion, respiratory chlamydial infections appear to be involved in chronic inflammation of lung and airways. Despite changes occurred on a sub-clinical level, pulmonary dysfunctions persisted in calves until the age of 7 months. Data obtained in this study proved new pathogenetic information about the impact of ubiquitous subclinical infection on the respiratory system.

14.3 CELLULAR IMMUNE RESPONSE OF CALVES TO MANNHEIMIA HAEMOLYTICA LEUKOTOXIN

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The aim of the study was to evaluate the effect of *Mannheimia haemolytica* leukotoxin on cellular immune response in clinically healthy calves. There was investigated the alternations of peripheral blood leukocytes using flow cytometry method (FCM) in calves experimentally challenged by intravenous administration of 25 µg of *M. haemolytica* A1 leukotoxin per animal. The experimental calves (Gr. I) were compared with the controls (Gr. II) before (0) and after the challenge (1, 2, 3, 4, 5, 6 and 24 hrs). The following parameters were assayed: white blood cell count (WBC), percentage of polymorphonuclear leukocytes (PMNL), mid-size leukocytes (MID), i.e. total value of monocytes, eosinophils and basophils, total percentage of lymphocytes (LYM) and their subsets: CD2+ (T lymphocytes), CD4+ (T helper lymphocytes), CD8+ (T suppressor/cytotoxic lymphocytes) and WC4+ (B lymphocytes). The obtained results of the study shown, that in peripheral blood of the experimental calves (group I) WBC and the percentages of MID, PMNL, LYM and their some subpopulations (CD2+, CD4+, CD8+ cells), were significantly lower ($P < 0.05$) in compared with the control group during the first of third hours of the experiment.

Key words: *Mannheimia haemolytica* leukotoxin, bovine leukocytes

14.4 THE EFFECT OF GROUPING ROUTINES ON THE HEALTH AND GROWTH RATE OF PRE-WEANED DAIRY CALVES

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In swine, poultry and intensive beef production, batch-wise rearing has been a successful means to reduce the level of infectious diseases. However, such a system may be difficult to fully implement in many dairy herds. The aim of the present study was to evaluate the effect of batch-wise rearing, adopted on pen-level, on the health and growth rate of pre-weaned dairy calves. The experiment was performed on a commercial dairy farm with 300 cows in south west Sweden and used 403 Swedish Red and Holstein calves. The calves were housed individually until approximately 12 days of age when they were transferred to one of 8 group pens for 5-8 calves equipped with automatic milk feeders. Four of the pens were used for continuous rearing (C pens) and 4 pens were used for batch-wise rearing (B pens). C pens always housed 5-8 calves in dynamic groups. Two of the C pens (welcome pens) housed calves aged 12-42 days, whereas the other 2 (weaning pens) housed calves aged 42-70 days. Hence calves were transferred from the welcome pens to the weaning pens at 42 days of age and were then further moved at 70 days. The B pens were filled one at a time and once a group of 5-8 calves was formed, the group was kept stable until the calves were moved out at 70 days of age. Calves were weaned at approximately 67 days of age. The experimental design was based on age blocks of 7 consecutively born calves. Within each age block, 2 calves were randomly allocated to each of the 2 welcome pens and 3 calves were allocated to a B pen. The immunoglobulin content of the first meal of colostrum given to each calf was measured indirectly using a colostrometer. The calves were weighed at 12 and 70 days of age. The calf caretakers were instructed to record on individual health forms all identified disease conditions in calves 0 to 70 days of age. Every third week the herd was visited by the author, who clinically examined all calves, auscultated their lungs and completed the health records. The effect of grouping routine on growth rate and incidence of respiratory disease was evaluated by linear or logistic regression models with age block and pen as random effects, and occurrence of diarrhoea or respiratory disease before transfer to group pens, season, year, and colostrum quality as extra explanatory variables. The model of growth rate also included bodyweight at 12 days of age, sex, and breed. There was no difference in growth rate between the 2 groups of calves; mean growth rate of calves reared continuously (n=240) was 660 (SD: 0.18) g/day and of calves reared batch-wise (n=163) 690 (SD: 0.17) g/day. Calves in C pens had significantly higher risk of respiratory disease (P=0.01; OR: 1.7). It was concluded that housing calves in stable groups is preferable from a health perspective.

14.5 SUCCESSFUL COMBAT OF THE PORCINE ENZOOTIC PNEUMONIA IN SWITZERLAND

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Successful combat of the Enzootic Pneumonia in Switzerland
Problem Porcine enzootic pneumonia (EP), caused by *Mycoplasma hyopneumoniae*, is an epidemic disease leading to economic losses in the swine production worldwide. Switzerland decided to combat EP by partial or full depopulation strategies. Treatment against *Mycoplasma hyopneumoniae* or vaccination is therefore not allowed. Rapid and robust diagnostic methods for *M. hyopneumoniae* infections are a prerequisite to eradicate the disease. A real time PCR had been developed for this aim by Dubosson et al. and was tested on nasal swab material of living pigs by Zeeh et al. and on lungs. The diagnosis previously used, based on a combination of gross pathology, histology, immunofluorescence and epidemiological tracing, was sometimes longsome and sometime uncertain. Aim The new PCR was implemented in the combat strategies and was thought to enhance the running eradication.

Methods: As notifiable epidemic, clinical signs of EP have to be reported immediately to the farm veterinarian or the Swine Health Service and finally to the Cantonal Veterinary Office. It orders appropriate clarification measures. The rtPCR described by Dubosson et al. was conveyed in laboratory routine. In cases of suspicion of acute EP (coughing pigs), 10 nasal swabs per farm are taken and tested for *Mycoplasma hyopneumoniae* DNA. Slaughterhouses have to forward lungs with bronchopneumonia. If the test is positive, appropriate measures are undertaken. Fattening farms are depopulated immediately or are allowed to fatten their pigs until slaughter weight if there is no potentially farms at risk in close neighbourhood. Breeding farms have to undergo a partial depopulation which means a 14 days young animal free interval with oral medication of the remaining adults (older than 9/10 month). During eradication at farm animal transport is restricted. The success of the eradication program is monitored by on farm visits and by slaughter controls. Results EP suspicious samples are regularly send to the laboratories. Nasal swab sampling and sampling of altered lungs has become routine. In 2003 208 cases of EP were reported and 56 cases in 2004. In 2005 there were 22 and in 2006 17 affirmed EP outbreaks. In 2007 until March 1 EP case was reported.

Conclusion: It is possible to eradicate EP area- or nationwide, if all pig related institutions cooperate, an adequate diagnosis exists and measures are conducted consequently.

15 INFECTION, INFECTION DISEASES AND IMMUNOPROPHYLAXIS

15.1 ENTERIC INFECTIONS IN PIGS – AN UPDATE ON DIAGNOSTICS, TREATMENT AND VACCINATION STRATEGIES TO CONTROL BACTERIAL AGENTS

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Abstract

The ban on the use of antimicrobials as growth promoter in pigs resulted also in an increase in enteric diseases prevalence. In Central European countries, the change manifested itself mainly in increased prevalence of swine dysentery and porcine proliferative enteropathy. In spite of increasing prevalence of swine dysentery (SD) in the Central European region (Czech republic, Germany, Hungary, Slovakia) principal information are unknown. Until now molecular SD pathogenesis remains unclear. We do not know what is the key to the infection onset and development. Key properties of *B. hyodysenteriae* connected with virulence as toxins production and mechanisms effecting increase of MIC values for pleuromutilins are completely unknown. The demand for a vaccine as a means of SD prevention grows with the spread of antibiotic multiresistance of *B. hyodysenteriae*. There is a growing body of evidence that *B. hyodysenteriae* is gradually becoming resistant not only to tylosin and linkomycin but also to pleuromutilins based on results obtained by examining isolates also from the Czech Republic, Germany, Hungary and Italy. In the Czech Republic alone, the number of farms where tiamulin and valnemulin MIC levels exceed 16 mg/l at the same time were described as increasing from 1 (1999) up to 18 farms during the year 2002. Tiamulin is a last choice for treatment, but decreased sensitivity of isolates on some farms are the reason for increasing dosages, but also of control and eradication expenses. Where pleuromutilins concentrations are in excess of 16 mg/ml, a programme based on their use does not seem to have a realistic chance of success, and the method of total depopulation/repopulation need to be used. Rather than haemorrhagic diarrhoeas in neonatal piglets associated with type C *Clostridium perfringens*, enteritis are much more wide-spread in Central European countries. The course of the disease is milder, and *C. perfringens* type A, carrying the gene *cpb2* that encodes the production of a novel toxin referred to as β 2-toxin has been identified as the only suspect agent. The new vaccine based on β 2-toxoid can be expected to provide protection for piglets not only against infections caused by type A *cpb2*+ isolates but also to confirm the hypothesis that β -2 toxin is the major factor of virulence of these strains.

Key words: pig, infection, prevalence, antimicrobial resistance, swine dysentery, porcine proliferative enteropathy, *C. perfringens*

Introduction

Enteric infections in pigs belong among important diseases with a negative impact not only on economics of pig finishing operations but also on piglet production. Contrary to the porcine respiratory diseases complex (PRDC), however, enteric infections are not considered a dominant health problem in a majority of intensive pig production countries. PRDC is a permanent threat also because to date very little is known about viral agents like the PRRS virus and porcine circovirus type 2 (PCV-2) (Allan and McNeilly, 2006). It was considered that we are still unable to deal with the PRRSV variability or explain the pathogenesis of the disease caused by PCV-2. Therefore swine producers in many countries continue to experience severe PRRS (Zimmerman, 2006) as well as PRDC losses.

When we take a closer look at the issue of porcine enteric infections in different types of pig operations in the recent past (grower/finishing/ breeding pig farms), we will conclude that they are not lagging very far behind PRDC. In addition to studying new bacterial agents of porcine enteric infection described in the mid 1990s, (*Lawsonia intracellullaris*, *Brachyspira pilosicoli*), it is necessary to elucidate the aetiological role of PCV-2 in colitis. From among classical causative agents of swine enteritis, *Salmonella* serovars especially those not host-adapted to pig (*S. Typhimurium*, *S. Infantis*, *S. Derby*) are, moreover, beginning to pose a growing problem in majority of EU member states. Not because of their primary clinical effect in pigs, but because of their fundamental zoonotic importance in public health. It is a reality that in the case of some bacterial causative agents of porcine enteric infections, the major concern is not their effects on health status of the pigs on farms, but mainly on populations of pork consumers, i.e. on people. As proven in several EU member states, various serovars of *Salmonella* enter the food chain and may be the cause of food-borne diseases. In this respect, the interest of the general public and public health authorities in the causative agents of porcine enteric infections is extraordinary because they want to be certain that the food they buy is safe. Current hot topics are EU programmes focusing on *Salmonella* prevalence in fattening as well as breeding pigs, and programmes of *Salmonella* prevalence control in the near future. Furthermore pigs are potential reservoirs of other enteric bacteria that cause infections in humans i.e. *Campylobacter* spp. (*C. coli*), *Yersinia enterocolitica* and comensals as *E. coli*, in which resistance to a variety of antibiotics may occur (McEwen, 2006). Transmission trough food chain is believed to be most important.

Economic importance of enteric disaeses

Enteric infections are an example of endemic diseases whose economic importance has been assessed repeatedly. At the individual farm level, performance data are often available. Most of common performance indicators, however, reflect the health status of the animals concerned only indirectly. The link between disease and its effect on performance needs to be established for each disease individually (Thomson et al., 2007). Because the majority of porcine enteric infections need not be reported to

veterinary authorities, data on enteric disease must be collected, e.g., as part of research projects. The ban on the use of antimicrobials as growth promoter in pigs resulted not only in a small reduction in production as documented in Denmark but also in an increase in endemic diseases prevalence. In Central European countries, the change manifested itself mainly in increased prevalence of swine dysentery and porcine proliferative enteropathy (PPE).

Swine dysentery (SD): re-emerging diseases in the Central Europe

Aetiology and prevalence. The disease is caused by the spirochaete *B. hyodysenteriae* described 36 years ago in England. Its typical manifestation is severe muco-haemorrhagic diarrhoea, which, if untreated, may be fatal. As an infectious disease, it primarily affects grower and finisher pigs, and currently occurs in most pig-producing countries except the USA and few EU countries (France, Denmark, Sweden). In the Central Europe region high prevalence of SD is observed mainly in the Czech Republic (Lobová et al., 2004), Germany (Herbst et al., 2007, Rothkamp et al., 2005), Hungary, Italy (Meriardi et al., 2005) and Slovakia, compare to Austria (Weissenböck et al., 2007) and Poland where the prevalence of SD is low.

The incidence of SD in the Czech Republic has been monitored since 1978 by the State veterinary authority. Following the liberalization of the trade in pigs in 1990 and the withdrawal in 1995 of SD from the list of contagious diseases subject to the reporting obligation, the number of farms with a clinical form of SD increased significantly during the mid-1990s (Fig.1). Another increase in the number of pig farms with clinical cases of SD was observed after 1998, when nitroimidazols and the growth promoter olaquinox (which has anti-dysenteric effects) were banned for the treatment of SD. Up to that time, tylosin, lincomycin and tiamulin were also used in the treatment of SD in the Czech Republic, but as in other countries, *B. hyodysenteriae* isolates resistant to tylosin and lincomycin were frequently reported (Cizek et al., 1998). The situation worsened in 1999, and led to the introduction of targeted treatment with pleuromutilins on farms with an endemic SD. At the time it was also common to use feed containing preventive medication in at-risk herds.

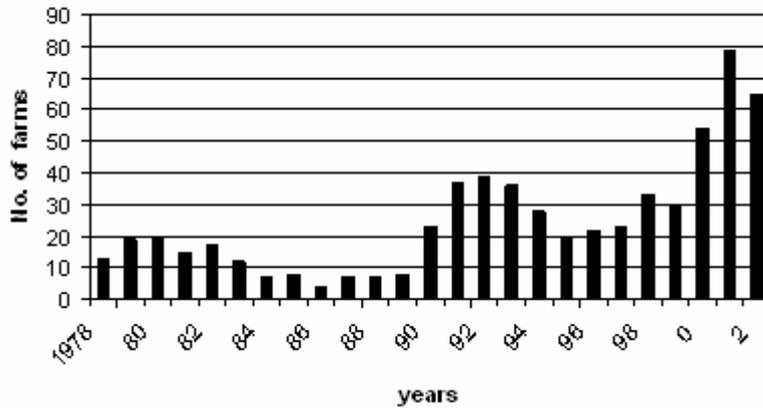


Figure 1. The number of pig farms in Czechia, where SD was recognised by culture method during a period 1978-2003 (Lobová et al., 2004).

New information on the agent is primarily expected from the genome sequencing, which will be completed shortly. The principal goal is to identify factors that are responsible for the pathogen's virulence, and also to find protective antigens that could be used for the design of vaccines. The problem, still unsurmounted in spite of intensive research in both Europe and Australia, is the failure to differentiate between strains of different virulence on the molecular level in order that the major factors of virulence could be identified. To surmount the problem, strains with specifically developed gene mutations responsible for, e.g., haemolysin production, are particularly needed. Discovered a long time ago and considered one of the main candidates for virulence, this toxin remains little understood especially from the point of view of the infection's molecular pathogenesis. In addition to the toxin, other key virulence factors include structures responsible for the strains' adhesion to colon enterocytes present on the spirochete surface. Their role in the process of pathogenesis has not been clarified, either. Due to the absence of such key data, methods have been developed that allow the assessment of isolate virulence in their total in an experimental infection model. Recent experimental pig infections in Sweden and Australia have confirmed differences in the infectiousness between hitherto commonly at the international level used strain B 204 and the new isolate X 384. In the experiments, the so-far most frequently used strain B204 induced infection on average in 20 (65%) of 31 pigs (Janson, 2007) with incubation period between 7 and 31 days, the new isolate from Australia proved infectious in 100% (Trott, 2007), and its incubation period was very short (5.3 days in directly infected pigs and 7.5 days in sentinel pigs). Although infectiousness of most of field isolates is much lower than infectiousness of these selected strains of *B. hyodysenteriae*, infections on farms occur on a regular basis. It is therefore highly probable that presence of some other predisposing factors, e.g. high percentages of soybean meal in the ration, is necessary for an outbreak of swine dysentery with its characteristic clinical symptoms. We have confirmed that assumption recently in our experiments in which the strain B204 failed to cause swine dysentery in 20 conventional pigs that were fed a diet containing no soybean meal. In

another experiment with the same strain and the same dose, a single modification, i.e. an introduction of soybean meal once daily, resulted in clinical manifestations of infection in 70% of pigs.

Immunological response. New findings identified the presence of proinflammatory cytokine IL-1beta and the production of serum amyloid A in pigs with manifestation of SD. In that period, the numbers of blood monocytes, neutrophils and CD8 α + lymphocytes increased. The susceptibility of pigs to infection appeared to be related to low levels of CD8 α + lymphocytes and high levels of $\gamma\delta$ T cells in blood prior to inoculation. (Jonasson et al., 2006). The presence of the anti-inflammatory cytokine IL-10 and the production of *B. hyodysenteriae* specific antibodies against lipoproteins SmpA and/or BmpB were associated with the recovery of sick animals. The importance of co-occurrence of specific antibodies and recovery from a *B. hyodysenteriae* infection remains unclear and needs to be addressed further (Jonasson, 2007).

Disease and diagnostics. The main clinical manifestations of a typical form of the acute disease are muco-haemorrhagic diarrhoea that lasts several days followed by diarrhoea for 2 to 14 days. Some of the pigs infected, however, do not show these typical symptoms, and under both standard conditions on farms and in experiments they only suffer milder symptoms with non-haemorrhagic diarrhoea. From the microbiological assay point of view, the most important information is that pigs with clinical symptoms shed *B. hyodysenteriae* continuously over the entire dysenteric period. As the pigs recover, the structure of their faeces returns to normal. On average, shedding stops at day 8 from recovery, but some pigs may continue to shed the causative agents, usually in an irregularly and in numbers that may be below the detection limit. In some cases, cultivation is successful in spite of the fact that no clinically clear-cut demonstration of enteric infection was observed. This usually happens in connection when other antibiotics than pleuromutilins are administered, or with low dose of pleuromutilins medication.

From the microbiological assay of *B. hyodysenteriae* point of view, the requirement of cultivation on a selective medium is still very important. It remains the golden standard for the confirmation of SD diagnosis, because PCR lacks the necessary sensitivity for the faeces testing. PCR-based diagnostic was described as suitable in faecal samples with high percentage (22,5- 31,1) of positivity in Germany (Herbst et al., 2007). In herds with endemic swine dysentery, an asymptomatic course of the disease with no visible diarrhoeas has sometimes been reported (Herbst et al., 2007). The most frequent findings in the survey focused for nondiarrhoeic pigs made in the Czech Republic (Šperling et al., 2007), in which 18 rectal swabs were collected from finishers with no clinical symptoms of diarrhoea on each of the 22 randomly selected farms, were *Brachyspira spp.* isolates. From that *B. hyodysenteriae* was detected by cultivation on 3 of the 22 farms monitored, just as *Salmonella enterica*. While *L. intracellularis* was demonstrated in rectal swabs of pigs from 7 farms (31.7%).

There are no commercially available kits for the detection of antibodies specific only against *B. hyodysenteriae*, due to cross reactivity with antibodies against antigens of other *Brachyspiras*. Based on the results of several preliminary studies, it is very likely that a test for the assay of antibodies against the unique recombinant antigen Bhlp 29.7 will be designed (La et al., 2007; Lobová et al., 2007), which would make it possible to introduce a herd serological test for the demonstration of swine dysentery. Such an examination would be particularly useful in cases where the causative agent was not demonstrated at the time of clinical manifestations, when the clinical picture was atypical, or at purchase of animals.

Therapy. Increasingly frequent field reports of reduced clinical efficiency of pleuromutilins in the treatment of SD highlighted the need for a laboratory assessment of pleuromutilins efficacy in the Czech Republic. In the period under investigation (1997-2001), minimal inhibitory concentrations (MICs) to both tiamulin and valnemulin increased, with differences between the period 1997-98 and 1999-2001 being statistically significant ($P < 0.001$ and $P < 0.0001$) (Table No 1.) Between 1997 and 2001, the MIC₅₀ and MIC₉₀ of tiamulin increased from 0.062 and 0.25 µg ml⁻¹ respectively to 1.0 and 4.0 µg ml⁻¹ respectively. Valnemulin MIC₅₀ and MIC₉₀ were ≤ 0.031 µg ml⁻¹ in 1997, and by 2001 were 2.0 and 8.0 µg ml⁻¹, respectively. The increase in MICs of tiamulin and valnemulin demonstrated in this study (Lobová et al. 2004) reflect the intensity of pleuromutilin use in the treatment of swine dysentery in the Czech Republic.

There is a growing body of evidence that *B. hyodysenteriae* is gradually becoming resistant not only to tylosin and linkomycin but also to pleuromutilins based on results obtained by examining isolates also from Hungary (Molnár, 1996), Germany (Karlsson et al., 2002) and Italy. The Central Europe is considered as the region with a particularly rapid development of antibiotic multiresistance and the growth in MIC values, primarily in view of endemic occurrence of SD and the absence of eradication programmes. In the Czech Republic alone, the numbers of farms where tiamulin and valnemulin MIC levels exceed 16 mg/l at the same time were described as increasing from 1 (1999) up to 18, during the year 2002 (Šperling et al., 2006).

For tiamulin breakpoint rather than being only the limit for laboratory resistance determinations, it is also a concentration at which clinical effectiveness begins to fail. The failure of the drugs may have, however, been caused by a number of factors related either to the host, technologies or the environment. The proposed clinical breakpoint of tiamulin resistance (Rønne and Szancer, 1990), particularly with regard to per-oral administration, seems to be in need of some adjustment. However it is necessary to stay in agreement with recent suggestion (Bywater et al., 2006) to retain term “breakpoint” solely for clinical breakpoints and distinguish this from the “epidemiological cutt-off value” which shows that a change away from the wild-type population (sensitive) may have occurred in a subpopulation, which may indicate emerging resistance. In spite of all the optimism in veterinary medicine, we are of the opinion that *B. hyodysenteriae* might be the first causative agent of animal infections against which no effective antibiotics registered for swine dysentery treatment will be

available. In the Czech Republic, and probably also in other countries where the situation is similar, the problem of MIC increase prevention and of effective therapy is addressed by combining pleuromutilins and tetracyclines and/or doxycycline. The fact that in spite of all that *B. hyodysenteriae* becomes less and less sensitivity to pleuromutilins has an enormous impact not only on treatment alone. For that reason it is necessary to abandon bad habits, such as administering pleuromutilins at preventive doses. What, therefore, is the priority? To stop the increase in MIC of pleuromutilins, which currently are the last drugs of choice. It is important to monitor the development of MIC not only in regions but particularly on farms, where they rely on pleuromutilins for most of their treatment efforts, and where there is no interest - because of a lack of funds - in swine dysentery eradication.

Table 1. Pleuromutilin MICs for 100 Czech *B. hyodysenteriae* isolates between 1997 and 2001 (20 isolates examined per year) (Lobová et al., 2004)

Antimicrobial agents	MIC ₅₀ , MIC ₉₀ and range of MIC ($\mu\text{g ml}^{-1}$)					Total 1997-2001	
	1997	1998	1999	2000	2001		
Tiamulin	MIC ₅₀	0.062	0.125	0.125	0.5	1	0.25
	MIC ₉₀	0.25	0.25	1.0	2.0	4.0	2.0
	Range	≤ 0.031 - 1	0.062 - 2	≤ 0.031 - 1	0.062 - 4	0.125 - 16	≤ 0.031 - 16
Valnemulin	MIC ₅₀	≤ 0.031	≤ 0.031	0.062	1	2	0.125
	MIC ₉₀	≤ 0.031	0.125	2	4	8	4
	Range	≤ 0.031 - 2	≤ 0.031 - 2	≤ 0.031 - 4	≤ 0.031 - 8	0.062 - 16	≤ 0.031 - 16

Although several antimicrobial agents effective against *B. hyodysenteriae* are available, the farmers because of influence of economic crises/insolvency choose rather alternative and inexpensive approach to control swine dysentery with additives as ZnO or more recently with zinc chelate. It is very well known that zinc plays an important role in bacterial metabolism of various species as well as in anaerobic bacteria. Additionally, the prevalence of postweaning diarrhoea and mortality caused by enteropathogenic *Escherichia coli* is significantly reduced in piglets fed with 2500-3000 ppm of ZnO (Jensen-Waern et al., 1998). It was found that exposure of *B. hyodysenteriae* to 0.1 mM (16.1 mg/l) ZnSO₄ completely inhibited production of haemolysin (Dupont et al., 1994), which is probably major virulence factor involved in the pathogenesis of SD. The usage of additives like zinc oxide or metal-amino acid chelates (with Zn and Cu) in water became very frequent in dysenteric farms during last few years. The point is that those additives are not able to treat the SD with the same efficacy as antibiotics. According results of our experimental study and field experience the main effect is associated with prophylactic effect. Dietary supplementation of water with zinc reduced the recovery rate of *B. hyodysenteriae* and

provided partial protection against development of cecal lesions in pigs. Nevertheless we should have to calculate that *B. hyodysenteriae* is not fully eliminated from the host as well as from the herd and farm environment.

Immunoprophylaxis. Until now there is no effective commercial vaccine available on the market. New findings in immunology have suggested new directions for the research into and development of recombinant vaccines in projects funded by pharmaceutical companies. Another approach is to use natural isolates with low virulence for live vaccines (Thompson et al., 2005). Such isolates may be available in laboratories but they are useless nowadays without comprehensive molecular characteristics that would facilitate their selection for animal experiments during the period of field trials. However vaccine development will certainly continue because successful prevention, control and eradication of swine dysentery without vaccination are impossible in the current adverse epidemiological situation.

Eradication in practice The fact is that SD is mainly controlled by the use of expensive eradications programmes and by antibiotic treatment. Tylosin and linkomycin resistance is common and wide spread and they cannot be considered reliable alternative medications. Tiamulin is a last choice for treatment, but increasing MICs of isolates on some farms are the reason for increasing dosages, but also of control and eradication expenses. Where pleuromutilins concentrations are in excess of 16 mg/ml, a programme based on their use does not seem to have a realistic chance of success, and the method of total depopulation/repopulation need to be used.

Principal SD problems. Molecular SD pathogenesis remains unclear. We do not know what is the key to the infection onset and development. Properties of *B. hyodysenteriae* produced toxins and mechanisms effecting increase of MIC values for pleuromutilins are completely unknown. The demand for a vaccine as a means of SD prevention grows with the spread of multidrug resistance of the SD causative agent.

Spirochaetal colitis

Aetiology and prevalence. The diseases (spirochaetal colitis/ intestinal spirochaetosis) pose an ever-growing problem in pig herds in Central Europe. It has been stated repeatedly that the etiological role of *B. pilosicoli* is clear, compared with that of other species (*B. intermedia*, *B. murdochii*) where it still remains to be defined. The diseases affects mainly pigs in herds with good health status (SD-free farms and SPF farms), with only infrequent use of pleuromutilins, macrolides or lincosamides for treatment. Typical clinical symptoms include non-haemorrhagic diarrhoeas that occur in individual pigs, rather than in total herds. For diagnosis, the optimum approach at present is necropsy of selected poorly performing individuals, where the principal finding is colitis, which, if untreated, may transform into necrotic colitis. Close monitoring of SD-free pig farms that failed to reach the expected parameters in their finishing operations revealed a high prevalence of intestinal spirochaetosis in the Czech Republic (Šperling et al., 2007), but not in Germany or in Austria (Weissenböck

et al., 2007). In most cases, it was the first time that that diagnosis, concerning enteritis in growers, was made on the farm.

Therapy. Rather than a therapeutic use of antibiotics, a comprehensive approach is recommended in solving this problem. In spite of the fact that in *B. pilosicoli* isolates changes in the MIC of antibiotics of choice compared with *B. hyodysenteriae*, are not very dramatic. Sensitivity to pleuromutilins (tiamulin and valnemulin) remains high, while majority of *B. pilosicoli* isolates exhibit a generally high MICs to tylosin, acetylisovaleryltylosin and linkomycin (Čížek et al., 2004, Šperling et al., 2005). Increased MICs of pleuromutilins were recorded mostly in isolates from pig farms with SD history as well as high consumptions of antidysenterics. According to some studies, clinical symptoms in pigs with spirochaetal colitis can be significantly improved by changing from wheat-based to barley-based rations (Thomson et al., 2007). Immunoprophylaxis of finisher pigs in connection with *B. pilosicoli* infections is not yet considered.

The role of some other brachyspiras isolated from pigs with colitis also remains unclear. More frequent than findings of *B. pilosicoli* are the isolation of the species *B. murdochii*, *B. intermedia* and *B. innocens*, whose isolates, as reported by some recent studies, are also pathogenic for pigs. According to the results of those studies, it is quite probable that species like *B. murdochii* will be considered potentially pathogenic. A brand new candidate among brachyspiras with a pathogenic potential for pigs may be the provisionally designated species “*B. suanatina*” (Rasback et al., 2007), which has been described as the causative agent of an SD-like disease in Sweden, Australia and Denmark. In experimental studies using isolates of “*B. suanatina*” weaners developed haemorrhagic diarrhoea. Until now a PCR for “*B. suanatina*” has not been reported. The results of a number of recent studies have clearly indicated how important it is to study and further clarify the role of brachyspiras in the porcine colon in view of the clinical relevance of such data.

Clostridial enteritis in neonatal pigs

Aetiology and prevalence. *Clostridium perfringens* type C is a much-feared causative agent of haemorrhagic diarrhoeas in piglets in the perinatal period characterized by high mortality in litters affected. In some countries in which the disease has spread to the majority breeding farms (Denmark), vaccination of sows with type C toxoids is absolutely necessary. That was, after all, demonstrated by an outbreak of new infections following the end of registration of vaccines, and thus the end of their use there. The vaccines that were to replace the previously used proved less effective. It was the changes in the use of vaccines that underlined the delicate balance during the controls of haemorrhagic enteritis among piglets caused by *C. perfringens* type C, in view of permanent exposure of sows and piglets to type C strains in certain herds.

Contrary to Denmark, type C strains did not spread to many farms in the Czech Republic because important nucleus and multiplier herds had not been affected. A

critical situation, however, developed in a commercial operation with 800 sows where the first outbreak of haemorrhagic enteritis of piglets occurred 1995 and affected most of litters. The disease and the causative agents were eradicated only after 5 years of careful monitoring, vaccination and partial depopulation with negative gilts.

Rather than haemorrhagic diarrhoeas in neonatal piglets associated with type C, enteritis are much more wide-spread in Central European countries. The course of the disease is milder, and *C. perfringens* type A has been identified as the only suspect agent. Diarrhoeas affecting the small intestine appear about 2 or 3 days after birth and last several days, usually until the age of 8 or 10 days. Piglets usually do not die, but those that have suffered of diarrhoea for several days will lag behind in growth and will not compensate for the loss of weight before weaning. In the Czech Republic, these strains spread very quickly particularly to breeding sow herds. Type A isolates from diarrhoeic piglets are especially noted for the fact that they carry the gene (*cpb2*) in the plasmid DNA that encodes the production of a novel toxin referred to as β 2-toxin, which is similar to the β 1-toxin of pathogenic strains of serotype C. A problem is that, compared with type C infections, cultivation findings of type A *cpb2*⁺ are also very frequent in piglets with no clinical manifestation of diarrhoeas. Moreover, sows in most herds with a prevalence of clostridium enteritis in suckling piglets are vaccinated with commercial vaccines against type C containing also the α -toxoid, which induces the production of antibodies also against the α -toxin of type A. For that reason, molecular analysis and subsequent study of pathogenesis for newborn piglets are essential for the clarification of the role of type A isolates.

Therapy. Therapeutic effect in the treatment of diarrhoeas with the isolation of *C. perfringens* type A *cpb2*⁺ in the field practice was observed mainly after per oral application of not only aminopenicillins but also certain macrolides. Antibiotics proved effective particularly when administered early before clinical symptoms were fully developed. However, C (Masaříková, 2006). The current therapeutic failures need to be related to decreased sensitivity but also of resistance to antibiotics used in the treatment of other porcine bacterial infections than enteric. According to our hypothesis, one of possible reasons for the spreading of these isolates between 2000 and 2006 is the increase in MIC and the development of resistance, which gave these isolates a selective advantage in sow intestines as well as in newborn piglet intestine.

Immunoprophylaxis. Contrasting with the efficacy of commercial vaccines against infections caused by type C is the absence of such efficacy against infections caused by *C. perfringens* type A *cpb2*⁺ (Masaříková and Smola, 2004). One of the main objectives is therefore the design of a vaccine that would protect piglets also against infection caused by isolates of type A *cpb2*⁺. The development of a new toxoid β 2-based vaccine is in the focus of interest of pharmaceutical companies. The new vaccine can be expected to provide protection for piglets not only against infections caused by type A *cpb2*⁺ isolates but also to confirm the hypothesis that β -2 toxin is the major factor of virulence of these strains.

Differential diagnostics. In contrast with the situation in the USA, in Europe and also in the Central Europe region the role and importance of toxinogenic strains of *C. difficile* in the diarrhoeic syndrome of suckling piglets in the first week of their life has never been defined. It follows from some studies that diarrhoeas of this etiology might make up to 10% of all piglet diarrhoeas observed in the first week of their life. The ongoing objective therefore must be a comprehensive differential diagnostics of neonatal piglet diarrhoeas, complete with a demonstration of the presence of toxins A and B in faeces.

What is needed? It will be necessary to explain the etiological role of isolates of type A *cpb2+* in the outbreak of newborn piglet enteritis, and, at the same time, to develop a new vaccine capable of protecting newborn piglets against infection. In order to design an effective therapy, the development of isolate resistance in individual herds will have to be monitored. Irrespective of its aetiologic role, *C. perfringens* type A will have to be classified as an indicator species for resistance development monitoring on pig farms.

Porcine proliferative enteropathy (PPE).

Aetiology. A wealth of new information on the causative agent of PPE, the gram-negative, obligatorily intracellular, bacterium *L. intracelularis* is expected when the results of its genome sequencing will be published. In this respect, another fundamental assumption has been revised, i.e. the existence of plasmids whose role is still unclear. Genome sequencing is expected not only to differentiate the basic areas of the bacterium genome, but also to identify pathogenicity islands encoding the basic factors of strain pathogenicity and virulence (Gebhart, 2006).

Diseases prevalence and diagnostics. In the past decade, a world-wide existence of PPE (i.e. including countries like Brazil where PRRSV has not been demonstrated) has been confirmed. European countries with traditional herd management but also with SPF herds have gradually confirmed high prevalence (95-100 % of herds), which means that there are no herds free of the causative agent. In retrospective it is clear that the number of clinical forms of PPE peaked in 1999-2000, i.e. shortly after the ban on the use of antibiotics as growth hormones was passed. Since then, clinical diagnostics of the disease has been modified in some respects due mainly to the spread of pathogenic brachyspiras in pig herds. In 1999, it was still possible to distinguish between symptoms of chronic ileitis and acute haemorrhagic enteritis with a high degree of certainty. However with an explosive spread of swine dysentery in Czechia when 60 to 80 new farms were being affected annually, the clinical picture of dysentery in grower/finisher pigs as well as in conventional sow herds became the dominant one. From 2002 onwards, reliable clinical diagnostics was limited to swine dysentery-free farms, the number of which has critically decreased. There was a spread of the complex of enteric infections of grower/fattener pigs, which is made more complicated by post-weaning multi-systemic wasting syndrom with high prevalence. The endemic incidence of swine dysentery, ileitis and intestinal spirochaetosis in the

majority of herds with a frequent medication with pleuromutilins, tetracyclines, macrolides and lincosamides creates a very labile situation. Co-infections occur, and their timing is determined by mistakes in the herd management or the rigidity of antibiotic therapy.

Microbiological diagnosis of PPE. Direct assay of *L. intracellularis* by nested PCR proved very important even before because of its high sensitivity (Tomanová and Smola, 2004; Gebhard 2006), and it is exceptionally important particularly wherever growers are in medication programmes. Today, there are also other highly sensitive methods (Tomanová et al., 2004) for the assay of *L. intracellularis* but the isolation of this agent by cultivation on cellular cultures will remain unattainable (Gebhart, 2006). We must bear in mind that in culture collections of microorganisms there are currently only 12-14 isolates of *L. intracellularis*, and only few of them come from pigs. For that reason, comparison of different strains will only be possible at the level of DNA extracted from clinical material.

Rather than PCR results, the current ileitis prevalence among grower and fatter pigs in individual herds can be estimated on the basis of serologic testing results. Antibodies against various *L. intracellularis* antigens found in porcine serum serve to show that there are no infection-free herds in Czechia (Tomanová and Smola, 2004) as well as in Hungary. Serological results obtained in weaned piglets (36,4%) also indicate a high seropositivity of sows in operations at different levels, including breeding and nucleus herds. In finisher pig herds, the serological prevalence is up to 55,4 % in 16 week-old pigs, and 91,4% in 20 week-old pigs. Two commercial systems (indirect fluorescent antibody test and ELISA) for the antibodies assay marketed by two pharmaceutical companies are currently available. Few months ago an interlaboratory study was performed to establish quality- control for serology testing. The ring test showed high level of results, which were correct even if both methods were used separately. Their results provide information on the status of the herd, rather than of individual pigs. Serological profiles of herds are routinely established and, after evaluation, they become the basis for the decision to use either preventive therapy or vaccination.

Immunoprophylaxis. Peroral administration of a live attenuated strain in water after weaning marks important progress towards the reduction of clinical forms of PPE.

Results showed that this can bring benefits not only at the end of fattening but also in the case of an early infection in the nursery. Vaccination also reduces the overall amount of antibiotics administered on pig farms by inducing immunity for approximately 17 weeks, and repeated medications are therefore unnecessary.

Besides therapeutic use of antibiotics eradication of *L. intracellularis* by medication has been shown to be possible in sow herds (Johansen et al., 2002).

Conclusion

Principal SD problems. Molecular pathogenesis in swine dysentery remains unclear. We do not know what is the key to the infection onset and development. Properties of *B. hyodysenteriae* produced toxins and mechanisms effecting increase of MIC values for pleuromutilins are still unknown. The demand for a vaccine as a means of SD prevention grows with the spread of multidrug resistance of the SD causative agent.

Few studies documented a continual decrease in susceptibility *B. hyodysenteriae* to both pleuromutilins. Increasing frequency of multi drug resistant isolates was observed in Czechia during a period 2001 – 2003. The fact that in spite of all that *B. hyodysenteriae* becomes less and less sensitivity to pleuromutilins has an enormous impact not only on treatment alone. For that reason it is necessary to abandon bad habits, such as administering pleuromutilins at preventive doses and to stop the increase in MIC of pleuromutilins, which currently are the last drugs of choice. It is important to monitor the development of MIC not only in regions but particularly on farms, where they rely on pleuromutilins for most of their treatment efforts, and where there is no interest - because of a lack of funds - in swine dysentery eradication. New alternatives to antibiotic, which are based on usage of non-antibiotic substances seems to be efficient for prophylactic effect instead for treatment.

It will be useful to find out which of the other *Brachyspira* species (*B. murdochii*, *B. innoces* and *B.intermedia*) are involved in swine colitis, which is connected with isolates of *B. pilosicoli*.

Enteritis caused by the *C. perfringens* type A (*cpb2+*) range among the diseases of the newborn piglets with a major economic impact recently. The results based on the antibiotic treatment and on the usage of the commercial vaccines against type C provided contradictory data. The causes underlying the spread of the infection are unclear. Our hypothesis suppose a relation with the absence of previously used antibiotic growth promotores, and possible decreased sensitivity of isolates to antibiotics frequently used in therapy of various bacterial infections in herds. In the retrospective study, decreased sensitivity of isolates to tylosin was expected compare to tiamulin. An increase of tiamulin MICs values in *C. perfirngens* type A (*cpb2+*) during the past 3 years was detected.

Porcine proliferative enteropathy became global problem for the pig industry. There are many topics for research in the field of molecular pathogenesis and molecular subtyping of *L.intracellularis*. The main objectives however is, to learn how to create pig herds that would be free of *Lawsonia intracellularis* in the future.

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15.2 DUAL-TARGETING - A NEW STRATEGY TO MINIMIZE SELECTION OF BACTERIAL RESISTANCE

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Introduction: Prevention of bacterial resistance to antimicrobials remains a challenge. The inherent genetic diversity in bacterial populations regularly provides for a spectrum of viable variants or mutants. This spectrum also includes mutants with reduced susceptibility to antimicrobials. Fluoroquinolones have two independent targets of activity in bacterial cells, DNA-gyrase and topoisomerase IV, which possess different sensitivities to drug action. Mutants with reduced susceptibility have developed an alteration in the first, more sensitive target, DNA-gyrase. Because the second target is less sensitive to drug activity, a higher fluoroquinolone concentration is required to eliminate such mutants. In a wild-type population, this concentration results in dual-targeting which ensures reliable elimination of the entire spectrum of viable variants. To establish a minimum dose level that provides for dual-targeting, the sensitivity of both targets needs to be considered. The sensitivity of the first target is defined by the Minimum Inhibitory Concentration (MIC), whereas that of the second target is determined by the Mutant Prevention Concentration (MPC). The MPC is the concentration of a fluoroquinolone that is inhibitory for mutants with an altered first target. Therefore, the MPC is a microbiological parameter that helps to develop a dosing concept that may considerably reduce the risk of emergence of bacterial resistance.

Material and methods: To determine the MIC and MPC values of Baytril® (Enrofloxacin) for *Escherichia coli*, agar plates containing serial dilutions of the active ingredient were incubated with 10^5 (MIC) or $>10^9$ CFU (MPC). The MIC and MPC were the lowest agar concentration of enrofloxacin that inhibited bacterial growth at 37°C over 24 and 120 hours, respectively. MIC and MPC values were produced for four wild-type isolates in four independent experiments. Results The MICs of Baytril® (Enrofloxacin) for *Escherichia coli* ranged between 0.03 and 0.06 µg/ml. The MPC values turned out 7 to 10 times higher and fell between 0.3 and 0.4 µg/ml.

Conclusions: The results will be compared with published MIC and MPC values as well as the pharmacokinetic profile of Baytril. MPC values of Baytril for wild-type isolates of *Escherichia coli* and *Mannheimia haemolytica* suggest that the recommended standard dose level of Baytril provides for dual-targeting at the site of infection. This will result in rapid pathogen elimination and the prevention of resistance selection over the dosing interval of 24 hours.

15.3 RESISTANCE TO CHALLENGE WITH EIMERIA ZUERNII IN CALVES TREATED WITH DICLAZURIL DURING PRIMARY INFECTION

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Problem: *Eimeria* (*E.*) *zuernii* coccidiosis induces enteritis, which is hemorrhagic in severe cases and may even be lethal. After primary infection calves are generally protected from subsequent coccidiosis. However, it appears possible that abrogation of the parasite life cycle by metaphylactic application of potent anticoccidial drugs may impair development of immunity. **Aim:** The present study explores whether calves that are treated metaphylactically with diclazuril at the approved dose during a primary infection with *E. zuernii* display sufficient resistance against a secondary challenge infection.

Methods: 28 male Holstein Friesian calves were allocated to 3 groups (A, B, C) as follows: A (n = 11): no treatment, primary infection, challenge; B (n = 11): 1 mg diclazuril /kg body weight 14 days after primary infection, challenge; C (n = 6): no treatment, primary infection with challenge dose. A dose of 250,000 sporulated oocysts of *E. zuernii* was chosen for primary infection of groups A and B and 7 weeks later these calves were challenged with 500,000 oocysts. The calves of group C remained naive and untreated until challenge when they were infected with the higher dose. The efficacy of treatment and the course of primary and challenge infection were analysed by clinical (diarrhoea) and parasitological (oocysts per gram faeces, opg) data. The study was performed strictly according to the principles of Good Clinical Practice (GCP).

Results: Following primary infection oocyst excretion was observed in 7 calves and diarrhoea on at least 3 consecutive days in 8 calves of group A. In contrast, all group B calves remained parasitologically negative and incidental cases of diarrhoea were not attributable to coccidiosis. After the challenge (A, B) or primary (C) infection with the higher dose, low to moderate opg were recorded in all groups. Highest opg counts of up to 14650 were observed in group C. All but one calf of group C developed liquid diarrhoea, partly with blood and tissue, whereas only one out of eleven treated calves (B) and none of the untreated calves (A) displayed clinical coccidiosis after challenge. Statistically diarrhoea was less frequent in groups A and B than in group C whereas groups A and B did not differ significantly.

Conclusions: Diclazuril efficiently controls primary *E. zuernii* coccidiosis. Oocyst excretion can be provoked in immunised calves by massive challenge but clinical disease after challenge is suppressed irrespective of treatment. It is concluded that diclazuril treatment of *E. zuernii* infected calves does not impair development of immunity.

15.4 BLOOD CHEMICAL PARAMETERS AND ACID-BASE BALANCE IN EIMERIA ZUERNII INFECTED CALVES

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Problem: *Eimeria zuernii* coccidiosis is an important diarrhoeal disease in calves and occurs worldwide. *Eimeria zuernii* infection may lead to severe clinical coccidiosis including haemorrhagic diarrhoea, dehydration, weight loss and reduced growth. **Aim** The pathophysiology of *Eimeria zuernii* infection is further characterized by a wide-range screening of blood parameters.

Material and methods: Infection trials were performed to investigate the effects of experimental *Eimeria zuernii* coccidiosis on clinical blood chemistry and acid-base balance in affected calves. Three groups of calves were formed: group C (n=14) served as uninfected control group, group A (n=11) was infected with 150,000 sporulated *E. zuernii* oocysts per calf, and group B (n=16) was infected with 250,000 sporulated *E. zuernii* oocysts per calf. Faecal samples were taken two to three times per week during the prepatency and daily during patency. Faecal consistency and oocyst excretion (quantified by modified McMaster method) were determined from each sample. Blood samples were taken contemporarily on days with faecal sampling. Serum samples were analysed for blood chemical parameters using Hitachi 912 technology (Roche Diagnostics, Basel, CH). Acid-base balance and electrolytes were assayed from heparinized whole blood samples using AVL Opti CCA technology (Roche Diagnostics, Basel, CH).

Results: Measurements revealed a marked influence of *E. zuernii*-infection on the following parameters: 1. total protein, albumin, urea, bilirubin, creatine kinase, and free fatty acid concentration which increased by coccidiosis; and 2. cholesterol, sodium, chloride, pH, base excess, standard bicarbonate, total carbon dioxide, and partial pressure of carbon dioxide which decreased by coccidiosis. Alterations in most parameters were most pronounced in the high-dose infected group. No significant and/or distinct changes after infection could be detected in blood glucose concentration, potassium, anion gap and oxygen saturation.

Conclusion: The pathophysiology of *Eimeria zuernii* coccidiosis is mainly characterised by diarrhoea and associated losses of blood, water, nutrients, electrolytes and alkaline buffer substances. This is reflected in aberrations in various blood parameters. Dehydration, lipolysis, endogenous protein catabolism, electrolyte imbalances, and a (mostly respiratorily compensated) metabolic acidosis could be identified as main aspects of systemic disease.

15.5 LABORATORY EVALUATION OF THE APPROPRIATE VACCINATION PROGRAMS AGAINST NEWCASTLE DISEASE IN BROILER CHICKENS UNDER FIELD CONDITIONS IN MOSUL PROVINCE (IRAQ).

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Four experiments were conducted to examine the efficiency of 4 different Newcastle disease (ND) field vaccination programs. For all experiments young broiler chickens were obtained from breeder flocks regularly vaccinated against ND. The following vaccination programs were applied to 128000 broilers (four broiler flocks programs ,with of about 8000 broilers flock):1- Vaccination at 1 day of age with lentogenic Hitchner B1/spray with oil emulsion vaccine /SC; 7,14,25,35 and 45 day old with Lasota/drinking water; 2- Vaccination at 1 day of age with oil emulsion vaccine /SC; 7,14,25,35 and 45 day old with Lasota/drinking water; 3- Vaccination at 1 day of age with hitchner B1/spray 7,14,25,35 and 45 day old with Lasota/drinking water;4- No vaccination at 1 day of age ; 7,14,25,35 and 45 day old with Lasota/drinking water. In all experiments, 20 randomly chosen birds from each of 16 broiler flocks were bled just prior to vaccination and tested for Newcastle disease antibody by hemagglutination inhibition test(HI).

Serological data indicated that the greatest response was found by applying the first program, when 1 day old chicks were vaccinated with combined Hitchner B1/spray and oil emulsion /SC among the 4 vaccination programs.

15.6 SPRAY DRIED EGG AS A RICH SOURCE OF IMMUNE GLOBULIN IN DIETS FOR WEANED PIGS - METABOLIZABLE ENERGY DETERMINATION

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The high concentration of IgY in spray dried chicken egg (SDE) was shown by Rose et.al.,1974(European J. of Immunology) and the efficacy against E. coli was shown by Shimizu et.al., 1988 (J. of Food Science). SDE is included in nursery diets for pigs as a source of immune globulin as well as essential nutrients. Two trials have been conducted with a total of 238 pigs weaned at 16 to 20 days of age to evaluate the efficacy of SDE added at zero and 5% of the diet. Both diets were balanced to meet minimums for amino acids and energy based on duck values. Each diet contained dried whey and fish meal. The experimental diets were fed for 10 days post weaning. Combining the data from the 2 studies, the inclusion of 5% SDE resulted in a significant increase of 18% in daily gain, 11% increase in feed intake, and 3% increase in feed efficiency. In trial one, daily gain,feed intake,and gain/feed values were 204 g, 204 g, and 1.00 g/f for the pigs on zero SDE and 243 g, 236 g, and 1.02 g/f for pigs receiving 5% SDE. In trial 2, daily gain, feed intake, and gain/feed values were 181 g, 253 g, and 0.71 g/f for pigs receiving zero SDE and 204 g, 263 g, and 0.78 g/f for pigs receiving 5% SDE. A digestion/metabolism study was conducted to determine the metabolizable energy value for pigs for this energy rich feed ingredient that contains more than 30% fat. Digestibility was determined for dry matter, protein, fat,energy, and NFE with 10 kg pigs for a 4 day adjustment period, followed by a 3 day collection of feces,urine,and wasted feed. Feeding, collections, sampling, and lab analyses were conducted under standard operating procedures using Parr adiabatic bomb calorimetry for energy, and Leco analytical equipment for protein and fat analyses. Digestibility values were as follows: gross energy, 84.1%; dry matter, 84.5%; fat, 89.2%; crude protein, 85.6%; and NFE, 95%. The energy values for dietary formulation are: metabolizable energy, 5092 kcal/kg; digestible energy, 5267 kcal/kg; and TDN, 113.1%. Spray dried egg is an excellent effective source of IgY, energy, and protein for use in providing immune globulins and critical nutrients for nursery pig diets fed in the first 3 to 4 weeks post weaning.

15.7 INTRADERMAL SINGLE AND BILATERAL VACCINATION AGAINST ACTINOBACILLUS PLEUROPNEUMONIAE IN PIGS

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Intradermal vaccination in pigs brings great advantages in administration and minimizing of postvaccination complications. In many herds vaccination against *Actinobacillus pleuropneumoniae* (APP) is still necessary to prevent pigs against several pulmonary changes and economical loss, caused APP worldwide. New intradermal techniques of APP vaccination help to improve efficacy and safeness of vaccination. Intradermal vaccination in pigs is provided generally at any of the part of dorsal surface of the pig, anatomically divided into the thoracic and lumbar part. Lymph from cranial and caudal parts of skin streams down to four of main surface lymph node - subscapular and inguinal. Lymph brings antigens down to the lymph node, enables communication and activation between present B lymphocytes, T lymphocytes, dendritic cells, macrophages and incoming antigen, included in lymph. Organs responsible to the major production of immunoglobulin are spleen and lymph nodes. Different results obtained after administration of antigen at different sites show consequence between number of competent lymph nodes and volume of titer of IgM and IgG. The aim of the paper was to declare significance of relevant superficial lymph node(s) in quantity of humoral immunobiological reply to antigen stimulation. In the experiment were used 6-week-old 42 piglets, divided at 7 groups, without colostrum-derived antibodies against APP. Two groups were vaccinated by two doses, two groups by four doses and two groups by eight doses; control group was not vaccinated. In each of paired groups was one group vaccinated at one site, and the second group was vaccinated at both sides of the body, to afflict two lymph nodes. The standard intradermal (i.d.) vaccine and dosage (0,2 ml/dose) was used, contained proteins and lipopolysaccharids, equal mixture of APX I,II,III inactivated APX toxins, commercially registered product Suivac APP id, Dyntec,CZ. Piglets were vaccinated and revaccinated by the same dose and site in each group under experimental protocol in age of 6 and 9 weeks respectively. Blood was taken weekly, analyzed by ELISA. IgM and IgG antibody values were measured.

Results from IgM and IgG show alike, obtained titers were higher in groups where one dose was administered bilateral in compare to groups where doubled dose was administered unilaterally; i.e. results from groups, where bilateral administration was provided, were generally better than results of unilateral vaccinated pigs with doubled dose of vaccine (doubled, quadrupled and eight folded dose were tested). The results indicate that vaccination may develop higher titer of IgG and IgM protecting antibody after dividing the intradermal vaccination dose at more places.

15.8 PARATUBERCULOSIS HERD SANITATION: FIRST EXPERIENCES WITH INACTIVATED VACCINE SILIRUM

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Since summer 2003 a new inactivated Johne's vaccine (SILIRUM, CZ Veterinaria Corp.) has been applied to five chronically paratuberculosis infected dairy herds in Rhineland-Palatinate. According to the results of the first examination in antibody ELISA (IDEXX), only the youngstock up to the age of one year was vaccinated in three herds. About half of the fourth herd (selected by breeder's point of view) and all animals of fifth herd were vaccinated. In all herds blood and fecal samples were collected once a year to examine in IDEXX antibody ELISA and microbial culture. Hygienic measures were also part of the program: quick culling of clinical cases, no breeding up of the calf from cows with clinical paratuberculosis, parturition only in clean and disinfected calving parlors, application of dam's colostrum only, hygienic feeding and closed herds if possible. Vaccination was done by local veterinarians. Up to now, the results of four herd examinations are available. Some animals got granulomas at the injection site. When vaccinated in first four weeks of life, granulomas disappeared in four to six weeks. If older cattle were vaccinated, granulomas sometimes persisted and reached up to apple size. In the fourth and fifth herd with a higher number of vaccinated animals, 33.3% and 51.2% of vaccinated animals showed positive results in antibody ELISA in the second herd examination (about one year after vaccination). At the third herd examination (another year later) the part of positive vaccinated animals in antibody ELISA was 27.3 and 22.5 %. Three years after the start of the experiment the proportion was 13.5 and 14.4 %. Shedders of MAP were found among vaccinated and non vaccinated animals – up to 6.3 % or 6.1 %. In four herds, there seems to be a decrease of clinical Johne's cases. In the complete vaccinated herd the number of clinical cases decreased only slightly after vaccination and increased again in the fourth herd examination three years after start of the experiment. According to the results of this examination and former experiments, the combination of hygienic measures, fecal examinations and culling of shedding animals as well as vaccination reduces sustainable prevalence of MAP in infected herds. The vaccination with SILIRUM causes smaller local reactions in comparison with live vaccine NEOPARASEC. Vaccination with SILIRUM seems to increase antibodies faster - but with shorter persistence. The role of "culture and cull" in herd sanitation should not be underestimated. Up to now the effect of vaccination with SILIRUM is not completely evaluable, further examinations are necessary. The success of an enduring sanitation is depending on strict compliance with hygienic measures.

15.9 FAECAL SHEDDING OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS BY CATTLE WITH SUB-CLINICAL PARATUBERCULOSIS IS INFLUENCED BY INDIVIDUAL HOST FACTORS

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Problem: Paratuberculosis is a chronic inflammatory intestinal disease of ruminants caused by *Mycobacterium avium* subsp. paratuberculosis (MAP), with high economic consequences especially in dairy cattle. In general, the disease is only considered a health problem by the producers when clinical cases become obvious. However, considerable amounts of MAP organisms are already shed by sub-clinically infected cows and contribute to the spread of the disease within a herd. Therefore, the identification and removal of faecal shedders is one prerequisite for a successful control of the disease. Identification of faecal shedders is hampered by the fact, that MAP is often excreted only intermittently in the latent state of the disease. **Aim:** It was the aim of the study to identify individual host factors of sub-clinically infected dairy cattle which influence faecal shedding of MAP in order to give recommendations for the optimal time point of sample collection for detection of faecal shedders by culture or PCR.

Material and methods: In two dairy herds with an average of 245 and 400 lactating cattle, respectively, faecal samples were collected five times every 5 to 7 month from all lactating cows and heifers of more than 18 month of age. Bacteriological culture was performed for the detection of MAP. Individual data of each animal included in the study were obtained from the herd records, i. e. age, number of lactation, lactation state, milk yield, milk quality parameters and others. For all animals with a bacteriologically positive faecal sample, individual factors at the respective time point were analysed for their contribution to the risk of faecal shedding.

Results: A higher proportion of faecal shedders can be detected in older animals and in animals in the first or third trimester of lactation. The risk to become faecal shedder is higher in offspring of cows which were also identified as being paratuberculosis infected. There was no clear relation between milk yield and milk quality parameters and the risk of faecal shedding.

Conclusions: Sample collection for the detection of faecal shedders should be performed in the first or last trimester of lactation. Although older animals have a higher risk of MAP excretion, faecal culture is also successful in younger dairy cattle and these animals should not be excluded from examination.

15.10 ACUTE PHASE RESPONSE IN CHICKENS WITH E.COLI INFECTION AND EIMERIA TENELLA INVASION

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Objective: The aim of this study was to investigate the effect of the experimentally induced infection with *Eimeria tenella* and *E. coli* on acute phase response in broilers.

Materials and methods: The chickens were divided into 4 groups of 40 birds each. The chickens from Ist group were administrated with *Eimeria tenella* on day 12th of their life. The birds from IInd group were inoculated with *E. coli* on the day 16th. Chickens from IIIrd group were infected with the both agents - *Eimeria tenella* and *E.coli*. The birds from IV group serve as control. Blood samples were taken on day 20 of their life. The following acute phase proteins were determinated: ceruloplasmin (Cp), haptoglobin (Hp), fibrinogen (Fb) and albumin. The total protein and globulin and albumin/globulin ration were also estimated.

Results: In all experimental groups the plasma concentration of Fb, Cp and Hp were significantly ($P < 0.001$) higher than in control group. The plasma concentration of Fb and Cp were most markedly affected in broilers infected with *E.coli*, while the highest values of Hp were found in broiler infected with both *E.coli* and *E.tenella*. The infection with *E.coli* and combination of *E.coli* and *E.tenella* caused a significant increase of plasma concentration of total protein and globulin. In the same groups a marked ($P > 0.05$) decrease of plasma albumin was observed.

15.11 THE USE OF ACUTE PHASE PROTEINS IN TREATMENT MONITORING IN CATTLE NATURALLY OCCURRING THEILERIOSIS

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Serum concentrations of acute phase proteins can provide valuable diagnostic information in the detection, prognosis, or monitoring of the disease. It was aimed in this study to investigate the use of serum amyloid-A and haptoglobin - acute phase proteins- in monitoring the treatment in cows naturally occurring theileriosis. For this purpose; 15 cows, 11 with theileriosis (group 1) and 4 healthy (group 2) were used. Cows in both groups were administered 2,5 mg/kg buparvaquone and serum samples were collected before and 1, 4 and 7 days after the injection in order to evaluate and compare serum amyloid-A and haptoglobin concentrations. SAA concentrations before the treatment were significantly higher than the healthiest. SAA concentrations have been significantly decreased after the treatment procedure. While some cows with theleriosis were associated with markedly decreased plasma haptoglobin levels, the others were found markedly increased.

It was concluded that SAA can be used in monitoring of the treatment of theileriosis.

15.12 OUTBREAKS OF CARDIOMYOPATHY DUE TO FOOT AND MOUTH DISEASE IN LAMBS

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Foot and mouth disease is a highly contagious viral disease of ruminants, caused by aphthovirus, Picornaviridae. The disease is clinically characterized by high fever, vesicle and ulcer in the oral cavity and interdigital space. Although the disease has largely controlled in cattle by vaccination in Iran, sheep population is still at risk and need more attention. During December 2006 to April 2007, 5 outbreaks of foot and mouth disease were diagnosed in Khorasan province with high mortality in lambs associated with cardiac lesions. There was no classic lesions of the disease in ewes. Short period of weakness, inappetance and cardiac arrhythmias followed by death in neonate lambs. Mortality was 7 to 32%. Post mortem examination revealed mottling of pericardium and endocardium and different degrees of myocardial cell necrosis. During winter close contact of sheep and presence of susceptible lambs lead to outbreaks of the disease, mortality and economic loss. Vaccination of sheep against foot and mouth disease as performed in cattle, may prevent the outbreaks and mortality in lambs.

15.13 SERUM BIOCHEMICAL CHANGES IN BOVINE EPHEMERAL FEVER

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Bovine ephemeral fever (BEF) is an infectious disease of cattle which characterized by inflammation of mesodermal tissues. The disease is caused by an insect- borne, unnamed rhabdovirus. Among domestic animals only cattle are known to be naturally affected but antibodies can be found in wild ruminant. During an enzooty of bovine ephemeral fever in dairy farms of Tehran province in Iran two series of blood samples were taken from 50 infected dairy cattle. One blood sample was taken with EDTA for virus isolation and detection of virus genome by PCR, and another blood sample without EDTA for measurement of serum biochemical parameters. Blood samples from 35 normal and no infected cows were also taken as control group. Serum calcium, phosphorus, alkaline phosphatase and creatine phosphokinase concentrations in infected and no infected cattle were measured.

Results of analysis in this study showed that serum calcium concentration in infected cattle were in normal range for cow but significantly lower than normal group ($P < 0.05$). Serum creatine phosphokinase concentration was also higher than no infected cows but the difference was not statistically significant. Concentrations of phosphorous and alkaline phosphatase in infected cows were also not significantly different from no infected cattle.

15.14 BACTERIAL PATHOGENS OF CATTLE AND PIGS - ACTUAL SUSCEPTIBILITY STATUS OF ENROFLOXACIN IN GERMANY

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Problem: Antibiotics are essential for the therapy of bacterial infections in both animals and humans. To ensure long term efficacy of the drugs and safety of food animal products, knowledge on the actual resistance status is important. Bayer established several resistance monitoring programs starting 1991 concerning veterinary pathogens as well as zoonotic bacteria. Aim This poster presents the actual susceptibility status for enrofloxacin, the active ingredient of Baytril, in respect to major pathogens relevant for cattle and pigs.

Material and methods: Bacteria were isolated from samples collected during 2000-2006 in three regions of Germany. The Minimum Inhibitory Concentration (MIC) was determined by agar dilution according to principles of the Clinical and Laboratory Standards Institute (CLSI). The enrofloxacin breakpoint of 2 mg/l was used to calculate the percentage of resistant bacteria.

Results: 665 bovine isolates covering the most important bacterial diseases were investigated. The resistance rates of the respiratory pathogens were 0% for *Pasteurella multocida* and 1.7% for *Mannheimia haemolytica*. None of the mastitis isolates was resistant (*E. coli*, *Klebsiella* spp., *Staphylococcus aureus*). For enteritis, all *Salmonella* strains were determined as susceptible whereas 24% of *E. coli* isolated from calves and 17% from adult cattle were calculated to be resistant to enrofloxacin. In the case of *Arcanobacterium pyogenes* the resistance rate was 8.5%. For swine, 1158 bacteria frequently involved in infections were investigated. Respiratory pathogens showed resistance rates of 0.5% for *Pasteurella multocida* and 2.6% for *Bordetella bronchiseptica*. Of the *Staphylococcus* spp. strains isolated from skin samples 8.8% were resistant to enrofloxacin. Pathogens from the urogenital tract/MMA showed resistance rates of 5.9% (*E. coli*) and 4.8% (*Staphylococcus* spp.). All *Salmonella* isolates derived from enteritis cases were susceptible whereas 7.2% of *E. coli* from piglets and 4.4% from pigs were resistant. The resistance rates and the MIC₉₀ values showed only minor deviations to other monitoring programs. Reasons for the differences might be the number of investigated isolates and the proportion of samples collected from treated animals. Bovine enteric *E. coli* showed relatively high resistance rates for enrofloxacin and a number of other commonly used antimicrobials. Therefore, testing of antimicrobial susceptibility prior to treatment is regarded essential.

Conclusions: The data presented here demonstrate that the resistance rates of enrofloxacin are relatively low. Prudent and rational use of antibiotics is crucial for maintaining low resistance rates. Ongoing monitoring will allow to detect emerging risks and to take adequate management measures.

15.15 REGULATORY PROCESSES IN LYME BORRELIOSIS: EFFECT OF TRANSFORMING GROWTH FACTOR-BETA

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Introduction: *Borrelia burgdorferi*, a bacterium of the order Spirochaetales, is the causative agent of Lyme borreliosis, a frequently diagnosed infection in humans and animals. After transmission by ticks, the bacteria cause acute as well as chronic inflammatory reactions, especially in joints, in the pericardium and meninges. TGF-beta (Transforming Growth Factor-beta), an immune modulating cytokine, plays a central role in controlling the activity of different cells of the immune system. Thereby, the pleiotropic factor elicits both immune suppressive as well as stimulating effects. The project aims to determine the influence of TGF-beta susceptibility and TGF-beta 1-production of T-cells on the borreliosis infection by means of transgenic mouse models. In addition the effect of TGF-beta on the development of Th17-cells, a recently described type of t-helper-cells, during Lyme borreliosis is being identified.

Methods: Mice were infected intradermally with *Borrelia Burgdorferi sensu stricto* N40, P4. Disease was observed up to 56 days past infection. Used mice strains are C3H/HeN WT (not transgene comparison group for *B. burgdorferi* infection), FVB/N WT (not transgene siblings of the transgene test mice), hCD2-TGF-beta (T-cell-specific overexpression of TGF-beta), hCD2-dnTGF-beta-RII (T-cell-specific inhibition of the TGF-beta-receptor II).

Results: 1. Evidence for the susceptibility of FVB/N WT-mice for the induction of Lyme borreliosis. 2. Evidence for the susceptibility of cell-specific TGF-beta-transgene mice (FVB-background) for the induction of Lyme borreliosis. 3. Significant increase of active TGF-beta during Lyme borreliosis. 4. Significantly more CD4+/IL-17+-cells by hCD2-dnTGF-beta-RII-mice than hCD2-TGF-beta-mice.

Conclusion: The transgene mice strains presented in this poster are an appropriate model for studying the Lyme borreliosis-disease as well as the influence of TGF-beta on the pathogenesis in vivo. Lyme borreliosis is connected with a significant increase of TGF-beta-concentration in sera of infected mice. Furthermore, the T-cell-specific modification in TGF-beta-activity and -sensitivity causes significant differences in the number of CD4+/IL-17+-lymphocytes in ex vivo restimulation experiments. The cytokine essential for the generation of Th17-cells thereby appears to have an inhibitory effect on the amplification of these cells.

15.16 LISTERIA MONOCYTOGENES KERATOCONJUNCTIVITIS IN DAIRY HEIFERS ASSOCIATED WITH FEEDING WRAPPED BALED GRASS SILAGE

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Dairy farmers probably remember *Listeria* sp. in particular, as a sporadic cause of abortion or as a meningitic condition that arose from ingestion of poor quality silage. *Listeria* is a bacterium that is capable of causing severe food poisoning in man. Clinical incidence of Listeriosis (encephalitis, abortion) in ruminants is increasing in Slovenia. Only few reports about ocular mucosal surface infection in ruminants without associated abortion or encephalitis related to feeding silage exist in literature. During the winter housing period (2005/2006) 4.1 % incidence of an unusual eye problem in cattle associated with the feeding of wrapped baled grass silage was observed. In a herd (n = 98) with loose housing system outbreak of clinical signs which included hyperaemic conjunctiva, lacrimation, photophobia, and cloudy cornea was observed in four high pregnant heifers in March 2006. Whole opened baled grass silage harvested in 2005 was fed at libitum to a group of free housed pregnant dairy heifers and cows. One week before clinical signs were observed the weather was cold (- 10o C). Harsh wind opened the panel of external stable wall and dispersed the rock wool isolation mixed with scattered sawdust bedding. About one week later four heifers were presented with watery ocular discharge and apparently blind. On examination all four heifers were affected with either unilateral or bilateral ocular lesion. Keratoconjunctivitis signs with watery ocular discharge, photophobia, varying degrees of corneal clouding, and anterior uveitis. Following the clinical report by veterinarian the four ill heifers were re-examined and conjunctival swabs, blood samples, row individual milk samples (n = 78), and corn and hay silage samples were collected. *Micrococcus* sp., *Enterococcus faecalis* were isolated from one swab and *Listeria monocytogenes* from three cases. All heifers with corneal clouding were negative on CF serological test for antibody to Listeriosis. Individual row milk samples, hay silage and corn silage were negative to *Listeria monocytogenes*. Chemical analysis of wrapped baled grass silage showed high concentration (>160 g/kg DM) of ash. *Listeria monocytogenes* were only isolated from eyes of two heifers with keratoconjunctivitis, uveitis and hypopyon symptoms. Three heifers had moderate leukocytosis. Affected heifers were confined to dark loose boxes and four days systematically treated with antibiotics (oxytetracycline) and ophthalmic ointment (garamycin). All heifers recovered within a fortnight.

On the basis of clinical findings and laboratory data we are of the opinion that ocular form of Listeriosis in heifers has been related to feeding baled grass silage and traumatic injures which occur with contaminated air with sawdust and rock wool.

16 ALTERNATIVES TO ANTIBIOTICS

16.1 EFFORTS TO REDUCE THE AMOUNTS OF ANTIBIOTICS USED IN LIVESTOCK, FOCUSED ON YOUNG FOOD PRODUCING ANIMALS (PIGS/POULTRY)

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Introduction

For more than three decades antibiotic feed additives were used, especially in young fattening animals to promote growth rate and improve feed conversion rate, but also, and this should be emphasized, to stabilize “gut health” (Kamphues 1992; Kamphues and Hebel 1999). With regard to the last point the rate of losses due to gastrointestinal disturbances, i.e. within the post weaning period in piglets or within the fattening period should be reduced. With the beginning of 2006 the use of antibiotic growth promoters is no longer allowed throughout the EU. Purpose of enacting this ban was to reduce the amounts of antibiotics used in animal production; impulses for this decision were the fear of developing bacterial resistances and of adverse effects due to interactions of feed additives and therapeutic drugs, but also the intention to diminish the risks for residues in food and for cross contamination. In non-EU-states, where antibiotics in feed are still allowed, demands for withdrawing antibiotically active substances also arise initiated by food companies, as a reaction of consumers’ expectations (Dahiya et al. 2006).

However, to reduce in-feed-antibiotics and to prescribe higher amounts of antibiotics for therapeutical purposes contradicts to the aims of this ban and to aspects of food safety. Furthermore, in the interest of human and veterinary medicine in general a prudent use of antibiotics is required (Kamphues and Hebel 1999). There is no doubt, that managing piglets and chicken without antibiotic growth promoters represents a challenge. Worldwide there are investigations in pig and poultry production to combat this new situation (Dahiya et al. 2006; Pettigrew 2006; Stein and Kil 2006). These efforts are often summarized under the title “alternatives to antibiotics”, however this phrase should be avoided for several reasons. If there are clinically observed obvious bacterial infections in herds of swine or poultry there is a need for using antibiotics, and not for so-called “alternatives”. Alternative solutions that enable a reduction of antibiotics, by improving the health status of the stock (Pettigrew 2006) have to be developed and established. According to many publications (papers, text books) enteral disturbances are the primary challenge in managing young pigs and poultry. In pigs these problems are mainly related to *E. coli* (Fairbrother et al. 2005), whereas in broilers and turkeys *Cl. perfringens* (and its toxins) is the primary threat (Dahiya et al.

2006). Therefore this contribution is focused on dietary tools that might improve the health status, or reduce the incidence and severity of these infections.

In recent years several excellent reviews focused on managing swine and poultry without using antibiotic growth promoters and without replacing them by an increased use of medicated feeds were published. In papers of Papers of Pettigrew (2006), Stein and Kil (2006) and Fairbrother et al. (2005) dietary tools and prevention strategies established in swine production are summarized. In spite, reviews of Patterson and Burkholder (2003) as well as of Gauthier (2003) or of Dahiya et al. (2006) are focused on potential strategies used in the feeding management of broilers in the post-antibiotic era, the last one with special emphasis on controlling and avoiding necrotic enteritis. In this short review it is unfeasible to cover the whole broad spectrum of tools and measures that exist (i.e. competitive exclusion-concepts, probiotics, prebiotics and enzymes). Also, newer “alternatives” like bacteriophages or nucleosides are not presented here. Therefore only some special tools were elected. All of them could act like a draft to demonstrate the complexity of the theme and the advances in knowledge, science and feed preparation resulting in manifold consequences, last but not least for veterinary practitioners. Not quoting the other tools does not mean that they are inefficient in reducing amounts of antibiotics used in food producing animals. However, own experimental experiences provide further and more profound comments on provisions presented here.

New concepts in breeding and genetics to enhance animals’ capability to resist microbial infections

All over the world efforts are made to develop new concepts in breeding and to change animals’ genetics to enhance their capability to resist specific microbial infections, respectively. In pigs, for example, the susceptibility to *E. coli* infections is related to the *E. coli* F18 receptor (Vögeli et al. 1997), which gives a chance for improving the health of pigs in the postweaning period, when oedema disease and postweaning diarrhoea are a main threat. Further in breeding of broilers and turkeys there is an increasing interest in the immune response of modern lines (Cheema et al. 2003, 2007) comparing potentially genetically based differences. Due to its relevance for food borne diseases in human beings, genetic resistance to *Salmonella* infections is of increasing interest (Girard-Santosuosso et al. 2002).

Optimizing the housing and further exogenous factors/ conditions including management

Madec (2005) emphasized that hygiene, management concepts and provisions play an important role in diarrhoea occurring within the postweaning period of pigs (i.e.: number of pigs per pen; stress-induced shedding of ETEC when weaned piglets are mixed). Furthermore it is necessary to avoid faults in diets’ composition (like oversupply with minerals, protein or demixing processes, lacks in hygienic quality of

feeds a.s.o.) that predispose especially young animals for gastrointestinal disturbances, not seldom followed by bacterial infections (Kamphues 2002). In general there is a higher risk for disturbances within the alimentary tract of younger animals when feeding intensity is increased (i.e. due to impaired acidification of stomach content when feed intake increases; Kamphues 1992).

Increased use of non-antibiotic feed additives

Organic acids and probiotics

For more than twenty years several organic acids have been widely used in rearing of piglets, but also in raising and fattening of broilers and turkeys. Without any doubt, organic acids have preserving effects on feedstuffs, i.e. within the diet. Furthermore, these acids foster the acidification of the gastric content and diminish the counts of different bacteria in the cranial part of the gastrointestinal tract (Kulla 2001; Papenbrock 2004). Recapitulating, the use of these additives results in improved performance (Kirchgessner and Roth 1988; Freitag et al. 1998) and besides, there are various studies pointing out the prophylactic effects against some pathogens (i.e. *E. coli*; Kirchgessner et al. 1992; Franco et al. 2005).

Current analyses of published data (in particular done by meta-analysis) support the beneficial effects of organic acids regarding the mentioned aspects (see review of Pettigrew 2006). Therefore, it should be asked, which aspects and ideas are new in this context regarding specific enteral disorders. In particular the following key aspects have to be mentioned:

- 1) enhancement of the gastric barrier function [especially tested under terms of experimental infections (e.g. *E. coli* and *S. Derby*)]
- 2) impact of volatile fatty acid concentrations in the chyme on the regulation of the etabolism of intestinal microorganisms
- 3) use of medium chain fatty acids to prevent specific enteral disorders

In recently performed studies (Hassan 2007; Neu 2007) a pelleted diet was supplemented with 0.9 % of a mixture of formic and propionic acid (fpa; 75/25) and 1.2 % of potassium diformate (pdf), respectively. Each piglet received a single dose of 1.5×10^9 - 3.3×10^{10} cfu *S. Derby* or 7.8×10^9 - 7.1×10^{11} cfu haemolytic *E. coli* included in the diet. Four to five hours later the animals were sacrificed and chyme was obtained from various localisations of the alimentary tract. In these samples the counts of *S. Derby* or *E. coli* were determined (Tab. 1).

Table 1: Counts of *S. Derby*/*E. coli* in the chyme of different locations in the gastrointestinal tract of reared piglets after oral infection (Hassan 2007; Neu 2007)

	piglets (n)	10	10	10	7	8	7
additives	fpa	-	+	-	-	+	-
	pdf	-	-	+	-	-	+
	orally applied:	<i>S. Derby</i>			<i>E. coli</i>		
cfu/g chyme	stomach	1.1x10 ⁶	1.4x10 ⁵	6.2x10 ⁴	3.8x10 ⁷	9.3x10 ⁵	4.1x10 ⁵
	small intestine	1.5x10 ⁷	6.4x10 ⁵	7.5x10 ⁴	7.4x10 ⁸	7.9x10 ⁸	1.2x10 ⁹
	caecum	1.2x10 ⁷	1.6x10 ⁶	9.1x10 ⁵	1.8x10 ⁹	6.1x10 ⁸	3.7x10 ⁸

The results indicate that both added substances reduced markedly the counts of applied bacteria in stomach content, but there was also a tendency for reduced counts in the intestine. However, this effect depends on the type of bacteria. Especially some species of gram-negative bacteria are susceptible to higher concentrations of formic acid or formiate in the surrounding substrate (i.e. chyme). Adding 1.2 % potassium diformate to a coarse diet reduced significantly counts of Salmonella in stomach and small intestine contents of piglets that were orally infected 4 – 6 hours before (Kamphues et al. 2007).

New insights regarding effects of short chain fatty acids concentrations in the chyme on the regulation of the metabolism in intestinal microorganisms, especially of those with pathogenic nature, are of special interest. In the past, the mode of action (concerning counts and activity of pathogenic microorganisms) of organic acids was referred to effects of the pH and the anion, respectively. However, scientists recently become more and more aware of the fact, that short chain fatty acids also act on the molecular level, meaning, that specific concentrations of short chain fatty acids trigger definite reactions of bacteria (Lawhon et al. 2002; Gantois et al. 2006; Van Immerseel et al. 2006). The potential to influence the regulation mechanisms of bacteria on the molecular level might give an explanation for unforeseeable observations, i.e. made for specific dietetic concepts (feed structure).

When coarsely ground diets were offered to pigs higher amounts of starch entered the large intestine and forced the concentration of butyric acid deriving from the fermentation of the starch was altered. In a field study (Visscher 2006) an enhanced particle size of the diet resulted in higher amounts of starch in the caecal content of

slaughtered pigs. Also the concentrations of butyrate at this location were higher. At the same time the *Salmonella* prevalence was reduced markedly.

Regulatory acting concentrations of certain volatile fatty acids in the cranial part of the gastrointestinal tract might be reached through supplementation of these acids to the diet. In the large intestine, the host's own intestinal microflora has the ability to produce these acids if an adequate substrate is available (Amtsberg 1984). In the future further research activities on these regulatory mechanisms might give explanations for advantageous or disadvantageous effects of specific feeding practices. Up to now, it is unknown why i.e. a diet based on cooked rice and meat (Pluske et al. 1998) protects against infection with Brachyspirae. So far the circumstances leading to the production of α -Toxin by *Cl. perfringens* in poultry are also not known.

These observations really point out that the development of dietetic concepts has to be based on the knowledge of metabolism regulation mechanisms (namely on molecular level) of the combating microorganisms. Therefore it is impossibility to create a single dietetic concept that would be effective against numerous pathogens ("golden bullet"). Looking ahead, dietetic concepts will not only be focused on gut health but instead on certain pathogens in the gastrointestinal tract. Like for the therapeutical use of antibiotics, a definite diagnosis of the pathogenic agent also in this case is a "conditio sine qua non".

In recent years, the interest in medium chain fatty acids, consisting of six to twelve carbon atoms (C₆: caproic acid, C₈: caprylic acid, C₁₀: capric acid, C₁₂: lauric acid) increased (Nakai and Siebert 2002; Van Immerseel et al. 2004; Mahu et al. 2006). In particular with regard to their antibacterial activity, it is very likely that these medium chain acids are superior to short chain fatty acids. When performing in vitro studies, caprylic and caproic acid appeared to be very effective against bacteria like *Salmonella* (Mahu et al. 2006). Especially with regard to exploding numbers of *Cl. perfringens* infections (primarily causing necrotic enteritis in poultry), the use of medium chain fatty acids (or triglycerides containing a corresponding medium chain fatty acid, in combination with its proper lipase) might be of interest (Decuypere and Dierick 2003; Skřivanová et al. 2005). In the following table information gathered regarding inhibitory effects of various fatty acids against *Cl. perfringens*.

Table 2: Inhibitory concentrations of C2 - C18 fatty acids* against *Clostridium perfringens* strains CCM 4435T and CNCTC 5459 grown on glucose (Skřivanová et al. 2005)

Fatty acid	CCM 4435 ^T	CNCTC 5459
C ₂ , C ₃ , C ₄ [#] , C ₅ [#] , C ₆	>5	>5
C ₈	1.92 ± 0.10	0.68 ± 0.06
C ₁₀	0.28 ± 0.05	0.27 ± 0.06
C ₁₂	0.04 ± 0.01	0.04 ± 0.01
C ₁₄	0.08 ± 0.02	0.18 ± 0.01
C ₁₆ , C ₁₈	>5	>5
C _{18:1}	0.48 ± 0.01	1.48 ± 0.05
C _{18:2}	>5	0.48 ± 0.30

* IC₅₀, fatty acid concentration (mg ml⁻¹) at which 50 % of the initial glucose was utilized within a 1-day incubation. Mean values of the three measurements ± SD

including branched isomers

Phylogenous feed additives, extracts of herbs and spice preparations

In the last decade lots of experiments were performed to test the efficacy of phylogenous feed additives, especially after the ban of antibiotic feed additives. The most important advantage of this “alternative” group is a high “social acceptance”. These products are claimed to enhance performance, to stabilize the intestinal flora and to improve the immunity of young animals (Stein und Kil 2006). Westendarp (2006) published a review on the use and effects of phytogenic feed additives in poultry. Most of the cited studies were performed with broilers, their results are quite different and not seldom contradictory. Amazingly high improvements of performance as well as negative effects (sometimes due to reduced feed intake) were observed. Furthermore, phytogenic substances in feeds of broilers are reported to reduce pathogenous bacteria in the intestine. There are various reports in literature regarding antibacterial effects of *Oreganum vulgare*, *Piper nigrum* or *Thymus vulgaris*. Especially essential oil components like thymol, carvacrol or cumicin should have inhibiting effects on Clostridia and *E. coli* as well as on Salmonella, Listeria and Yersinia (Dahiya et al. 2006). There are some published studies indicating antibacterial effects, but most of the results were achieved by in-vitro-studies.

Kamphues et al. (2001) performed various investigations on the potential effects of oreganum extracts (oil) on protein digestibility and the intestinal flora of reared piglets, but also on piglets' health experimentally infected with haemolysing *E. coli* (F18+). Digestibility of the diet, nitrogen balance, feed conversion rate as well as concentrations of lactate, volatile fatty acids and ammonia within intestinal contents were not influenced significantly. Counts of *E. coli*, Streptococci and Lactobacilli in intestinal contents did not respond in any way to the feed additive in the diet (2 g of the product per kg diet; the product contained 5 % of oreganum oil, within 81 % carvacrol). However, in studies, where reared piglets were exposed to haemolysing strains of *E. coli* orally, the number of animals developing clinical symptoms was

reduced (7 of 15 in the control group; 3 of 15 in the group fed the oreganum containing diet). Also, when oreganum extracts were added to diets of broilers in order to prevent necrotic enteritis (due to *Cl. perfringens* and its toxins) contradictory results were observed (Dahiya et al. 2006). Therefore, it is highly speculative to comment any perspectives in the future of these feed additives. Nevertheless, especially under aspects of feed safety and transparency in the food producing chain there is a need to exactly define the constituents of the products used as additives. Furthermore the fate of these substances in the alimentary tract has to be investigated (are they digested, metabolized, absorbed?) to avoid any discussion on potential residues in the food. All phyto-genous products of interest have to be tested like other feed additives.

Physical form of the diet

In former times grinding intensity of ingredients, i.e. particle size of the diet was considered (Kirchgessner et al. 1985) under the aspects of digestibility (nutritive value), localization (small vs. large intestine) and way of digestion (auto-enzymes of the animal vs. enzymes of microorganisms). In order to improve the digestion rate in the small intestine in general, a high grinding intensity was recommended combined with the need for pelleting the diet. However, increasing the grinding intensity and feeding pellets, increased the rate and score of gastric ulcers (Amory et al. 2006). Therefore, it was recommended and accepted to set up limits for the grinding intensity of diets for pigs, by the occurrence of gastric alterations.

During the last decade it became more and more evident that the particle size in a pig's diet was related to the prevalence of *Salmonella* and further pathogens (lowering grinding intensity results in reduced prevalence). Therefore, there is a need to re-evaluate the question of grinding intensity under the aspects of gut health and enteral infections. Combining a reduced grinding intensity and adding potassium diformate (1.2 % in the coarse diet) lowered significantly the rate and duration of *Salmonella* excretion in reared piglets that were orally infected with *S. Derby* (Kamphues et al. 2005).

About ten years before, Brunsgaard (1998) published an interesting and thought-provoking paper on experiments in pigs fed coarse or fine diets based on wheat or barley. His work focused on morphological characteristics, epithelial cell proliferation and lectin binding patterns in the large intestine. Independent of the type of cereal Brunsgaard (1998) found marked effects of the diets: In pigs fed the coarse diets larger crypts, a higher epithelial cell proliferation (mitoses), as well as differences in the amount and type of mucins were observed (more sialic acid α -2,3 neuraminic acid, less mannose in pigs on coarse diets). So finally, the conclusion of the author was "that pigs fed a coarse diet are better protected against intestinal infections than pigs fed a fine diet".

Comparing the fine wheat diet with the coarse barley diet there were also differences concerning mannose residues on the intestinal surface in the hind gut of pigs. In

chickens those findings were correlated to a higher adhesion rate of Salmonella in the intestinal tract (Baba et al. 1993).

Hedemann et al. (2005) continued to investigate the impact of feed particle size and feed processing (pelleted vs. non pelleted diet) on pigs' intestinal tract including the small intestine. The most interesting findings in the distal small intestine were a greater production and secretion of mucus in pigs fed the pelleted diets (independent of the diets' grinding intensity). In a cell culture model the changed mucus production in the distal small intestine was accompanied by a higher incidence of Salmonella when pelleted diets were fed to pigs. Recent studies of Liu et al. (2006) in broilers were focused on the effects of particle size and physical form of the diet (mash vs. pellet) on the epithelial layer of the small intestine of chickens. Here the density of mast cells, the concentration of histamine as well as the concentration of stem cells were estimated. The distribution (localization) of mast cells and their counts were markedly influenced by the physical form of the diet (lower numbers in animals fed coarsely ground diets). Also the histamine content was markedly lower when chicken were fed the coarsely ground diet. The authors referred the effects on the number of mast cells and the histamine content in the small intestine to the regulation of the stem cell factor concentration (= growth factor of mast cells) depending on the particle size and diet form. These findings are of special interest because mast cell numbers are correlated to the production and release of active modifiers that regulate the movement of intestinal smooth muscle (forced and also reduced gut motility interfere with clinically obvious symptoms like diarrhea and also constipation, a problem well known in feeding of sows). Warzecha et al. (2006) found marked changes in feces quality of pregnant sows fed diets that differed in particle size only (due to different sieves in the hammer mill). Reducing grinding intensity resulted in lower values of hardness and dry matter in the feces in spite of an identical crude fibre content.

Oral application of antibodies produced in animals (serum, colostrum, eggs) or in genetically modified organisms (bacteria, yeasts, plants)

Uptake of colostrum leads to a passive immunization in several neonatal mammals. By this mechanism the neonate gets passive immunity while its own immune response is not fully developed and therefore its self-active, specific, humoral and cellular immunity must be considered as insufficient (Stefaniak, 2006). Even after permeability of the intestinal epithelium for antibodies as well as immunoglobulins is lost, antibodies deriving from mature milk are still important for immunity of the young animal. Although concentration of antibodies in milk decreases distinctly with time after parturition it contains a remarkable content of antibodies. These antibodies act locally in the gut of the youngs and cause a local immunity. Against this background, the aim of oral application of antibodies to young animals – that is performed since decades - becomes clear (Berghman et al. 2005). Oral application of antibodies is performed especially in phases in which the animals are at greatest risk to sicken due to infection with infectious pathogens of the gut (first days of life and in the postweaning period).

The mode of action of the different pathogens differs (some are noxious for the epithelium while other pathogens causes severe diarrhea by secretion of toxins without any structural changes on gut epithelium). If antibodies are present in the lumen of the gut the pathogen is neutralized by specific binding. Due to this binding the mode of action of the pathogen is inhibited by neutralization, immobilization or agglutination which makes it impossible for the pathogen to bind to the gut epithelium. By this the negative effects of pathogens (inflammation, alteration, damage of epithelium, proliferation, production of toxins) are minimized or reduced.

Concerning the origin of the antibodies for oral application a differentiation on origin seems to be expedient (Stefaniak, 2006): allogenic antibodies (obtained from the same species / pertaining to antigenically distinct animals of the same species) and xenogeneic (antibodies produced in other species). Most published studies concerning oral application of antibodies deal with antibodies originating of the following origin:

- colostrum or milk of cattle
- plasma or serum of pigs
- eggs or yolk of immunised hens

Stefaniak (2006) gave a detailed characterization of the immunoglobulins of the class IgG (resp. IgY from eggs) that occur in these animal products. There are notable differences within the different IgG of the IgG-class (i.e. regarding sensibility of inactivation, proteolytic digestion or increasing temperature). If oral application of antibodies is intended in the field conditioning, preparation, processing of antibodies must be done carefully. Furthermore, it is important, that the products are stable, easy to store and handle and that the preparing of the antibodies is not too expensive (Berghman et al. 2005). Most antibody preparations contain freeze-dried immunoglobulins which are applicated solely or together with the feed to the animal.

Conventionally spray-dried porcine plasma (8 % in the diet) failed to protect reared piglets that were infected orally with pathogenic *E. coli* (van Dijk et al. 2002), but led to improvements of feed intake, daily gains and faecal condition scores. Pettigrew (2006) reviewed experiments on the use of spray-dried porcine plasma in reared piglets and concluded that there are marked effects on performance in general and that there is some evidence for protection against *E. coli* related diseases. Also immune egg products were used in young animals of different species to protect them against viral and bacterial infections (i.e. against *E. coli* in piglets or in chicken against *Cl. perfringens*; Pettigrew 2006; Dahiya et al. 2006). There is good evidence that egg antibodies could protect young animals, but production and preparation of those products are quite expensive.

With recent advances in genetic engineering a new way of antibody production gained worldwide great attention and increasing interest: By using genetically modified organisms production of designed antibody proteins became possible. Production of antibodies (IgG) in transgenic plants was first described in 1989 (Hiatt et al.). For this

antibody production no immunised animals are needed after genetically modified plants are established and large quantities of “plantibodies” can be produced much cheaper than in animals. Stoger et al. (2002) compared production levels in transgenic crops (rice, wheat, pea and tomato) as well as production levels in different tissues of the plants. Hagemann (2006) tested the effect of antibodies produced in yeasts and peas that are directed against F4 of ETEC in reared piglets challenged by an experimental infection.

Up to now efficiency of plantibodies can not be evaluated finally. Available studies (especially after experimental infection) gave contradictory results. There is need for further investigations on stability of the plantibodies in the alimentary tract. In vitro experiments showed that a low pH (important factor for passing the stomach) did not effect stability of antibodies produced in peas (against F4 of ETEC) as much as suggested (only slightly reduced activity), while incubation with native chyme of small intestine from reared piglets led to a very strong decrease in activity (antibodies could not be detected using ELISA technique, Hagemann, 2006). If suggesting an oral uptake of pathogens in the piglets binding of the antibody to the pathogen in the stomach might be a possible way to prevent infection of piglets by ETEC due to neutralisation of ETEC in the stomach (Möbeler et al. 2007). If adherence of ETEC at the host cells is avoided through bound antibodies the pathogenic ETEC could not colonize the gut and an infection would be prevented.

Further investigations are needed but one critical point should be mentioned: even though transgenic antibody producing plants have been established and great amounts of plantibodies could be produced theoretically using these plants, the amounts of plantibodies produced in plants are still limited, because of distinct restrictions of cultivation of these plants (“social acceptance” of GMO?).

Production of antigens in plants for oral vaccination or anti-colonisation

Development of plant biotechnology allows also production of antigens in plants (Mason et al. 1992). Using this method there is no risk of contamination from potential human pathogens. Additionally no expensive process of purifying the recombinant antigen-protein is needed if the plant tissue is used as a carrier (oral uptake of the whole plant or parts of it). In the last decade at least 45 antigens have been produced in plants (Arntzen 2005). Huang et al. (2003) showed that FaeG produced in plants could trigger a specific serum antibody response in mice. Anti-colonisation is another way on which antigens can act against infections: by binding of FaeG-protein on the receptor the binding of the F4 ETEC to this receptor is prevented (Joensuu et al. 2004). In 2006 Joensuu et al. used Alfalfa for production of F4 (K88) fimbrial adhesion FaeG. Intra-gastrical immunization of piglets using this FaeG caused a weak F4-specific humoral response which was increased by co-administration of adjuvants.

Conclusion

The key challenge of intensive animal production is to maintain the health status of the young animals, and not their performance as it used to be in former times. It has to be avoided that the amounts of antibiotics that were used as feed additives in earlier decades, are now applicated as medicated feeds. Non-antibiotic dietary tools are necessary to improve the health status and to avoid intestinal disorders, especially due to infections with *E. coli* in piglets and with *Cl. perfringens* in poultry (broilers, turkeys).

Further bacterial infections seem to increase after withdrawal of in-feed antibiotics. The development of efficient dietary strategies to control these enteral infections and disorders depends on the understanding of interactions between the diet, the animal, its intestinal flora and the pathogenous bacteria. Effective non-antibiotic prevention of disorders and reduced performance will only be attained by means of multidisciplinary research efforts. A diet is not only an energy and nutrient containing substrate, it has a marked impact on the gut wall, the intestinal flora, the reaction of the animal and on the survival of exogenous bacteria as well. This complexity was demonstrated as an example by describing effects of the physical form of the diet (its particle size). The production of volatile fatty acids in the intestine exerts regulatory effects on resident as well as exogenous bacteria that enter the alimentary tract (butyric acid against salmonella).

In the future ingredients of a diet for food producing animals may be used for active immunization against specific bacteria (as demonstrated by recombinant plants that express special proteins of *E. coli* fimbrial antigens) or as a source of antibodies (“plantibodies”) against infectious agents (i.e. antibodies against F4 + *E. coli*). Last but not least animal derived protein (like egg powder, dried blood protein products) earn special attention not only as a source of essential amino acids but also as a source of immunologically active constituents. This could be opposed to existing legislative efforts (intra species feeding?) in the EU, which should be re-evaluated critically (conflicting aims). It seems that the post-antibiotic era could develop to an era of dietetics, meaning that diets for special purposes have to be created for animals suffering from special diseases or confronted with special pathogens (to avoid infections).

Finally, it should be emphasized that a distinct dietary tool, effective and advantageous in one direction could result in drawbacks concerning another pathogen or disturbance. Eventually new parameters are necessary to characterize feeding concepts or dietary tools concerning their impact on the health status of food producing animals (amounts of prescribed antibiotics per kg of carcass or edible food). That could help to characterize the quality of the whole food production chain including management, feeding, housing and further environmental influences.

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16.2 THE INHIBITORY EFFECT OF PROBIOTIC E. COLI NISSLE 1917 ON EPEC INFECTION IN A PORCINE IN VITRO MODEL IS MEDIATED BY ITS ADHERENCE VIA F1C FIMBRIA

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The probiotic *E. coli* strain Nissle 1917 (EcN; O6:H1:K5) is thought to be effective in the treatment of intestinal disorders caused by various pathogenic bacteria. Recently, *in vitro* studies described strong inhibitory effects of EcN on adhesion and/or invasion of intestinal pathogenic bacteria. In this study we determined the ability of EcN to influence host cell infection by enteropathogenic *E. coli* (EPEC), an important diarrheogenic pathogen in humans and animals, in an *in vitro* porcine intestinal epithelial cell model (IPEC-J2). We focused our attention on a detailed phenotypic description of alterations in single steps of EPEC infection such as adherence and the formation of micro-colonies, as well as attaching and effacing lesions. Pre-incubation of IPEC-J2 with EcN was found to drastically reduce the infection efficiency of EPEC in a concentration dependent manner, suggesting EcN interferes with early interactions of EPEC with host cells. While attachment and formation of micro-colonies was reduced, adherent bacteria still formed attaching and effacing lesions. Further studies revealed that EcN adherence to porcine epithelial cells is largely mediated via F1C fimbria. This adhesion mediates the probiotic effect since a non-adherent EcN Δ foc mutant did not reduce EPEC infection. A commensal *E. coli* wild type strain lacking F1C fimbriae and not showing strong adherence or a probiotic effect showed strong adhesion and an inhibitory effect on EPEC infection after introduction of a complementing plasmid harbouring the foc gene cluster. In addition to F1C fimbriae, we showed that EcN flagellae are also involved in the adhesion process. The H1 flagellae of EcN forms a bacterial network on the host cell surface. A non-flagellated EcN Δ fliA mutant showed reduced adhesion to the host cell surface and a reduced capacity to inhibit EPEC infection.

In conclusion, the inhibitory effect of EcN on the infection of porcine intestinal epithelial cells with EPEC is mediated by its adherence via F1C fimbriae and H1 flagellae.

16.3 EFFECT OF PROBIOTIC (*SACCHAROMYCES CEREVISIAE* CNCM I-1079) ON BLOOD PARAMETERS, GROWTH AND HEALTH OF NEONATAL HOLSTEIN CALVES

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Probiotics are used to control or maintain a constant state of intestinal bacteria. When the bacterial populations are altered by stress or antibiotic therapy, animal health and performance may decline. This experiment was design to study the effects of feeding a Probiotic (*Saccharomyces cerevisiae* CNCM I-1079) on growth and health of neonatal holstein calves. Forty -five neonatal Holstein calves were selected from one dairy farm of Mashhad suburb. After birth, calves were randomly assigned to three groups. Calves in group I (control group) received no probiotic. Calves in group II (experiment I group) was given probiotic at 1gr/day with colostrums and then with milk during the first 2 weeks of life. Calves in group III (experiment II group) was given probiotic at 1gr/day with colostrums and then with milk during the first 3 weeks of life. At the third week, milk was replaced with milk replacer. The body weight and skeletal growth (Heart girth, body length and weithers height) were measured at birth and repeated every week until 5 weeks of age. Blood samples were collected via jugular vein puncture at the time of weighing and analyzed for hematological indices , plasma total protein concentration and plasma fibrinogen concentration. Fecal scores (fluidity) were monitored and evaluated. Daily health was also recorded. Data were analyzed by using ANOVA, Kruskal-Walis and Chi-square procedure with SPSS 13. There was not significant differences between groups for hematological indices , plasma total protein concentration and plasma fibrinogen concentration, skeletal growth average daily gain, the incidence of neonatal diarrhea and the days of the treatment; however, fecal scors at third weeks of age between groups I ($1/8 \pm 0/26$) and III ($1/07 \pm 0/07$) was significantly different. The results of this study indicated that under the circumstance of this investigation, Probiotic (*Saccharomyces cerevisiae* CNCM I-1079) was not effective in improving calf performance and health but under nutritional stress condition was effective in maintaining fecal score.

16.4 EFFECTS OF ORAL APPLICATION OF ANTIBODIES OF MICROBIAL OR VEGETABLE ORIGIN (PRODUCED IN YEAST AND PEAS) DIRECTED AGAINST F4 OF ENTEROTOXIC E. COLI (ETEC) IN EXPERIMENTALLY INFECTED WEANED PIGLETS

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Problem: In weaned piglets infections with enterotoxigenic *Escherichia coli* (ETEC) are a main cause of diarrhoea and one of the most important reasons for piglet losses. Alternatives to antibiotic treatment of this disease are of major interest because of the great economic importance and according to the problems of resistance of bacteria as well as food safety. Aim of this study was to test the effect of orally applied antibodies (directed against the F4 fimbriae of ETEC) in experimentally infected piglets. Antibodies used in this trial were produced by transgenic yeasts and plants (peas).

Material and methods: The study was performed in weaned piglets (n=64). Most of them (n=48) were experimentally infected orally with ETEC via a bouillon containing about 10¹⁰ cfu per piglet. Short chain fragment variable antibodies (ab) were given to the animals via top dressing to the diet which was offered three times a day. In a further trial peas containing the ab were included into the diet as a feed component (10 %) and this diet was offered ad libitum. Parameters used to characterise diarrhoea were: dry matter (dm) content of feces, fecal excretion of ETEC, sodium and chloride content in feces and clinical symptoms. Additionally some piglets were sacrificed to collect digesta samples of different parts of the gastrointestinal tract. Detection of antibodies was done using ELISA and Western blot technique.

Results: Experimental infection was successful and manifested in decline of dry matter content of feces and increase of sodium and chloride in feces. The ETEC used for experimental infection was detected via rectal swap at least once in 47 of 48 exp. inf. piglets. Fecal expulsion of the applied ETEC correlated significantly with weakness (p=0.0003), consistency of feces (p>0.0001), decrease of dm-content (p<0.0001) and increased concentrations of sodium and chloride in feces (p<0.0001). There was no positive effect of ab application on intensity or duration of diarrhoea after sole application of ab as top dressing. In piglets receiving ab continuously via transgenic peas as feed component an infection could not be avoided but lower intensity of diarrhoea was observed.

Conclusions: Further research on transgenic peas as an ingredient or feed component seems reasonable but today is limited due to the lack of adequate amounts of such peas. Addition of so called plantibodies might be an interesting alternative for prophylaxis of infectious diseases in weaned piglets and could be useful to minimize amounts of antibiotics used in pig production. Compared to other sources of antibodies plant produced ab are much cheaper to produce and much easier to apply to the animals.

16.5 IGY TECHNOLOGIES - ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTING FACTORS

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After weaning, the gastrointestinal tract of piglets is colonised by all microorganisms present in the new environment. This postweaning period of one to two weeks is manifested by decreased performance and by outbreaks of diarrhoea that may be lethal. It is just this period when the effects of growth promoters, or preventive feed medication, are most marked. The using of IgY technologies could be alternative. We tested the use of yolk antibodies for the control of the intestinal microbial population of clinically normal piglets. The experiments were conducted using the dried egg mass containing egg yolks antibodies to porcine rotavirus, E.coli, and other agents. In the experiment, the monitoring of performance was completed by investigation of effects of yolk antibodies on the digestibility of nutrients. Faeces were collected from the floor of pens, digestibility was calculated by conversion to hydrochloric acid-insoluble ash. 20 piglets of two clinically normal litters were pooled and allotted to two groups of six animals to provide equal starting conditions for both. All piglets were fed the commercial feed mixture and those of the experimental group received egg yolk antibodies 3 g per animal per day from day 1 to day 7. Rectal swabs were collected and examined for the presence of haemolytic E. coli. The results of the experiment have confirmed higher weight gains and more effective feed utilisation in piglets treated with egg yolk antibodies. The highest difference was recorded at the end of the 1st week, when the weight gain in the experimental group was 167 and in control group 131 g per day. Mean daily weight gains increased during the subsequent weeks to 388 g in controls and 420 g in the experimental group at the end of experiment. Better feed conversion ratio in the experimental group 1.715 kg (control 2.101 kg), is supported also by digestibility data - the experimental group showed higher digestibility of crude protein (79.53 %, control 76.58%), crude fibre (64.34 %, control 61.64 %), and crude fat (56.43 %, control 54.01 %). Bacteriological examination demonstrated haemolytic E. coli at weaning in two piglets of each group.

The findings were negative in all piglets of the experimental group at post-weaning day 5 already, while one piglet of the control group was positive still on day 14. Higher weight gains and more effective feed utilisation were observed in the piglets not only during the period of supplementation, but also in the subsequent period when both the experimental and the control group were feed identical diets. This effect can be explained by the fact that the change in the composition of the intestinal microbial population runs rather chaotically under uncontrolled conditions. Supported by NAZV 1G 46085.

16.6 THE EFFICACY OF PROBIOTIC ADMINISTRATION TO SOWS FOR THE BENEFITS IN SUCKLING PIGLETS

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Primary goal of the work was to evaluate the efficacy of probiotic administration in sows for the benefits in suckling piglets. There were 2 x 16 hybrid sows included into the trial. The sows included in the trial reflected the composition of parity number seen in conventional production sow herds. Control and experimental groups were balanced according to the sows parity number. Both groups had the same genetic background. The trial started about 2 weeks before farrowing and lasted until weaning, at the age 28 days after farrowing. Control group (n=16) was fed with standard feed for lactating sows (12.76 MJ DE). Experimental group (n=16) was fed with control feed (same as that of the control group) with 400 g BioPlus 2B/t feed, which equals 1.28×10^6 CFU/g feed. BioPlus 2B (Chr. Hansen A/S, Hoersholm, Denmark) is probiotic preparation containing equal amount *Bacillus licheniformis* and *Bacillus subtilis* at the total concentration 3.2×10^9 CFU per gram of preparation. Blood samples were collected from all sows on day 1 and day 15 of lactation. Parameters, which were measured from blood serum: urea, total proteins, albumin, cholesterol, total lipid level, haemoglobin. During the experiment parameters concerning piglets were investigated: number born alive, number stillborn, number weaned (about 4 weeks), individual weight at birth, 14th day, at weaning. The increase of total serum proteins was higher in experimental group, though not significantly. The albumin level was relatively uniform during the experiment and did not deflect from the reference range in both groups. Serum urea level increased in both groups on day 15 in comparison with day 1. Level of urea in blood ranged from 3.66 to 4.64 mmol.l⁻¹. No significant differences were observed between groups in individual sampling. Level of cholesterol in blood of experimental group increased. On the contrast, cholesterol level in control group was constant in both sampling. As a result, significant higher cholesterol level was found in experimental group compared to control group on day 15 (P=0.038). Similar to cholesterol, total lipids in blood of experimental group was significantly higher in comparison to control group on day 15 (P=0.007). Significant higher value of haemoglobin in experimental group was observed in comparison with control group on day 15 (P < 0.05). No significant differences were revealed in number of piglets born alive, stillborn and number of weaned pigs between groups of sows. The suckling piglets in experimental group reached better weight already on day 14 of their lives and this state persisted up to the end of the experiment. The differences in weight of the experimental group and control group were highly significant at the end of the trial (P=0.002).

16.7 THE EFFECT OF DIETARY MANNAN ON THE OPIOID AND SOMATOTROPIC SYSTEMS ACTIVITY IN GROWING LAMBS

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Introduction: Alternative to antibiotics are natural factors like mannan oligosaccharide components (MOS), derived from cell wall of yeast (*Saccharomyces cerevisiae*). Many investigators reported that supplementation of animals diet with MOS inhibited the pathogens, prevented attachment and colonization of enteric bacteria to the intestinal wall and stimulated the activity of the immune system. It is known that immune system acts via other systems such as: opioids, nervous and endocrine. Growth hormone (GH) is secreted throughout life, and is implicated as an important factor in the aging process. The release of GH is stimulated by the hypothalamic GH releasing hormone and by ghrelin. Recent results suggest that ghrelin plays an important role in energy homeostasis, regulation of immune system, body weight and food intake. On the other hand, an opioid peptide, Met-enkephalin and its synthetic analogs also stimulate the release of GH through the same receptor as ghrelin. **Aim:** Thus, the present study was undertaken to investigate the effect of mannan oligosaccharides, strong immunological stimulators, on the plasma levels of Met-enkephalin, ghrelin and GH in growing lambs.

Materials and methods: The experiment was carried out on the female lambs between 30 and 60 days of life. Animals were divided into standard (fed with standard diet) and experimental group (fed with standard diet plus 0.2% of mannan components). Blood from the jugular vein was taken on days 1, 14 and 30 of experiment. Plasma levels of ghrelin and GH were estimated by radioimmunological method using commercial kits. Met-enkephalin was measured by RIA using own method (Pierzchala and Van Loon, 1990).

Results: Met-enkephalin plasma level in standard fed animals was significantly decreasing with age ($P<0.05$). The mannan treatment significantly lowered the plasma level of endogenous opioid on day 14 of experiment ($P<0.01$). Dietary mannan modulated the plasma level of ghrelin throughout the experiment ($P<0.01$). Unexpectedly, the effect of mannan on the growth hormone plasma level was observed as early as after 14 days of treatment ($P<0.01$). Unpublished results showed that parallel with the changes of opioid peptide level, mannan affected the white blood cells amount and the ratio of in vitro lymphocytes proliferation

Conclusion: The results suggest that mannan oligosaccharide components may be considered as a strong modulator of immune system by interfering with opioid and endocrine systems in growing lambs. **Acknowledgments:** Grant from Ministry of Science and Higher Education No. 0763/P01/2006/30.

16.8 EVALUATION OF A TREATMENT OF DAIRY COWS WITH RETAINED PLACENTA WITH PROTEOLYTIC ENZYMES.

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Veterinary products containing proteolytic enzymes are approved for the treatment of mastitis in dairy cows. Proteolytic enzymes have also been tested for the treatment of chronic endometritis.

The objective of the study was to evaluate, if the treatment of cows with retained fetal membranes (RFM) with proteolytic enzymes decreases the proportion of cows experiencing fever as a sign for acute metritis. This could reduce the amount of antibiotics used for the treatment of acute metritis. Furthermore, effects of the treatment with proteolytic enzymes on reproductive performance were analyzed in the current lactation. The application of proteolytic enzymes was tested in addition to two different strategies for the management of RFM. The field trial was conducted on 40 commercial dairy farms in Germany. Cows that retained their fetal membranes for >12 h postpartum were assigned in alternating order to one of four treatments. Because of concerns of the owners, it was not possible to evaluate the treatment with enzymes as exclusive treatment or to include untreated controls. Strategy CEF (n=54) was based on the systemic application of Ceftiofur (1 mg/ kg BW, 3 to 5 d) only if cows showed fever ($\geq 39.5^{\circ}\text{C}$). In group CEF+E (n=63) all cows received proteolytic enzymes (4800 FIP-E of Chymotrypsin, 480 FIP-E of Trypsin, 60 FIP-E of Papain/ 100 kg BW, i.m.) on 3 consecutive days, beginning at the day of enrollment. Cows with fever received Ceftiofur as described for CEF. In groups CEF and CEF+E no attempts were made to remove the fetal membranes manually and no intrauterine treatments were performed. Strategy IUT (n=74) was based on the manual removal of the fetal membranes and an intrauterine treatment with 6000 mg of Tetracycline for 3 consecutive days. In case of fever cows received 6000 mg of Amoxicillin (i.m., 3 to 5 d). In group IUT+E (n=73) all cows received proteolytic enzymes as described for CEF+E in addition to strategy IUT. All cows were examined for signs of chronic endometritis 28 to 34 d postpartum and treated with 500 μg of Cloprostenol. Proportion of cows with fever as a sign for acute metritis did not differ between CEF and CEF+E (72.2 vs 78.0%) and IUT and IUT+E (17.6 vs. 24.7%). Prevalence of chronic endometritis did not differ between CEF and CEF+E (30.0 vs 37.7) and between IUT and IUT+E (32.4 vs 42.3%). No significant differences were found in reproductive performance parameters (days to first service, days open, conception rate) between the groups.

In conclusion, this study failed to show any beneficial effect of the additional treatment with enzymes in the dosage used in this trial in both strategies CEF and IUT, respectively. Further research is required to evaluate the effects of proteolytic enzymes in untreated cows with RFM.

16.9 EFFECT OF A SPECIFIC BLEND OF PLANT EXTRACTS (CINNAMALDEHYDE, CARVACROL AND CAPSICUM SPP) ON ACID-BASE AND SERUM L-LACTATE IN FINISHING BULL CALVES.

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Introduction: Natural plant extracts have been proposed as an alternative to ionophore antibiotics as a dietary supplement for reducing ruminal disturbances in feedlot cattle. **Aim:** This study investigated effects of a blend of plant extracts (cinnamaldehyde, carvacrol and capsicum) on blood acid-base balance and serum L-lactate levels in an 86-day feedlot experiment with 24 double-muscled Belgian Blue bull calves.

Materials and methods : Animals were allotted randomly to one of two experimental groups: 1) a control group (no supplementation, n=10), and 2) a group receiving dietary supplementation with a combination of plant extracts (100 mg/kg -DM basis-, composed by capsicum oleoresin 2mg/kg, carvacrol 5 mg/kg, cinnamaldehyde 3 mg/kg) [n=14]). Animals were fed with a high-grain ration, typical of diets fed commercially to feedlot cattle in Spain. Productive data (average daily gain, feed intake and feed:gain ratio) were also considered as complementary evaluation. Blood venous pH, pCO₂, HCO₃⁻, Base Excess and serum L-lactate were determined.

Results: Beneficial productive response to supplementation was observed. No effect of the additive or additive x time interactions were observed in acid-base balance. Beneficial time x treatment interaction was observed on serum L-lactate. The time course of L-lactate during the study is in accordance with the antimicrobial activity of cinnamaldehyde on gram-positive bacteria: supplemented animals showed significant lower values than control.

We conclude that under the conditions of this study, the specific blend of natural plant extracts composed by capsicum oleoresin, carvacrol and cinnamaldehyde can be a proper alternative as growth promotants, although further research is needed about the effects on different metabolic parameters.

16.10 EFFECT OF A SPECIFIC BLEND OF PLANT EXTRACTS (CINNAMALDEHYDE, CARVACROL AND CAPSICUM SPP) ON METABOLIC PARAMETERS IN FINISHING BULL CALVES FED A HIGH-GRAIN DIET

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Problem: Natural plant extracts have been used for centuries for various purposes (traditional medicine, industrial applications, food preservatives) because of their antimicrobial properties and because most of them are categorized under GRAS (Generally Recognized as Safe) for human consumption. The use of natural plant extracts appears as one of the most natural alternatives to the antibiotic use as a supplement for reducing ruminal disturbances in feedlot cattle. **Aim.** This study investigated effects of a commercial blend of plant extracts (cinnamaldehyde, carvacrol and capsicum) on metabolic parameters in a 86-day experiment with 24 double-muscled Belgian Blue bull calves.

Materials and methods: Animals were allotted randomly to one of the two experimental groups: 1) control group (no supplementation, n=10, CTR), and 2) supplementation with 100 mg/kg - DM basis- mixed plant extracts: capsicum oleoresin (*Capsicum annum*) 2mg/kg, carvacrol (*Origanum spp*) 5 mg/kg and cinnamaldehyde (*Cinnamomum spp*) 3 mg/kg (n= 14, XT). Metabolic parameters were determined: glucose, NEFA, triglicerydes, total proteins, albumin, BUN, creatinine, GOT and γ GT. Productive data were also considered as complementary information associated with supplementation.

Results: Beneficial productive response to supplementation was observed. No direct effect of treatment was observed in the studied parameters although significant time x treatment interactions were detected for NEFA, triglicerydes, total proteins, creatinine, and γ GT.

Conclusions: Under the conditions of this study our preliminary results show that natural plant extracts composed by capsicum oleoresin, carvacrol and cinnamaldehyde can be a proper alternative as growth promotants improving the benefits of a barley-based high-grain diets.

16.11 EFFECTS OF MALIC ACID SUPPLEMENTATION (MALIC ACID OR A COMMERCIAL MALATE SALT) ON ACID-BASE BALANCE AND PRODUCTIVE PERFORMANCE IN FINISHING BULL CALVES

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Introduction: Increasing concerns over the widespread use of antimicrobial additives in animal feedstuffs, and the corresponding new regulations proposed by the European Commission have prompted an interest in possible alternatives, such as malic acid supplementation. **Aim:** The aim of the present study was to evaluate the effects of two chemical forms of malate (malic acid and a commercial malate salt) on acid-base balance and productive performance in cattle maintained in a commercial feedlot system, taking into account the finishing period of the productive cycle.

Material and methods: Thirty-eight Belgian Blue bull calves were utilized for a 86-day feedlot study. Bull calves were allotted randomly to one of three experimental groups: 1) control group (no supplementation, n = 10), 2) DL-malic acid supplementation (n = 14, 4 g/kg DM basis) and 3), supplementation with a commercial salt of DL-malic acid (n = 14, 4 g/kg DM basis). Blood pH, pCO₂, HCO₃⁻, base excess (BE), AG (aniongap), and serum L-lactate were determined.

Results: From the statistical analysis of the data the results are: 1) supplementation with the organic acid has beneficial effects, during the finishing period better results were obtained with the acid form; 2) generally, and taking the acid-base balance into account, the malate salt seems to be more effective than the acid form, due to the fact that malic acid tends to decrease blood bases (although the possibility that the malate salt may cause blood alkalization due to decreased ppCO₂ needs to be explored further); 3) animals fed a high-grain diet with high crude protein content showed stable blood acid-base values, but also decreased BE and higher levels of serum L-lactate, indicating that they may develop ruminal acidosis.

Conclusions: Based on experimental results obtained we can conclude that the malic acid is a valid growth promotant from a productive point of view. But, after internal medium analyse the salt malate utilization seems to be more safety than the acid one to preserve animal health. Therefore, further research is needed to study the alkalotic trend observed in this experiment.

16.12 FIELD EVALUATION ON THE CLINICAL EFFICACY OF YEAST KILLERTOXINS IN THE TREATMENT OF PIGS AND CALVES WITH CLINICAL SIGNS OF DIARRHOE (ENTERITIS)

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Introduction: The toxic effect of yeast killer protein has been suspected to be potentially useful as biotherapeutic agent to improve the health of humans or animals (Ponelli et al. 1993-1996). Biomass derived from non-pathogenic yeasts - Type Ascomycetes- (Williopsis Hanseniospora, Pichia, Saccharomyces) contains peptides, acids and aldehydes. These are metabolites of fungus metabolism which are able to act fungicidal and bactericidal. They are called 'Killertoxines'. The effect is caused by blockade to the toxic receptors of intestinal pathogenic germs. They are specifically effective against Enterobacteriaceae (e.g. E. coli).

Material and methods: The cultures, especially Williopsis and others, are cultivated in suitable medium. Hereby Killertoxins are secreted extracellularly into the culture medium. The Killertoxins are separated from cells by filtration and the activity is determined in units. For clinical application the whole supernatant is lyophilised, increasing the activity by ~ 30 times. The product is applied orally by injector. The dosage for piglets is 1-2 ml (about 1-3 times), calves get 10-15 ml (about 1-3 times) in intervals of 6-12 hours. The effect is based on a blockade of adhesion of E. coli to the intestinal receptors and probably on an enzymatic effect (Bauer, 1999).

Results: From 1996 - 2003 multiple patients (piglets and calves) with diarrhoea and other intestinal disturbances were treated. The product was used for therapy and prophylaxis: Results are reported as percentage of animals cured after 1 - 4 administrations. New born piglets (n=4500) had the best results 55 % were cured with 1 application, 30 % with 2 applications, 15 % with 3 applications. The rest was treated with other therapeutics. Older piglets (n=1500), age 3-8 weeks, 40 % were cured with 1 application, 35 % with 2 applications, 15 % with 3 applications. The rest was treated with other therapeutics. For prophylaxis (n=1200) 80 % were successful. Calves (n=105) 85 % were cured with 1-3 applications mostly within 2 - 5 days. For prophylaxis (n=100) 80 % were successful with 1-2 applications. The rest (20 %) was not successful.

Discussion: Almost 70 % of the diseased patients (piglets and calves) were housed in farms with endemic intestinal infections. A part of this patients, esp. calves, was pretreated about 1 - 5 times with antibiotics and other therapeutics. The effect of the treatment with the test product was mainly positive in infections suspected to be caused by E. coli and viruses (Rota-, Corona virus). A reduced effect was observed in infections caused by Clostridia. Deutsches Patent Nr. 199 12 439.6-09 Killertoxins derived from yeast to be used for pharmaceutical manufacturing

16.13 THE INFLUENCE OF PROBIOTIC ADMINISTRATION ON SELECTED METABOLIC PARAMETERS AND GROWTH OF WEANED PIGS

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The aim of the study was to observe the efficacy of probiotics, based on genus *Bacillus*, in weaned pigs. A total of 100 piglets divided into 2 trial groups of 50 pigs each, comprising an equal number of male and female pigs were used in the trial. The trial was carried out from weaning, at the age 28 days, and the following 10 weeks. All pens were assigned with pigs with the same distribution of genetic background. Average start weight was approximately 7.5 kg. All pigs were allocated to the trial within the same day. Control group was fed with standard diets for pigs in the range from weaning until 45 kg of body weight without additives and without antibiotics (including omission of zinc and copper at a growth promotion level). Experimental group was fed with control feed with 1000 g BioPlus 2B/t feed which equals 3.2×10^6 CFU/g feed. BioPlus 2B (Chr. Hansen A/S, Hoersholm, Denmark) contains *Bacillus licheniformis* and *Bacillus subtilis* in concentration 3.2×10^9 per gram of preparation. Blood samples were collected at the age of 42 days and analysed for serum cholesterol level, total serum lipids, serum urea content, total serum proteins, albumin and haemoglobin. Pigs were individually weighted at weaning, at the age of 42 days, at the age of 56 days, at the end of the trial. Feed conversion ratio was calculated in the weighing intervals. Serum levels of total proteins were in range 52.4 - 58.3 g.l⁻¹, serum proteins in experimental group were significantly higher compared with control group ($p < 0.001$). The albumin level in both groups did not deflect from the reference range. Serum urea level in both groups was below reference limit, without significant differences between groups. Level of cholesterol in blood of experimental group was significantly higher compared with control group ($p < 0.001$). In both groups serum cholesterol level was within reference limit. On the other hand, total lipids in blood of experimental group were slightly lower in comparison with control group. Total lipid level in experimental group was 2.48 g.l⁻¹, serum lipids in control group were 2.73 g.l⁻¹, but differences were not significant. The values of haemoglobin were in the reference limit in both groups of pigs. The weaned pigs in experimental group reached better weight already on day 42 of their life and this state persisted up to the end of the experiment. The differences in weight of the experimental group and control group were significant at the age of 42 days ($P = 0.038$). Average daily gains were significantly ($P < 0.001$) higher in experimental group compared with control group in the first two weeks of the trial. Though non-significantly, feed conversion was better in experimental group at the beginning of the trial.

17 ANIMAL BEHAVIOUR AND WELFARE

17.1 HOW TO DEFINE AND EVALUATE WELFARE IN MODERN DAIRY FARMS

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Abstract

Animal welfare remains a difficult task because animals cannot be “consulted”, while citizens tend to feel it according to their (human) needs. This suggests to join empirical information of animal scientists and philosophers to better define animal welfare. Welfare can therefore be defined as “good success (avoiding sufferance and sustaining fitness) achieved in coping with difficult conditions”. This means that its evaluation must consider the comfort conditions of breeding system, but the response is also essential to understand whether the possible sub-optimal comfort has been successfully overtaken and animals do not suffer but feel well. For the evaluation of breeding system, the indirect indices are used: housing, management, microclimate, hygiene, feeding etc.; to evaluate animal response, the direct indices can be utilized, particularly those regarding functions (fitness): physiological, behavioural, performance and health. In general, this last indices, have the scope to measure the effects of a chronic stress condition avoiding, as much as possible, the acute stress changes. Of the physiologic indicators, heart rate and body temperature could be useful if remote probes will be easily available. Some blood parameters (immune system and some metabolites) are also promising, while a better standardized measurement of cortisol (basal or after ACTH challenge) seems very helpful. Behavioural indicators are very sensible, but require time and some caution for the interpretation; among these there are indices of human-animal relationship, comfort behaviour, getting-up behaviour, social behaviour and stereotypies. Health indicators are extremely important and can be external (general appearance, skin aspect, legs-feet conditions, udder and particularly teat appearance, digestive functions, anomalous discharges from vulva, nose or mouth etc.) or based on milk-blood parameters. Besides somatic cell count, electrical conductivity and some electrolytes and enzymes in milk, of great importance are the parameters of acute phase response (positive and negative acute phase proteins). Particularly in peripartum period, these last proteins seems to allow a risk-card to monitor the consequences of inflammation in the transition period. Performance indicators, if milk yield and quality but also fertility and longevity are considered, can be also useful. The direct and indirect indicators are both useful to evaluate animal’s welfare, but their weight in the model can be different according to the aim: research, certification, farmer assistance. The models can be therefore different, but a very accurate one, mainly based on a wide range of functional indicators, would be needed as a “reference” model to validate the others.

Key words: welfare, definition, evaluation

Introduction

We totally agree with Fraser's (1999) statement: "The increasing convergence of the scientific and philosophical approaches may lead to a more integrated field of study and to a greater awareness that neither empirical information nor ethical reflection can, by itself, answer questions about our proper relationship to animals of other species". Also, according to Lund et al. (2006), animal welfare science has to work at the interface between natural and social sciences where scientific issues involve ethical, economic and political dimensions. It is in fact impossible to properly respect animal welfare rights without an ethical approach, but it is meaningless to define a welfare standard without a proper approach to the biological, behavioural and psychological aspects of different animal species.

Of course, it appears to us impossible to define all of these aspects, but we shall try to reach the best definition of welfare according to them. Then, we shall look for the best way to evaluate animal welfare objectively.

Basic philosophical and scientific aspects of animal welfare

According to the capacity for suffering of animals, they should have some rights, which are subordinate to human rights (Warren, 1983; cited by Pascalev, 2006); therefore, most western nations have promulgated laws and followed policies that acknowledge the sentient nature of animals, their capacity to experience pain, their awareness of themselves and the environment, and the need and obligation on the part of humans to treat animals (including farm animals) in a respectful and humane way (Pascalev, 2006).

Obviously, a "respectful and human way" remains to be defined. For the Catholic Church, this could mean to "operate within the God scope" (Giovanni Paolo II, 1991); but again, we agree with Fraser (1999) that both scientists and philosophers must provide the empirical information and the ethical reflection needed to define animal welfare, and to formulate rules to obtain it. Nevertheless, we cannot discuss welfare without a positive answer to the following questions: can the animal production and particularly the intensive one be acceptable? Can the environment in which the animals are kept be made suitable despite the lack of "natural" conditions? To what degree do the five freedoms of the Brambell Report (1965) have to be satisfied to obtain an acceptable level of welfare?

- Unfortunately, the answers can vary widely:
- the herdsmen point to the good animal health and good technical results (Blosser, 1987);

- the citizens tend to put more emphasis on the opportunity for the animals to socialize and behave naturally (Bock, 2007).

To find a proper compromise it seems essential to agree, at least, on the basis of welfare. They substantially correspond to the well-known five freedoms (Webster, 1995); it is, however, noteworthy that no breeding system can fully satisfy them; in fact, according to Webster (1994): “Absolute attainment of all five freedoms is unrealistic, indeed they are to some extent incompatible. Complete behavioural freedom, for example, is unhygienic for all us animals! In fact, all commercial husbandry systems have their strengths and weaknesses; therefore the five freedoms make more difficult to sustain a sense of absolute outrage against any particular breeding system”. Therefore, the optimal present and future welfare of any animal requires a compromise between conflicting present and future needs.

Unfortunately, our suspicion is that the majority of the stakeholders have a more romantic approach to the theme. Therefore, our wish is that -at least in the future- philosophical consumer concerns and scientific efforts allow us to recognize the real animal's perception of its own welfare and finally try to educate public opinion towards a perception of welfare that is as close as possible to that of the animals themselves (Webster, 1994). To objectively evaluate animal welfare is perhaps the first essential step to educate the public about it.

Fair definition of animal welfare

Of the “dozens” of definitions (Bono, 2001) that have appeared in the last 30-40 years, some outline the “feelings” aspect: e.g., Dawkins's remark (1980): “Absence of suffering. Suffering understood to be an unpleasant emotional state induced by fear, pain, frustration, exhaustion, loss of social companions”. Others are more empirical and incorporate the farmers' interests: “as long as the animal is growing normally, performing well, is properly nourished and free from diseases, and suffers no physical mistreatment, there is no cause of concern” (Blosser, 1987). For a fairer definition of welfare, Carezzi and Verga (2007, in press), suggest that “the most widely definition of animal welfare should comprehend the whole state of the organisms, considering together body and mind and their links”. Namely, “the welfare of an animal is determined by its capacity to avoid suffering and sustain fitness”. This is very similar to Fraser and Broom's (1990) suggestion that welfare depends upon the “...degree of success achieved in coping with difficult conditions”.

We consider this kind of definition fair (e.g. acceptable both by philosophers and scientists), because it confirms that a good level of welfare is not achieved merely by the absence of difficulties - as an oversimplified interpretation of the five freedoms might suggest - but by the herdsman's capacity to overcome them through genetics, management, feeding, hygiene, social environment, etc. (Bertoni and Calamari, 2006). Therefore, measurement of potential stressor stimuli cannot be utilized alone to define welfare because the same situation may be stressful or not according to genetics,

experience, physiology, etc., of the animals, in other words to its capacity to fight negative conditions. Finally, this kind of approach would avoid the risk of misinterpreting the breeding systems by consumers, who quite often are influenced by their subjective and anthropomorphic feelings.

Animal welfare evaluation

A pragmatic approach to welfare evaluation could begin from Dawkins' (1983, cit. by Veissier and Boissy, 2000) claim that "animal welfare is a subjective condition of the animal which needs an objective evaluation by man". Nevertheless, because the animal's mental experience, part of its welfare, cannot be measured directly but has to be assessed indirectly (Sandøe and Simonsen, 1992), there is a risk of anthropomorphic judgment (Veissier and Boissy, 2000). For this reason, of the three main criteria of welfare: feel well, function well and lead natural life, only physiological function is useful because objectively measurable (Duncan and Fraser, 1997).

Nevertheless, the breeding system has considerable importance and can be quite easily evaluated; therefore, Veissier et al. (2000), suggest the following objective indices:

- characterization of the environment against ideal conditions of housing, management, feeding, microclimate, hygiene, etc., which can guarantee comfort
- evaluation of animal response to the specific life environment by physiological, behavioural, performance and health indices (measurements of "functioning well")

The first are also called influencing factors or indirect parameters while the others are response (output) or direct parameters. Within the last parameters, an apparently small difference exists between Sørensen et al. (2001) and Winckler (2006), who suggest three directly measurable parameter types: physiological, behavioural and pathological, and other authors including also performance indices (Bertoni et al., 1999; Verga et al., 2000.; Veissier et al., 2000). The differences between these views are not small and can mean a different approach to the welfare issue in intensive –high-yielding systems; this will be discussed later on.

We assume that both indirect and direct parameters are useful for evaluating Animal Life Quality or welfare at farm level, but because the indirect parameters are more easily understandable and widely described (Bartussek, 1999, Bracke et al., 2002; Calamari et al., 2003a), we will not discuss them. Otherwise, direct parameters appear to better show the presence and the level of chronic stress conditions, the true causes of lower welfare; therefore they are believed the most reliable way to measure welfare status in single animals, particularly when different and accurate indicators are contemporaneously utilized. For the future, more attention would be paid to the possibility (not so easy) to evaluate of good welfare, i.e. positive affective status (Winckler, 2006).

Functional indices of welfare

In general, functional indices of welfare are preferred because more effective, reliable and sensible, compared to feelings indices; therefore, we will discuss them according to the following categories: physiological, behavioural, performance and health.

Physiological indices of animal welfare

For welfare we are not interested in the short-lived changes, occurring in acute stress situations (Broom, 2003); on the contrary, our interest is in indices of chronic stress. However, some parameters of physiological indices can be modified for both conditions; therefore, it is essential to discriminate between short-term and long-term variations.

The most widely-used physiological indices are:

1) *Heart rate* Increased heart rate (tachycardia) occurs in response to disturbing situations (i.e. transport, approach to an animal by unknown humans or animals, unfamiliar handling), and can remain higher for several hours after the beginning of stress. It is supposed to be an adaptation for an expected future flight response (Broom, 2003). Interestingly, some evidence suggests that the heart rate remains higher—at least in the pig—for several days during the psychological stress of isolation, while cortisol and ACTH levels consistently decreased in the same period (Schrader and Ladewig, 1999); this could imply that it is useful for chronic conditions as well.

2) *Breathing rate* Breathing rate increases rapidly when animals are disturbed. Unfortunately, this and other accompanying indicators (muscle tremor, foaming at the mouth, etc.) must be interpreted with caution because several other factors (acute or heat stresses) could influence their changes.

3) *Body temperature (rectal)* Body temperature is dysregulated during many stress conditions. For instance, it increases during infectious events. Therefore, only a new system of body temperature measurement (e.g. based on radiotelemetric technique), which provides easily obtained and reliable data, could increase interest in this indicator.

4) *Metabolic indicators* Metabolic indicators can be categorized according to the mechanism that induces their variation. First of all, haematocrit and glucose (and NEFA) levels can rise rapidly, due to catecholamines in acute stress situations. Some of these stressors (e.g., transport, fright) also cause vigorous exercise and sometimes muscle damage; thus, some enzymes increase markedly (e.g. creatine phospho-kinase, LDH, aspartate transferase). We are not interested in them as welfare indicators, but we wish to point out that a very stressful blood sampling – modifying them – can prevent proper interpretation of blood parameters. Other chronic and negative conditions that can be evaluated by metabolic indicators, if properly performed (Bertoni and Trevisi 1992; Bertoni et al., 2000), are energy shortage (high NEFA and BOHB), protein excess or shortage (urea), and some mineral deficiencies (phosphorus, magnesium,

selenium, iodine, copper, zinc). Other chemical plasma parameters that seem to be related to welfare status are creatinine, potassium and alkaline phosphatase. Creatinine is an index of body muscle mass (Fekry et al., 1989), and its level in lactating cows is an index of body mobilization (Bertoni et al., 1994). Potassium can be reduced in mildly stressed animals for a long time, and the mechanism of its reduction seems to be linked to the stress hormone oxitocin (Legros et al., 1988) and to catecholamine rise (Trevisi et al., 1992). Alkaline phosphatase also is reduced in some stressful conditions such as heat stress (Vazhapilly et al., 1992) or overcrowding (Calamari et al., 2003b), but no data are available to explain its changes.

5) *Immune function* Stress has long been associated with immune system impairment. In fact, stress conditions often exert a negative effect on several components of the immune system (e.g. lymphocyte apoptosis induction, proinflammatory cytokine production inhibition, peripheral mononuclear cell reduction, neutrophil chemotaxis, antibody production, natural killer cell production, neutrophil/lymphocyte ratio increase) (Lay and Wilson, 2004). Several kind of stress conditions, including disease, promote the release of pro-inflammatory cytokines which affect endocrine and metabolic status. Nevertheless, the main indices of disease stress (positive and negative acute phase proteins) will be discussed with the health indicators.

6) *Endocrine parameters* Endocrine parameters from the sympatho-adrenal and hypothalamic-pituitary-adrenal axis are frequently used in welfare evaluation. Some show a very short spike after acute stress events (i.e. CRH, ACTH, epinephrine, norepinephrine, β -endorphin, vasopressin) and so appear less useful, while cortisol (basal or change after ACTH challenge) is the preferred one (Sapolsky et al., 2000). Nevertheless, its interpretation for welfare evaluation requires caution because its level is affected by several factors such as circadian rhythms (Möstl and Palme, 2002); sampling (Negrão et al., 2004); restraint (Bertoni et al., 2005a); stage of lactation (Bertoni et al., 2006b); coitus and nursing (Manteca, 1998); and habituation (Von Borell, 2001; Smith and Dobson, 2002). To reduce the variability, cortisol evaluation have been proposed in faeces, saliva, milk etc., but available data are still insufficient. However, using well-standardized conditions, higher basal plasma cortisol in dairy cows have been observed in herds in less favourable situations (eg., larger group size, higher disease frequency, lower cleanliness score etc. [Trevisi et al., 2005b]).

The most widely recognized method of evaluating the adrenal cortex function is however the challenge with ACTH (its analogue ACTH₁₋₂₄ or tetracosactide) (Verkerk et al., 1994). Nevertheless, to avoid several disturbing factors: milk yield, age, ambient temperature and stage of lactation (Hasegawa et al., 1997; Bertoni et al., 2005b), as well as genetic factors (Weiss et al., 2004), a better standardization is needed: dose, time of bleeding, response measurements (Bertoni et al., 2005a).

Behavioural indices of animal welfare

The importance of behaviour as a tool for welfare assessment in animals was first underlined by the Brambell Committee (1965). Behaviour is animals' actions that aim to change and optimize their internal environment; thus, it provides information about their needs, preferences and internal status (Mench and Mason, 2001). Despite behavioural indicators are considered the most sensible among the indicators of animal response (Veissier et al., 2000), their use in captive and domesticated animals has generated much discussion and controversy particularly in domestic animals, where selection has produced changes in behaviour (Price, 1984).

As behaviour indicators, Sørensen et al. (2001) have suggested observations of: the human-animal relationship, the comfort behaviour such as getting-up behaviour, and social behaviour.

The human-animal relationship is influenced by many factors including genetic predisposition, housing conditions, the quality and quantity of human contact, and handling procedures (Hemsworth et al., 1990). Fear tests are considered to be a more direct measurement of the human-animal relationship (Hemsworth et al., 1996). Animals responding fearfully indicate a negative feeling which may be a product of previously strained conditions. The choice of test person should be considered very carefully, as the response to an unfamiliar person tends to differ from the response to the regular stock person (Rousing et al., 2001).

Comfort behaviour indicators focus mainly on resting behaviour. It has been shown that surface quality in cubicles affects lying behaviour and the number of skin lesions occurring at carpal and tarsal joints (Oertli et al., 1995 cited by Waiblinger et al., 2001). Several indices have been proposed to evaluate the comfort of resting area (Cook et al., 2005).

The resting positions of cows vary and could be used to evaluate the comfort of the resting area, particularly of the cubicle. The normal positions are well detailed by Anderson (2003). Getting up and lying down in the stall are other indicators suggested (Capdeville and Veissier, 2001; Sorensen et al., 2001) for evaluating the adequacy of the space available for cows lying down or standing up. Veissier et al. (2004) suggest, for every getting-up movement, to recording the number of intentions (the cow extends its head forward), and whether the movement was interrupted (the cow lies down again after lifting its hindquarters) or abnormal (the cow lifts its forequarters before its hindquarters).

Social behaviour refers to movement as well as to contact between congeners. The inclusion of social interaction has been recommended for on-farm welfare assessment protocols (Winckler et al., 2003). Winckler et al. (2003) consider agonistic (e.g. displacement, butting, threatening) and cohesive interactions (e.g. licking, head resting), and observed these behaviors between the morning and evening milkings. Unfortunately, these observations are time-consuming, and continuous observations for some periods during the day are necessary.

Cows grooming themselves (licking or rubbing) and grooming (licking) others were observed by Phillips and Rind (2001). Excessive grooming, particularly self-grooming, could be interpreted as an alteration of behaviour and may be a form of abnormal behaviour (Mench and Mason, 2001).

Abnormal behaviours, together with species-specific behaviour, are other aspects of behaviour suggested for animal welfare assessment (e.g. Veissier et al., 2000). Abnormal behaviour includes stereotypies (repetitive behaviour patterns with no obvious function), excessive licking and even eating of hair, wool or feathers, etc.. The stereotypies are a much-studied group of abnormal behaviour patterns.

Health status as welfare indicator

The negative relationship between health status and welfare is obvious, especially for the third freedom: "...from pain, injury and disease". Everybody knows that low health status is a cause of physical pain and psychological depression (see the effects of pro-inflammatory cytokines, by Johnson and Finck, 2001). Furthermore, health impairment is a consequence of chronic stress (Elsasser et al., 2000). Therefore, a vicious cycle can occur: low welfare, immune depression, disease, low welfare ... (Broom, 2006).

Any kind of pathology involves some degree of poor welfare (Broom, 2006). Pathological conditions can be caused by genetics; physical, thermal and chemical injuries; infections and infestations; metabolic abnormalities and nutritional disorders. The indicators of health can be individual (estimated on the basis of clinical examinations or based on milk or blood parameters); they can be also at farm level: the same as above on representative subjects but also frequency and type of health problems, case histories of culled animals, etc...

Good examples of external health indicators have been given by Rousing et al. (2000); however, in the case of welfare evaluation of individual cows, the following aspects and clinical parameters can be evaluated:

- General appearance: BCS, hair cleanliness and eye brightness;
- Skin: parasites, infections, lesions etc.;
- Legs: lameness, hoof care;
- Udder quality: clinical mastitis, teat lesions (with special attention to the teat score: Neijenhuis, 2000).

Outstanding systemic diseases can be diagnosed in single cows by looking at general conditions, body T°, respiration rate, anomalous discharges (nose and mouth, vulva), and digestive troubles (cud chewing rate, faeces score). Some of these indicators must be "adjusted" in their interpretation according to specific physiological and production stages of the cows; e.g., time from calving can be very important. Cows in their dry period, in early lactation, or long from calving can in fact be judged equally normal

even if they show completely different BCS, faeces score, udder aspect, etc.; furthermore, the vulva discharge can be peculiar immediately after calving.

Milk and blood parameters can be also useful for defining health conditions such as:

1) *metabolic diseases*: high ketone bodies, low protein/fat ratio, etc. in milk indicate negative energy balance, while urea is a satisfactory index of protein intake level. Ketone bodies and urea can be also measured in the blood with the same scope. Other blood parameters can be useful for evaluating the optimal metabolic status (blood minerals, other energy metabolites, liver enzymes, etc.), but more information has been given in the physiological parameters chapter;

2) *infectious diseases*, with special emphasis on mastitis and hence to the somatic cell count in milk (but also electric conductivity, Na, K and Cl contents, NAGase activity etc)

3) the so called “*disease or inflammatory stress*” (Elsasser et al., 2000), which is due to the effects of pro-inflammatory cytokines (IL-1, IL-6 and TNF α). They are in fact a source of inflammation phenomena that cause some pain and many systemic effects, no matter whether cytokines originate by infectious, parasites, trauma, endotoxins of digestive origin, oxidative stress, toxics, etc. Of interest to us are the increases in haptoglobin and ceruloplasmin, and reductions in zinc and calcium (Bertoni et al., 1989; Trevisi et al., 2005a). Except for ceruloplasmin, which remains higher for weeks to months, the other changes are “short-lasting” and can be observed only during the pathological event (whether clinical or sub-clinical). However, the effects of this stress can be recognized for a “longer” time, particularly because cytokines cause a diversion of liver synthesis (Bionaz et al., 2007) with a rapid reduction of some negative acute phase proteins (albumins, lipoproteins measured as cholesterol, Retinol Binding Proteins measured as vitamin A and paraoxonase), which afterward tend to return to normal values quite slowly.

The effects, more or less important according to the severity and length of inflammation, can vary according to physiological status; e.g., some are very evident and dangerous around calving (Bionaz et al., 2007, Ametaj et al., 2005; Bertoni et al., 2006a). Their negative effect on reproduction can be observed for long time (Bertoni et al., 2001; Bertoni et al., 2006c) and can be recognized with the above blood parameters. This suggests the possibility of arranging a risk-card (Bertoni et al., 2006d) using two or three blood samples in the first month of lactation to evaluate the severity of inflammatory phenomena around calving (such as a rise of the liver positive acute phase protein haptoglobin), and the response to these phenomena (reduction of synthesis of the liver proteins albumin, lipoprotein, RBP, and paraoxonase, as well as a reduction of bilirubin excretion with a raise of blood level). Cows can be ranked as being at low, medium or high risk having impaired reproductive activity (Bertoni et al., 2001, Bertoni et al., 2006c).

Performance as welfare indicator

It is much less obvious with respect to health to consider good performance as an index of welfare. On the one hand, it seems obvious because a proper covering of needs included in the five freedoms means a better chance for good animal growth, milk yield, reproduction, etc. (Broom, 1997; Rushen and Passillé, 1998). On the other hand, intensive milk, eggs, fish, etc., production is considered a welfare reducing factor (Rollin, 2004;). Unfortunately, the level of productivity can be considered as critical, and cannot easily be defined; however, it is not difficult to verify some negative relationship between milk yield and both health (Rauw et al., 1998) and fertility problems (Butler, 2000). Furthermore high genetic merit dairy cows seem more susceptible to metabolic disorders, particularly mastitis and lameness (Knight, 2001), despite it seems reasonable to exclude the possibility that high milk yield could modify the HPA axis with a reduction of adaptive capacity (and consequently an impairment of welfare) (Beerda et al., 2004).

This apparent contradiction is not surprising: in fact, McInerney (1991), cited by Newman (1994) and more recently by Appleby (2005), suggests that the relationship between productivity and welfare is complex: in the first step, both are raised, while much later, both are reduced. We think it is obvious, and we have in fact demonstrated - in some commercial farms - that high genetic merit cows, if properly managed, that means without excessive exploitation, have a welfare improvement and “consequently” they show an increase of milk yield and fertility (Trevisi et al., 2006; Calamari et al., 2003a).

Where is the point? In our view, good performance is a true indicator of good dairy cattle welfare (and this holds for other species as well); however, it cannot be limited to milk yield. Other aspects of performance, such as fertility and longevity, can confirm that the good level of production has been obtained in sustainable conditions for the cows (Appleby, 2005). Of course, it has to be underlined that high genetic merit animals can yield optimal answers if selection is not restricted to milk yield, and if nonproductive traits such as disease resistance, fertility, and longevity are also included (Essl, 1998; Darwash et al., 1999; Heringstad et al., 2000); furthermore, their superior needs have to be properly satisfied to maintain their optimal welfare (Ingvartsen et al., 2003).

Therefore, a proper evaluation of animal performance, that estimate actual milk yield and composition (in relationship with the supposed genetic potential and with the lactation stage), but also the susceptibility to diseases, to reproductive problems and in one word to culling rate, can be a useful indicator of welfare (cow or farm).

Welfare evaluation at farm level

Till now the approach to welfare evaluation has considered the general criteria, therefore applicable to single cow or to groups (farms). This corresponds to the aim of our contribution, nevertheless few words are needed to explain our view on the farm evaluation of welfare. Before entering the specific aspects, it appears worthwhile to remember that welfare is a continuum with two extremes: very bad and very good. The farmer cannot ensure maximum welfare; he can only provide good husbandry (Webster, 2001). These concepts are of enormous importance because they imply a composite scale for welfare measurement (Scott et al., 2001) and the definition of an acceptable range that the farmer must ensure to his cows (to be progressively improved).

Many contributions to the objective evaluation models for farms have been given by Bertoni et al. (1999), Bracke et al. (1999), Sørensen et al. (2001), and Capdeville and Veissier (2001). A model that considers both indirect (housing/management and feeds/feeding, each one representing 30% of the total score) and direct indicators (functional indices, representing the remaining 40%), has been adopted by our Institute (Calamari et al., 2003a) and partly validated in the field (Calamari et al., 2004). The model has showed good reliability, because some preliminary results have confirmed a good relationship between IDSW (Integrated Diagnostic System Welfare) scoring and the cortisol basal values (unpublished results); nevertheless the IDSW needs some adjustments, i.e. the weight of direct indices (40%) seems to need an increase (our unpublished results).

Nevertheless, it is noteworthy that the level of deepening of every model can vary according to the aim of welfare evaluation: research, certification, or farmer assistance. In the first case, the welfare measurement can evaluate every cow, and direct indicators can represent the largest part of the total weight; in fact cost and time are not the most important aspects. Furthermore, this kind of evaluation can be considered as “reference” model (for the lack of specific gold standard) to validate the others, which are more simple models (certification or farmer assistance). With this in mind, the model for farm evaluation can be simplified as much as possible to reduce time and costs, while ensuring reproducibility and repeatability, but first of all the objectivity: the scoring must discriminate the farms according to the real welfare of their animals as well as suggests the less positive situations to be improved.

Conclusion

Animal welfare evaluation is therefore a very complex issue: because “to objectively evaluate the subjective condition of the animals” is not an easy task for nobody, but also because the “animal’s perception of its own welfare may be very different from that of the human public”. This suggests that both scientists and philosophers must provide the empirical information and the ethical reflections needed to define animal welfare and formulate rules to obtain it.

For a shared definition of welfare we think that suggestions of animal right supporters or those of farmers must be taken with caution, on the contrary a fair interpretation of the five freedoms (e.g. good success achieved in coping with difficult conditions) can be accepted by everybody. According to this definition, the evaluation of animal welfare would consider both comfort level of environment (housing, feeding, management, microclimate, hygiene etc.) and the response of the animals to it. In other words, the breeding system is important, but the response of animals can be influenced by many factors not easily predictable and thus must be properly evaluated.

The comfort level of environment can be properly evaluated according to the quite well defined ideal standards for building, equipments, management, feed-feeding, hygienic-sanitary conditions etc.. Less easy is the evaluation of animal response which would consider the level of feeling (sufferance) and fitness (function). It is in fact widely accepted that only functional indices are enough reliable to guarantee an objective evaluation. Therefore, the animal response to the environment can be evaluated according to physiological, behavioural, performance and health indices (functional). To do this, the direct indices and the indirect one can be utilized in a different way and more or less deeply according to the scope: research, certification or farm assistance. However, it is noteworthy to point out that both practical models (reliable but simple and at low cost) and more accurate models are needed; the first for the welfare evaluation at farm level and the second for research and to validate the practical models to guarantee their objectivity.

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17.2 DIFFERENT METABOLIC STATUS OF PREGNANT DAIRY EWES WITH HIGH OR LOW DEGREE OF BEHAVIOURAL LATERALIZATION

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In the last years behavioural lateralization at the population level (i.e. right-left asymmetries showing a similar direction in more than 50% of individuals of the population) has been widely documented across vertebrate species. In sheep, behavioural and neurobiological methods have unraveled that the right and left sides of the brain differ in the recognition of familiar and unfamiliar faces. During pregnancy ewes could develop some metabolic disorders such as parturient toxæmia. The aim of this study was to investigate possible relationships between the degree of behavioural lateralization on some metabolic parameters during pregnancy of dairy ewes. The study was carried out on a flock of 57 Sardinian ewes. Lateralization was investigated by behavioural tests measuring left-right side preferences during turning in front of an obstacle to rejoin cagemates and clockwise-anticlockwise direction of jaw movements during rumination. On the basis of the behavioural tests sheep were divided in two groups: sheep with high degree of lateralization (HDL) and sheep with low degree of lateralization (LDL). During the pregnancy period, all ewes were monthly submitted to blood sample (for a total of four samples) to evaluate the following plasma parameters: insulin, glucose, NEFA, β -HB, bilirubin total and direct, GGT, ALT, AST, urea, creatinine, total protein, uric acid, Ca, Cl and K.

Results were analyzed by ANOVA using the statistic software SIGMA STAT 2.3 to verify the effect of the different type (HDL or LDL) on the blood parameters. The results revealed statistically significant differences between HDL and LDL groups for the following parameters: direct bilirubin (0.273 Vs 0.206 mg/dl), creatinine (0.92 Vs 0.88 mg/dl), Na (147.1 Vs 143.1 mEq/L), Cl (109.7 Vs 105.5 mEq/L), glucose (58.0 Vs 53.4 mg/dl), total protein (6.56 Vs 6.33 g/dl). Significant differences into the group with one fetus between HDL and LDL type, were recorded for direct bilirubin, creatinine, Cl and Na while less differences were observed in the group of ewes with a two fetus pregnancy (glucose and Ca). In all case HDL ewes were observed to show higher values in the estimated blood parameters. These preliminary results show some significant effect of the different degree of behavioural lateralization on the assessment of metabolic parameters during pregnancy in healthy ewes. These findings suggest that behavioural lateralization degree should be taken in account when blood parameters are used to evaluate an animal metabolic or health status. To confirm this hypothesis further study should be carried out in ewes with healthy problems in the late pregnancy.

17.3 DETERMINATION OF RESTING VALUES OF CORTISOL IN CATTLE IN A SELECTED HERD

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Aim: The aim of the study was to determine resting cortisol values in cattle of different production stages in a Red-Pied cattle herd.

Material and methods: In the herd under study, stress load induced by surgical dehorning using different types of anaesthesia was monitored in adult cattle. Cortisol levels were measured in 152 healthy animals of different ages and sexes. Blood samples were collected when animals were resting after the morning feeding, without excessive animal handling, at the same time of the day and under equal environmental conditions. It was confirmed that none of the animals included in the study were neither ill nor treated in the past two months. When the blood collection was finished, all the animals were subjected to clinical examination to reveal potential pathological changes. All the animals selected were regarded as healthy. Blood samples were withdrawn from the coccygeal vein without unnecessary fixation and handling the animals, using the Hemos HO tubes with disposable needles. The animal response to sample collection was minimal. 6 ml samples were collected and heparinized to obtain unclotted blood. Plasma cortisol was determined by enzyme immunoanalytic method. Resting cortisol values were measured in animals of 5 production stages, i.e. in heifers, pregnant and non-pregnant cows, bulls and calves. For each category, mean value of resting cortisol was determined.

Results: The highest levels were found in calves: 56.03 ± 40.57 nmol.l⁻¹. Based on the mean value, the reference range of 0-173,23 nmol.l⁻¹ was determined for the calves in this herd. In the heifer group, mean resting cortisol value was 18.43 ± 21.65 nmol.l⁻¹. Based on the mean value, a reference range of resting cortisol in heifers in the herd under study was determined as 0 - 61.55 nmol.l⁻¹. In non-pregnant and pregnant cows, resting cortisol levels measured were 62.08 ± 49.70 nmol.l⁻¹ and 23.89 ± 23.83 nmol.l⁻¹ respectively. From these values, reference values of 0 - 161,48 nmol.l⁻¹ and 0 - 71,55 nmol.l⁻¹, respectively, were derived for this herd. In bulls, resting cortisol levels measured were 17.25 ± 13.71 nmol.l⁻¹. From these values, reference range of 0 - 51,21 nmol.l⁻¹ for resting cortisol level in bulls in this herd was measured.

17.4 ANIMAL HEALTH OF VEAL CALF PRODUCTION IN FARMS WITH STANDARDS OF HIGH ANIMAL COMFORT: PRELIMINARY RESULTS

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Problem: In the Swiss veal calf production, the logistics (several stopovers), the overcrowding, the general animal health status, the use of antibiotics, the high death rate (3%), the feeding of milk replacer, and the husbandry (indoor livestock farming) represent serious production and animal welfare problems. **Aim:** The aim of the present study was to evaluate animal health status, use of antimicrobials, welfare status, and carcass data of calves kept under conditions of high animal comfort (group 'naturafarm').

Material and methods: The data collection started in July 06 and will end in October 07. The prerequisites for veal 'naturafarm' veal included short transportation time, groups of maximum 40 calves, indoor straw bedding, hay and water available ad libitum, permanent access to an outside pen and medical surveillance by a contract-veterinarian throughout the fattening period. After arrival at the farm, calves were examined by the veterinarian, and the medical findings were recorded. Data concerning management, stall system and feeding were collected by questionnaire during visits to the farm. Calves that died during the fattening period were examined at the Institute for Veterinary Pathology, Vetsuisse-faculty of Berne, Switzerland. At the end of each fattening period, medical treatment and carcass data were collected. For evaluation of the animal welfare, 100 abomasa originating from calves of the group 'naturafarm' and 100 abomasa from control calves (veal production under minimal standard conditions) were collected and evaluated for the presence of mucosal lesions. **Results:** So far, preliminary data exist on (a) 'health status after arrival of the calves', (b) 'causes of death' and (c) 'evaluation of animal welfare'. (a) Two thirds of 578 calves showed one or more pathological findings, from which more than 50% exhibited respiratory symptoms. (b) Up to now, 137 dead calves were announced by the farmers (death rate of 1.7%). In 108 of them, necropsy was performed by a specialized pathologist. In 58 of 108 calves (53%), the cause of death was localised in the GI tract, in 28 of 108 (26%) in the respiratory tract, and in 1 of 108 (1%) in the CNS; septicaemia was responsible in 4 of 108 (4%) and in 13 of 108 (12%), identification of the cause of death was not possible. (c) Evaluation of the lesions in the pyloric area revealed no difference between groups, whereas significantly more lesions in the fundic area were found in calves of the group 'minimal standard'. Before considering any correlation between animal welfare and abomasal lesions, data about the feeding of the calves must be collected.

17.5 EFFECT OF FLOORING AT THE WALKING AREAS ON LOCOMOTION, BEHAVIOUR AND CLAW STATUS OF DAIRY COWS

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To improve our understanding of the influence of flooring systems on the locomotor apparatus of dairy cows, three experimental studies were performed as a part of the EU financed project 'LAMECOW'.

To evaluate objectively locomotion of dairy cows on different floors, trackway analysis was used. A slippery surface resulted in stride shortening, a wider posture and an asymmetric gait. Using soft rubber mats made locomotion more alike that on a natural yielding surface. Lamé cows showed less gait asymmetry on soft floorings than on hard ones. Locomotion on hard floors with good slip resistance was also improved, compared to slippery hard floors, although not to the extent of yielding surfaces. Preference studies showed that the majority of cows preferred to walk and stand on soft rubber flooring rather than concrete flooring. The cows also showed a slightly stronger preference to walk on solid instead of slatted rubber mats, and to stand on extra soft rubber instead of soft rubber mats. However, lame cows did not show a stronger preference for soft flooring than non-lame cows. During a long-term study at an experimental dairy, cows were exposed to different flooring systems regarding softness and abrasiveness of walking and standing areas. Claw health was evaluated along with claw horn growth and wear rates, as well as biomechanical parameters. The growth and wear of the claws were greatly affected by flooring system. On a rougher flooring (mastic asphalt), an exaggerated wear of the claws was seen, resulting in a higher growth rate, lost concavity of the sole in lateral claws, and most weight exerted to the sole area of the claw. Because the contact area of claws on rough floors was larger, the average pressure exerted to the claw sole was lower. When rubber-equipped feed-stalls were used together with mastic asphalt in alleys, wear was reduced, net growth was positive and the loss of the concavity was reduced. Rubber mats on walking and feeding areas resulted in a lower growth and wear rate, an increased net growth, and a concave sole where the bulbar and wall areas of the claw carried most weight. No association could be shown between different flooring systems and the risk of claw lesions probably because of the low prevalence of claw diseases in all the compared flooring systems. Comparing with solid mastic asphalt, a solid rubber flooring decreased the risk of lameness in animals of parity three or higher.

It was concluded that a soft, hygienic and non-slippery flooring with a moderate wear of the claws is possible to establish, thus securing good cow locomotion comfort, health and welfare. Studies were also financed by the Swedish Farmers' Foundation for Agricultural Research.

17.6 CLAW HEALTH IN ORGANIC AND CONVENTIONAL DAIRY HERDS

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Housing, management and production intensity are factors known to affect the claw status of dairy cows. Management and production intensity are generally also aspects that distinguish organic dairy herds from conventional. It is therefore possible that the claw health of organic and conventional dairy cows is different, but to our knowledge this has not been studied to any great extent.

The aim of this study was therefore to investigate if there were differences in claw health status, recorded at routine claw trimmings, between organic and conventional dairy herds in Sweden. The data originated from a field study of 20 organic and 20 conventional dairy herds, where information on herd characteristics were collected by questionnaires administered at visits and from the official milk recording scheme. Data on claw health status was retrieved from routine recordings, but only trimmings from September 2005 to May 2006 was used in these analyses. Thus, records of 821 cows in 11 organic and of 755 cows in 12 conventional herds were used. A generalised linear mixed model with logistic link and herd included as a random effect, to account for clustering, was used to assess effects of explanatory factors on the individual cow risk for one or more lesions in any claw in any foot. Potential explanatory factors on herd level were type of herd (organic or conventional), stall type (free or tied), feeding regimen (total mixed ration or not), amount of roughage in early lactation, weeks at pasture during the preceding season, level of milk production and herd size, while potential explanatory factors on individual cow level were breed, parity, season at trimming and days in milk at trimming. Development of the multivariable model was by backward elimination of non-significant ($p > 0.05$) effects. Overall, claw lesions were more prevalent in organic (34%) than in conventional herds (29%), but the within herd variation was substantial, i.e. 3 to 91% and 8 to 67% in organic and conventional herds, respectively. The overall prevalence of dermatitis was 6 and 3%, of heel horn erosion 14 and 17%, of sole haemorrhage 23 and 12%, and of sole ulcers 3 and 2% in organic and conventional herds, respectively. The final multivariable model included the effects of type of herd ($p = 0.3$; forced into the model), feeding regimen ($p = 0.2$; confounder), weeks at pasture ($p = 0.2$; confounder), parity ($p = 0.001$), days in milk ($p = 0.01$) and season ($p < 0.001$).

Our results indicate that there is no significant difference in the overall risk for claw lesions between cows in organic and conventional herds.

17.7 COMPARATIVE STUDY TO THE HEAT STRESS OF HIGH PERFORMANCE COWS IN LIGHT CONSTRUCTION AND MASSIF STABLES

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The genetic progress as well as new keeping and feeding technologies ever make a herd milk yield of over 10000 l for animal and year. This requires a daily synthesis of >50 l of milk in the high lactation. Looked energetically an input of >300 MJ metabolic energy is required for this, more than 40% converted into warmth. To minimise the warmth disposal problem of the dairy cow, outdoor climate stables were recommended without warmth checked wall and roof. Despite far improved air change rate by the installation of ventilators it comes to the heat stress in the light construction stables among cows. This problem was examined in a light construction stable (A) and in a massif stable (B) to handle the radiation, rectal temperature and breath rate. A weather station and a station for the thermal comfort to ISO 7730 were installed at every stable. The note of the climate data (air and radiation temperature, air speed, air humidity) was carried out synchronously from April until September 2005. 1824 data sets could be used for the evaluation from 76 days. In every stable 25 cows were selected with a daily milk yield of >45 l and measured the radiation with an infrared camera, rectal temperature and breath rate in the warm periods. To example of a hot summer's day the consequences of the global radiation become represented on the heat balance of the stable buildings, on the stable climate sizes as well as on the physiological parameters of the cows. Significantly higher air and radiation temperatures as well as also a higher wind speed were measured at comparable outdoor climate data in the stable A. The air temperature rose from 17.2 to 33.8°C and the radiation temperature to 38.7°C. The cows doubled her breath rate (68.2 ±9.7 breaths/min) up to the evening hours, the rectal temperature rose (39.7 ±0.74°C) and the possibility of the radiation went against zero. The temperature courses in B were considerably flatter and reached only peaks of 31.3°C. As expected the radiation temperature remained on the level of the air temperature. The physiological parameters were not significantly increased. The radiation did not come to a standstill either. The warmth entry into stable buildings by the global radiation is considerable. For light construction stables all the more leads this to the charges of the thermal regulation mechanisms of high performance cows. At radiation temperatures above 36°C the radiation comes to a standstill, the breath rate doubles, the rectal temperature increases individually more than 40°C and the cow can only lead the metabolic warmth away about evaporation. It is recommended to respect stable making with a warmth checked roof, by construction elements storing cold or sprinkle plants for the reduction of the radiation temperature

18 BREEDING, GENOMICS AND PRODUCTION DISEASES

18.1 BREEDING AND PRODUCTION DISEASES

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Introduction

Production diseases have been initially termed as “man made” problems consisting of the breakdown of the various metabolic systems of the body under the combined strain of high production and modern intensive husbandry (Payne, 1972). This definition has been extended to a broader view to include in that complex infectious and non infectious diseases originating from interactions between the animals physiology and the corresponding production environment (Drackley, 2006). Simianer and König (2002) classified diseases in livestock into three groups: (i) infectious diseases: They are caused by biological agents like bacteria, viruses, fungi and parasites; (ii) anomalies and genetic defects: They comprise inherited anatomical (e.g. hernias, atresia ani, inverted teats) and metabolic (i.e. BLAD, DUMPS). abnormalities which lead to a wide variety of phenotypes including lethal outcome, In several of these diseases at least the predisposition to develop the disease is inherited. Generally the frequency of occurrence is rare, however increase of disease frequency can be observed in animal families; (iii) functional diseases: This group might be most similar with the term production diseases resulting from a functional overstraining by the required performance, were the environment conditions are not able to compensate the burdens, affecting negatively on the metabolic function (ketosis, milk fever) or the organ system (mammary gland, claws, legs etc). Common to many of the above mentioned diseases is that they have frequently a polygenic background. In most cases, a genetic component can be identified. However, heritabilities for disease resistance, especially including infectious and functional diseases are low in pigs (Henryon et al., 2001) as well as in cattle (Uribe et al., 1995). According to Simianer and König (2002), one reason for the low heritability is the permanent acting selection pressure on disease susceptibility. This trait is under natural selection even before beginning of domestication (in contrast to performance traits) leading to the reduction of genetic variation and fixation of alleles and haplotypes. Additionally, the development of diseases is dependent on environmental factors (infectious pressure, feeding, husbandry conditions) leading to a high environmental variance of the trait. Both elements (low genetic and high environmental variance) lead then to the low heritabilities observed.

Genetic parameters of production diseases

In dairy cattle breeding, indications of negative correlations of milk yield traits and production diseases is being recognized in the last decade (Bergfeld and Klunker, 2002). Functional traits play an important role in many countries. For example, in Germany, France, Denmark or Norway, 50% or more of the cumulative breeding index is explained by functional traits. In the German Holstein population, an increasing genetic trend in genetic gain has only been observed in production and exterior traits but not in traits like somatic cell score in milk, life time production performance and in the fertility complex (VIT, 2006). Frequencies of diseases show a high variation, depending on the definition of the traits and the intensity of data collection. For mastitis, Gröhn and Bruss (1990) reported a mastitis frequency of 7%, whereas Fourichon et al. (2001) reported mastitis frequencies varying between 29% and 51%.

The estimated heritabilities of several production diseases have been low. Using different models, heritabilities between 0.03 and 0.41 have been estimated were most of the estimates are in an interval between 0.02 and 0.09 (Heringstad et al., 2000, Heringstad et al., 2003, Heringstad et al., 2007, Kadarmideen et al., 2000, Van Dorp et al., 1998, Zwald et al., 2004). Reported heritabilities for fertility diseases are also generally low with most values being below 0.05 for metritis and ovarian cysts (Distl, 1992, Lyons et al., 1991, Ouweltjes et al., 1996, Van Dorp et al., 1998). Hinrichs et al. (2006b) clustered the diseases into four categories – fertility diseases, udder diseases, metabolic diseases and claw and leg diseases. Highest frequencies were observed for fertility and udder diseases. Estimated heritabilities varied between 0.02 and 0.05 for fertility diseases, 0.06 and 0.08 for udder diseases, 0.08 and 0.16 for metabolic diseases and 0.01 to 0.03 for claw and leg diseases. The heritability for milk fever was found to vary from 0.04 to 0.13 (Heringstad et al., 2007, Uribe et al., 1995, Van Dorp et al., 1998). Heritabilities for ketosis were described by Van Dorp et al. (1998), Uribe et al. (1995), Heringstad et al. (2007), Zwald et al. (2004), Lyons et al. (1991) and Simianer et al. (1991), values ranged from 0.06 to 0.39. Heritabilities for displaced abomasum were provided by Van Dorp et al. (1998), Uribe et al. (1995) and Zwald et al. (2004), and vary between 0 and 0.28. Retained placenta showed also heritabilities below 0.1 (Van Dorp et al., 1998, Heringstad et al., 2007). For leg and claw diseases, heritabilities were estimated by Simianer et al. (1991) and Lyons et al. (1991) and found to be between 0.02 and 0.17.

Genetic correlations between yield and diseases have been shown to be low (Rogers, 1993, Rogers et al., 1995). However, these estimates showed high standard errors making the interpretation difficult. Pryce et al. (1997) reported a genetic correlation between milk fever and mastitis of 0.64, Zwald et al. (2004) showed a correlation between ketosis and mastitis of 0.17. Heringstad et al. (2005) investigated genetic correlations between clinical mastitis, milk fever, ketosis and retained placenta and found low to moderate values (from -0.1 to 0.40). The largest estimates were between milk fever and ketosis, the lowest were between milk fever or ketosis and retained placenta. Because of positive genetic correlations between the diseases they suggest some general disease resistance factors with a genetic component. Similarly, Hinrichs

et al. (2006a, 2006b) found low genetic correlations between fertility, udder, metabolic and claw and leg diseases, with exception of a moderate (0.59) genetic correlation between fertility and leg and claw diseases.

Selection response for clinical mastitis, ketosis and retained placenta was different in cow groups selected for high and low milk production (Heringstad et al., 2007). The high milk production group showed increasing genetic trends for all three diseases, whereas in the low production groups the trends were decreasing. After five generations, the genetic distance between the selection group of high protein yield and low mastitis frequency was 10 percentage units clinical mastitis, 1.5 percentage units ketosis and 0.5 percentage units retained placenta. From this data, the authors concluded that increasing milk production will lead to an unfavourable selection response in disease resistance. Selection against clinical mastitis will lead to considerable genetic improvement in mastitis resistance. Moreover, a favourable correlated selection in ketosis and retained placenta can be expected.

In pigs, the length of the productive herd life of a sow (LPL) plays a major role in efficient piglet production. Increasing the LPL will reduce replacement costs and increase most probably the total number of piglet born alive during the life span of the sow (Serenius and Stalder, 2004). Thus, sow longevity depends on the ability to survive in the given environment to avoid involuntary and voluntary culling. Failure in reproductive performance including a low number of weaned piglets is one of the most important reason for voluntary culling. Reasons for involuntary culling are leg and claw diseases and complex infection diseases. Generally, commercial pigs differ in the production/reproduction performance, depending whether they are maternal or paternal breeds. Maternal breeds exhibit satisfactory levels of both reproduction and production traits, whereas paternal breeds like Pietrain have high meat yield but lower reproduction performance. Heritabilities for total number of piglets born, born alive, weaned and piglet survival up to weaning are low and difficult to improve by selection (Rothschild and Bidanel, 1998). However, moderate heritabilities are found for those reproductive traits which depend only on the female or male, like age at puberty, standing reflex, ovulation rate, weaning to oestrus interval or testis weight, sperm quantity and sperm mobility respectively. Studies on the heritabilities of length of productive life (LPL) and life time prolificacy (LTP) showed values between 0.05 and 0.12 (Serenius and Stalder, 2004). Leg conformation traits were moderately genetically correlated with length of productive life and lifetime prolificacy. Genetic correlations between average daily gain (ADG) and LPL/LTP were found to be low. However, backfat thickness was correlated moderately (0.22) with survival traits. Tarres et al. (2006) found also an influence of exterior traits (number of teats, and feet and leg score) on the risk of culling, the productive life expectancy and the annual replacement rate. Their data show the possibility to improve sow longevity via indirect selection for exterior traits which was indicated by an increase of LPL from 560 days in the year 2000 to 710 days in the year 2003 in their data set. Regarding leg soundness, the data of Yazdi et al. (2000) show low, but significant favourable correlations between breeding values for longevity and osteochondrosis. Higher osteochondrosis incidence was associated with higher risk of culling. Kadarmideen et al. (2004) found in a Swiss

breeding population a moderate unfavourable genetic correlation between osteochondrosis lesions on different long bones and daily weight gain, feed conversion ratio and percentage of premium cuts. These findings might have implications for pig breeding programs aiming for robust pigs.

QTL and candidate genes for production diseases

Van der Waaij et al. (2000) suggested that selection for production traits under infection pressure will improve genetic resistance and production potential which can be achieved in the absence of infection. Depending on the molecular information (identification of responsible DNA-variation or identification of trait linked DNA-marker), data of direct or indirect DNA-tests can be integrated in the selection process by marker assisted selection (MAS).

Two general methodological approaches have evolved to identify genes contribution to polygenic traits:

2) The candidate gene approach: Genes are designed to be candidates, when either their function (functional candidates) or their position in the genome (positional candidates) give rise to the hypothesis, that they play an essential part in the trait function and variation. Knowledge of function and existence of such genes is often obtained from heterologous species like human, mouse, rat or even microorganisms.

1) The genome scan approach: The hypothesis in this approach is, that within the whole genome loci exist, which have an effect on the trait variation. This effect is verified by a segregation analysis of DNA marker with performance traits. This analysis ends up with the identification of chromosomal regions exerting an effect of the observed trait. Such a locus is called QTL (quantitative trait locus). Since positions affecting traits are identified in the genome by the genome screen, this approach is also called positional genetics. After identifying chromosomal positions, candidate gene approaches can follow to identify the responsible gene.

Pig and poultry

The porcine genetic linkage map now has nearly 3000 loci, including several hundred genes, while the physical genetic map is also growing rapidly and has over 3000 genes and markers. Several recent quantitative trait loci (QTL) scans and candidate gene analyses have identified important chromosomal regions and individual genes associated with traits of economic interest (Rothschild, 2003). Functional genomics has been applied to the genetic dissection of immune response in different ways. The QTL detection based on experimental crosses between lines that differ in their innate and specific immune response is also one of many methods used. Using these methods, the experimental populations have to be custom bred and challenged to study genetic

differences in immune response and map genetic loci underlying these differences in most infectious disease study (de Koning et al., 2005).

Antibody response was one of the first immune competence traits to be examined by QTL analysis (Edfors-Lilja et al., 1998). Many QTL responsible for immune response variation could be detected on various chromosomal regions in studies in mouse, chicken and human (Siwek et al., 2003a, 2003b, Zhang et al., 1998, Zhou and Lamont, 2003a, 2003b, Zhou et al., 2003). By screening these chromosomal regions, evidences for significant association of candidate genes were found e.g. genes related to interferon which plays an important role for primary and secondary antibody response to different antigens as shown in chicken (Zhou et al., 2001). These results in chicken confirmed the genetic association between immune response and disease resistance, thus promoting the improvement of poultry immune competence using MAS (Yonash et al., 2001). The transforming growth factor beta 2 gene (*TGFB2*) was already suggested as candidate gene to improve antibody production in chicken which can be applied in MAS (Zhou and Lamont 2003a). Similar results were found in pig. QTL close to the mast/stem cell growth factor receptor (*KIT*) gene was detected and candidate gene analysis showed significant effects of this gene on the immune response-related traits (Wattrang et al., 2005). The QTL scan done within a Meishan x Large White F2-resource population, bred at INRA, France, revealed a QTL for base cortisol level which may be related to stress and perhaps immune response on chromosome 7 (Milan et al., 1998).

Other experiments focused on the identification of genes that reveal resistance or susceptibility to specific diseases. The major histocompatibility complex (MHC) genes are a group of genes encoding molecules which are involved in the control of immune response and disease resistance, which have been characterised and organised into three classes in mammals. The porcine MHC is called the swine leucocyte antigen (SLA) and has been mapped to the region that surrounds the centromere of SSC7 (Geffrotin et al., 1984). The role of the SLA complex in controlling the immune response and its association to immune traits has been confirmed by several studies. Vaiman et al. (1986) first reported the role of SLA complex on serum haemolytic complement levels, indicating CH50 levels were significantly associated with SLA complex differences. The initial immune response to the hen egg white lysozyme (HEWL) challenge was studied in SLA haplotypes H10 and H12 pigs, and the result indicated higher response in H10/H12 animals than H10 and H12 (Vaiman et al., 1978). The genes coding for the receptors (K88abR and K88acR) to which *E. coli* of the serotype K88 bind, has been mapped on chromosome 13 closely to the transferrin gene (Guerin et al., 1993, Vogeli et al., 1992). Animals lacking these receptors are resistant to diarrhoea caused by this *E. coli* serotypes. Resistance to another *E. coli* serotype causing weaning diarrhoea and oedema disease occurs when receptors to which the *E. coli* fimbria F18 fit are missing. The locus for this receptor has been mapped on chromosome 6 and is to a high certainty identical with the alpha-(1,2)-fucosyltransferase1 gene, *FUT1* (Bertschinger et al., 1993, Vogeli et al., 1996, 1999). The gene for Natural Resistance Associated Macrophage Protein 1 (*NRAMP1*), associated with resistance to Salmonella challenge in mice, has been recently mapped

to pig chromosome 15 (Sun et al., 1998). The causative mutation of many genes is still unknown but fine mapping and candidate gene analysis of the region is underway in many labs.

In our group studies focusing on immunologic parameters as indirect traits for general defence power against a number of pathogens and analyses aiming at the identification of loci associated with resistance to a particular disease in pigs are underway. As part of our porcine genome scan to identify quantitative trait loci (QTL), we examined 7 traits of the immune response in a Duroc x Berlin Miniature pig resource family (DUMI). Complement activity via classical (CH50) and alternative pathways (AH50), antibodies response to *Mycoplasma hyopneumoniae* (Myco), Tetanus toxoid (TET) and PRRS virus, the complement component C3c, and Haptoglobin (Hp) were utilized as phenotypes for linkage mapping. QTL analysis for the porcine immune responses was performed in the F2 and backcross generations of Duroc x Berlin miniature crossbred pigs. In the F2 population, 67 genome-wide significant QTL for complement activity and antibody response to Myco, Aujeszky and PRRS vaccines, including acute phase proteins were found especially on SSC1, SSC2, SSC4 and SSC16 (Wimmers, 2002). In the backcross population, 42 significant and 24 highly significant QTL were detected for all immune traits using the single traits. Most QTL were detected on SSC3, SSC16, and SSC18. No significant F-values were detected on SSC12 and SSC13. Highly significant QTL were detected for antibody response to Myco, TET and PRRS vaccinations, AH50, CH50, C3c and Hp concentration.

By using the candidate gene approach, we analysed the *C3* gene as a direct candidate for general defence power. We identified two polymorphic sites within the coding sequence. The *C3* alleles are segregating in our DUMI resource populations (Wimmers et al., 1999, Wimmers et al., 2001, Wimmers et al., 2003). Association analyses of the *C3* gene with measures of immune function (haemolytic complement activity in the classical and alternative pathway, C3c serum concentration, antibody titre measured prior and after vaccinations with Myco, TET, Aujeszky and PRRS vaccines) were analysed, indicating the *C3* gene were found to be significantly associated with AH50 and CH50. Animals with the more frequent haplotype present in Duroc and other commercial breeds exhibit higher AH50 and CH50 levels than the animals with haplotypes specific to some Berlin Miniature Pigs. The association of *C3* with complement activity reinforces the importance of *C3* as a candidate gene for natural resistance to microorganisms (Mekchay et al., 2003). Another complement factor, complement component 5 (*C5*), mediates potent inflammatory and cytolytic events after proteolytic activation by complement convertase enzymes. Association analysis between *C5* and the immunological parameters were carried out. The homozygote AA was found to be significantly different from the other two genotypes with respect to AH50 and CH50, whereas genotype CC was found to be significantly different from the other genotypes for C3c and HP levels. Association of *C5* with complement activity traits and acute phase proteins promotes pC5 as a candidate gene for innate disease resistance (Kumar et al., 2004). Mannose-binding lectin (MBL) mediates activation of the complement system via the lectin pathway. Two forms of MBL, MBL-A, are encoded by two distinct genes named *MBL1* and *MBL2*. A new single

nucleotide polymorphism of porcine *MBL2* was reported. Association study has indicated that *MBL1* genotypes differ in C3c serum concentration, i.e. in vivo complement activity, at $P < 0.1$. Correspondingly, linkage analysis revealed a QTL of C3c serum level close to the position of the MBL genes on SSC14. The study thus promotes the porcine MBL genes as functional and positional candidate gene for complement activity (Phatsara et al., 2007).

Cattle

Domestic cattle face different nutritional and management challenges which have impact on the incidence of several diseases or various health problems. The main production diseases in cattle are milk fever (hypocalcemia), grass tetany (hypomagnesaemia), ketosis (acetonemia), rumen acidosis, lameness, and fatty liver syndrome (Blowey, 1999). Many of these diseases are strongly linked to metabolic imbalance. Malnutrition clearly influences the ability of the immune system to function, affecting the incidence of some disorders such as milk fever, ketosis and mastitis (Goff, 2006). Most of the disease traits in cattle have low heritabilities, with the exception of abomasal displacement (0.28) and ketosis (0.39) (Uribe et al., 1995, Van Dorp et al., 1998). Use of QTL information in breeding strategies would potentially be advantageous and contribute to understanding of genetic-environment interactions on the molecular level. Mastitis is the number one health problem of the dairy cow, being more frequent than lameness, milk fever, ketosis and abomasal displacement (Goff, 2006). Schulman et al. (2004) have mapped QTL affecting mastitis on chromosome 14 and 18 (120 cM). Holmberg and Andersson-Eklund (2004) also investigated QTL affecting clinical mastitis in Swedish dairy cattle and they found regions on chromosomes 9, 11, and 25. Several QTL affecting clinical mastitis have been identified in Norwegian cattle on chromosome 6 and additional putative QTL on chromosome 3, 4, 6, 8, 14 and 27 (Klungland et al., 2001).

Several studies have mapped QTL for somatic cell score (SCS) or somatic cell count (SCC), an indicator trait for mastitis resistance, on chromosomes 2, 4, 5, 7, 9, 10, 18, 21, 22, 23 and 27 (Ashwell et al., 1998, Heyen et al., 1999, Holmberg and Andersson-Eklund, 2004, Kühn et al., 2003, Rodriguez-Zas et al., 2002, Zhang et al., 1998). In these studies, QTL regions for clinical mastitis and SCS are shown on chromosomes 9 and 27. Heyen et al. (1999) have detected putative QTL influencing mastitis or somatic cell score on chromosome 23 at position 26 cM. This QTL is the location of the bovine major histocompatibility complex (BoLA) which plays an essential role in the induction and regulation of acquired immune response and was mapped to BTA23q21. Sharif et al. (1998) reported a significant association of *BoLA-DRB3-2*16* alleles with low SCS in Holsteins, but with no effects in Jerseys. In addition, Rupp et al. (2007) reported that *BoLA DRB3.2*3* and **11* alleles were associated with low SCC, whereas alleles **22* and **23* were associated with high SCC. Thus, *BoLA DRB3* alleles may be considered as promising potential markers for mastitis resistance or susceptibility.

Sugimoto et al. (2006) found that cows susceptible to mastitis have a three-base insertion in a glycine-coding stretch of the gene for forebrain embryonic zinc finger-like (*FEZL*), a transcription factor with a role in neuronal development. In the same experiment, the *FEZL* region was mapped as a QTL on chromosome 22 and the polymorphism of the length of the glycine stretch of *FEZL* was shown to be significantly associated with SCS.

Youngerman et al. (2004) reported a significant association between *CXCR2* SNP +777 genotype and percentages of sub-clinical mastitis cases in Holsteins. Holsteins expressing genotype GG had decreased percentages of sub-clinical mastitis. However, genotype CC cows had increased percentages of sub-clinical mastitis. This bovine *CXCR2* gene has been mapped to chromosome 2, approximately at 90.3 cM.

A genome scan with microsatellite markers revealed four chromosomal regions that are associated with the lameness phenotype in different lactations stage in Danish Holstein cattle, for the first lactation on BTA 5 and 26 at 44.2 and 56.1 cM, respectively, and for the second lactation on BTA 19 and 22 at 12.1 and 8.1 cM, respectively (Buitenhuis et al., 2007). QTL approaching experiment-wise significance were located on chromosome 6 and 23 for general quality of feet and legs (Hiendleder et al., 2003). Almeida et al. (2007) reported the serum concentrations of cortisol and dehydroepiandrosterone (DHEA) of lame cows which showed a 23% decrease in serum DHEA ($P=0.01$) and 65% higher cortisol:DHEA ratio ($P=0.06$) compared to sound cows. However, in the same experiment, no significant differences were found in candidate gene (*POMC*, *IL-1 β* , *CD62L*, and *GR α*) expression between lame and sound cows. For milk fever, no QTL region is reported. However, genes encoding enzyme and factors involved in calcium and vitamin D may play an important role in development of the disease. Although promising heritabilities of ketosis and displaced abomasum (0.39 and 0.28) were estimated, no QTL results analysing this traits are available. Ketosis is accompanied by marked accumulation of triacylglycerol in liver. Understanding of lipid metabolism in liver, in particular fatty acid oxidation, may allow to develop genetic tools to prevent the development of metabolic disorders like ketosis.

Hypocalcaemia, metabolic alkalosis and negative energy balance, are factors associated with displacement abomasums. Metabolic alkalosis is mentioned as a risk factor for abomasum displacement and can be a cause of hypocalcemia via a reduced sensibility of the receptors for parathyroid hormone (Van Winden and Kuiper, 2003).

In our working group we are interested in the regulation of genetic aspects of fertility in early development since fertility measured as non return rate, birth rate or conception rate has a low heritability and breeding progress has not been not achieved in the last years in Holstein cows. Early embryonic loss is a major cause of low fertility in dairy cows (Morris et al., 2001). In order to increase our understanding of mechanisms leading to normal development, we have focused to elucidate regulation of embryonic survival in cattle. We applied large scale transcriptional analyses of bovine embryo biopsies in relation to pregnancy success after transfer to recipients (El-

Sayed et al., 2006). Based on the development of the biopsied embryos after transfer, biopsies were pooled in three groups: those resulting in no pregnancy (G1), resorbed embryos (G2) and those which developed to a normal calf (G3). Gene expression analysis was done using a home made bovine preimplantation-specific cDNA array (219 clones) and the Blue-Chip (with around 2000 clones). Fifty-two respective 58 genes were differently expressed between G1 vs. G3 resp. G2 vs G3. Biopsies resulted in calf delivery were enriched with genes necessary for implantation (*COX2* and *CDXC2*), carbohydrate metabolism (*ALOX15*), growth factor (*BMP15*), signal transduction (*PLAU*) and placenta specific (*PLAC8*). Biopsies from embryos resulting in no pregnancy were enriched with transcripts involved inflammatory cytosines (*TNF*), protein amino acid binding (*EEF1A1*), transcription factors (*MSX1*, *PTTG1*), glucose metabolism (*PGK1*, *AKR1B1*) and *CD9*, an inhibitor of implantation. Biopsies from embryos resulted in resorption were enriched in transcripts involved in protein phosphorylation (*KRT8*), plasma membrane (*OCLN*) and glucose metabolism (*PGK1* and *AKR1B1*). The genes identified in this study are promising functional candidate genes of influencing early development in cattle. Several of these genes have been implicated in other reports being associated with embryo loss or survival at the early stages of *in vivo* development. However, it is evident, that genetic mechanisms, occurring very early in the development (here at blastocyst stage) have impact on later events of establishing and maintaining pregnancy. It is not clear at this time, how and by which mechanisms the expression of the identified genes is influenced by the metabolic state of the cow. One could speculate that some production diseases will have an impact on oocyte and embryonic regulation of gene expression. Combining the knowledge of the pathophysiology of the diseases with this powerful genome wide transcriptome analyses might pave the way to understand and resolve the molecular pathways required for successful pregnancy und thus contribute to improve selection for fertility in cattle.

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18.2 ANALYSIS OF DNA SEQUENCE VARIANTS IN CANDIDATE GENES IN NORMAL AND BOVINE SPONGIFORM ENCEPHALOPATHY CATTLE

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Problem: With the occurrence of the BSE crisis, several studies have been started to evaluate sequence variabilities in the prion protein gene (PRNP) to assess their possible associations with BSE. In contrast to sheep, goat and humans no functional polymorphisms in PRNP leading to a susceptibility/resistance towards transmissible spongiform encephalopathies (TSE) have been reported in cattle. Recently, however, a promising association between polymorphisms in the regulatory region of PRNP and the disease have been reported. In parallel to these investigations, several genome-wide DNA marker scans have been performed and QTL regions that are significantly associated with BSE susceptibility/resistance (BTA 5 and 17) were detected. Aim Our project was focused on the molecular analysis of functional and positional candidate genes located in the bovine QTL region on BTA 17q23-q24. This region harbors marker INRA025, which is significantly associated with BSE susceptibility/resistance.

Materials and methods: DNA from 84 BSE cattle and 284 non-diseased control cattle (Holstein, Fleckvieh, and Braunvieh) were genotyped for mutations in the candidate genes coated vesicle membrane protein (RNP24), Proteasome 26S subunit, non-ATPase, 9 (PSMD9), and phosphatidylinositol transfer protein, membrane-associated 2 (PITPNM2). Prior to that, discovery of polymorphisms was done by heteroduplex cleaving (Tilling). **Results:** SNPs were detected in all three genes, and allele frequencies were calculated for each SNP stratified according to disease. A single silent SNP was detected in exon 2 (C153T) of RNP24. No significant association between this SNP and BSE susceptibility/resistance was found (Chi², p is equal to 0.5). Four SNPs were detected in PSMD9 (one SNP in intron 2, two SNPs in exon 3, and one in intron 3). The exonic SNPs (C184T and C193T) are silent, whereas SNP C184T is significantly associated with BSE susceptibility/resistance (Chi², p is less than or equal to 0.01). The significance we found also in SNP in intron 2 (Chi², p is less or equal to 0.01). A total of 12 SNPs was identified in PITNM2 (7 exonic, 3 intronic and 2 SNPs in the promoter region). The SNP in exon 4 (G32A) causes an amino acid exchange (R to Q). The SNP C113T in intron 9 revealed a significant association with BSE (Chi², p is equal to 0.01) and the SNP in exon 17 (C90T) also reveal significance (Chi², p is equal to 0.000).

Conclusions: A total of 17 SNPs have been characterized in three candidate genes and their association with BSE susceptibility/resistance was estimated. Chi-square calculations revealed significant associations between four SNPs and BSE. After analysis of further genes located in the targeted QTL region, haplotypes will be constructed to assess their possible effects.

18.3 INVESTIGATION ON RECORDING AND UTILIZATION OF HEALTH TRAITS IN DAIRY CATTLE

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Within the last years the performance level in milk production has increased tremendously. This has as a consequence that functional traits especially health traits could not follow this trend. At the moment there seem to be problems concerning the length of the productive life as well as culling because of health problems. In order to work on that field with methods of modern breeding exact recording of functional traits is essential. Therefore farms have been chosen, which are able to record the necessary information in good quality as well as ensure the assistance from the veterinarian. Data was collected on Saxonian farms from 2000 on resulting in information on about 10.000 active dairy cattle. The average 305 day performance of these farms is between 8.000 and more than 10.000 litre per cow. The veterinary diagnosis for clinical mastitis, mortellaro, panaritium, sole ulcer, endometritis, cycle disorders and cysts mentioned in the herd management program on a PC form the starting point for the definition of the traits. The diseases have been chosen according to the quality of the data and their importance for breeding and economics. The definition is made by a central diagnostic keycode. The exact duration of a disease could not be recorded. Therefore a specific duration of illness has been assumed as the basis for calculating the the sum of days of disease per lactation. The same time span was used to identify repeated therapies which did not result in a new manifestation of the disease. Genetic parameters have been estimated for the recorded traits. Single traits animal models have been used. They considered the following effects: a fixed herd - year - season effect, the number of lactation and the age at first calving as well as the time between two calvings as covariables. The covariables were nested within the first and the following lactations as linear regression. Beside the additive genetic effect of the animal a permanent environmental effect was included for the repeated records. The heritabilities are as low as expected, between one and six percent. The results for the permanent environmental effect of the repetition for the target definition are on the same scale. Since 2006 the estimation of total breeding values is conducted on the basis of these parameters. The results of this estimation will be available for the breeders. This is the first step to use more information about functional traits for breeding decisions in Saxony. In general it would be expected that such traits would be more important for selection decisions in the future.

18.4 INTEGRATIVE IMMUNOGENOMICS AND HEALTH OF THE DAIRY COW: SNPS AND CHIPS AND LATTE TO GO

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Abstract

Mastitis is an economically important inflammatory disease facing the dairy industry, with a complex etiology. Although a number of management strategies have been put in place by the industry, there is an interest in using genetic selection in conjunction with these management strategies to reduce the incidence of mastitis. Since mastitis resistance is a quantitative trait with low heritability, it has traditionally been difficult to make genetic progress for disease resistance. The availability of new genomic tools however, may make this a reality in the next several years. This manuscript provides an overview of some integrative immunogenomic strategies that are being utilized to identify genes conferring mastitis disease resistance. These strategies include establishing associations between polymorphisms in candidate genes and mastitis disease resistance phenotypes, identifying quantitative trait loci for mastitis disease resistance phenotypes by genome scan using single nucleotide polymorphism (SNP) chips, and using microarray analysis to identify novel pathways and candidate genes associated with the host immune response.

Introduction

Infectious diseases of food-producing animals are expensive and damaging to the agricultural industry with ramifications for both animal and human health. Mastitis is the major economically important inflammatory disease facing the dairy industry, causing decreases in milk quality and quantity that account for annual losses in excess of two billion dollars in the US, alone. While management strategies such as vaccination programs, improved milking hygiene, and dry cow therapy have helped to control this complex disease, selection for enhanced production traits has been demonstrated to reduce the genetic resistance of cow's to clinical mastitis (Heringstad et al., 2007). This, along with recent consumer concerns about drug residues in dairy and meat products, the development of antibiotic resistant pathogens, and increased risk of zoonotic diseases has fueled an interest in using genetic selection as a means to reduce the incidence of mastitis in dairy herds. While direct measures of mastitis resistance, such as bacteriology and recorded cases of clinical mastitis, are preferred for selective breeding programs, these measurements have low heritability estimates,

are costly to perform, and are not routinely recorded. Many countries have instead, opted to incorporate milk somatic cell score (SCS), an indirect measurement of mastitis resistance, into genetic selection indices to enhance dairy cow health (Miglior et al., 2005) on the basis of positive and moderate-to-high genetic correlations between milk SCS and clinical mastitis (Rupp and Boichard, 2003). Rupp et al. (2006) recently reported a proof of concept of this strategy using sheep. In their study, intramammary infections occurred less frequently after one generation of breeding for low milk SCS. The long-term impact of genetic selection for low milk SCS however remains to be determined, since reduced milk leukocyte numbers are likely to negatively impact pathogen recognition and initiation of the host immune response during intramammary infections (reviewed by Rupp and Boichard, 2003; Morris, 2006). An alternate strategy to reduce the incidence of mastitis, among other diseases, in dairy herds is to select breeding stock on the basis of enhanced immune responsiveness (Morris, 2006). This strategy has been tested with pigs (Magnusson et al., 1997; Wilkie and Mallard, 1999; Reddy et al., 2000; Crawley et al., 2005; Mallard and Wilkie, 2007) and is being investigated with dairy cows (Hernandez et al., 2003, 2006; Rupp et al., 2007).

Identifying genes and genetic variants that contribute to variation in these quantitative health traits is a focus of our research laboratories. Once identified, the frequency of different alleles that contribute to variation in these health traits may be adjusted within a population through selective breeding to reduce the incidence of mastitis within dairy cows. Resources that are being made available through the bovine genome sequencing project (<http://www.hgsc.bcm.tmc.edu/projects/bovine/>) are likely to have an enormous impact on the way strategies are implemented to improve polygenic traits such as disease resistance in cattle breeds. The recently published assembly of a Holstein-Friesian haplotype block map based on a panel of 15k single nucleotide polymorphisms (SNP) for example, will provide an initial platform for more efficient whole genome genotyping and quantitative trait loci (QTL) detection, though it is estimated that as many as 75-100k tag SNPs will be required to completely cover the bovine genome for genome-wide association studies (Khatkar et al., 2007). The following sections will highlight some of the integrative immunogenomic strategies that are being utilized to identify candidate genes, and genetic markers linked to QTL that may be used in breeding programs designed to improve mastitis resistance through genetic selection.

Bovine toll-like receptor 4, caspase recruitment domain 15, and major histocompatibility complex association studies with milk SCS, clinical mastitis, or immune responsiveness

Milk SCS are comprised primarily of mammary epithelial cells and blood derived macrophages in a healthy mammary gland. During intramammary infections however, blood derived neutrophils become the predominant effector cell population. Although neutrophils are important for eliminating infectious pathogens their effector function also contributes to tissue damage during mastitis. It is therefore, imperative that the host regulates neutrophil trafficking to, and function within the mammary gland. The

trafficking of these different cell populations from the blood to mammary secretions is dependent on genes that regulate pathogen surveillance, and cell adhesion and migration. Many of these genes are constitutively expressed in the healthy mammary gland, while numerous others are induced during intramammary infection. We have focused on identifying polymorphisms in genes regulating pathogen recognition; these genes are likely to contribute to variation in milk SCS and the host response to intramammary infection. Pathogen recognition by the pattern recognition molecules (PRMs) toll-like receptor (TLR)2, TLR4 and caspase recruitment domain 15 (NOD2/CARD15), and the major histocompatibility complex (MHC) class I and II molecules plays a key role in the initiation of the innate and acquired immune response. Since PRMs recognize highly conserved pathogen associated molecular patterns (PAMPs), they are required to initiate the host innate and subsequent acquired immune response (Figure 1). In contrast, MHC molecules present processed peptide antigens to T helper cells and are therefore, essential for the initiation of the host acquired immune response.

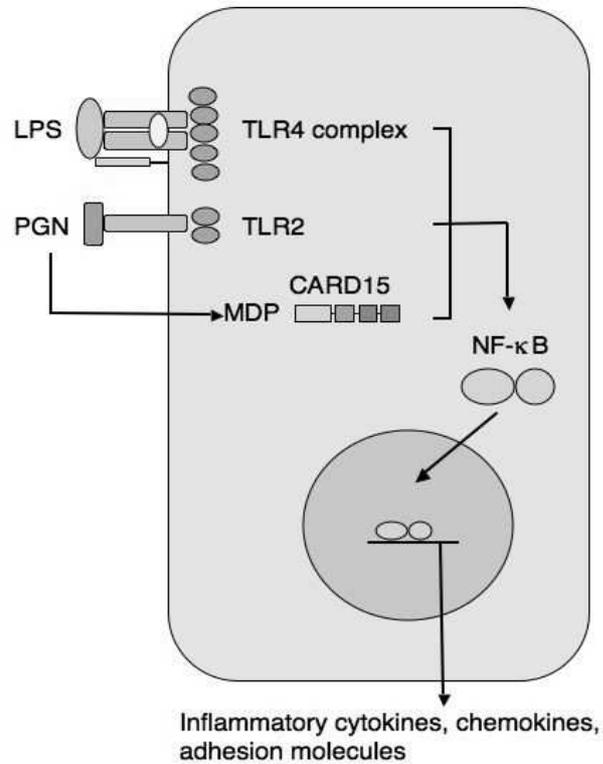


Figure 1: Recognition of bacterial PAMPs; peptidoglycan (PTG), lipopolysaccharide (LPS), and muramyl dipeptide (MDP) by PRMs; TLR2, TLR4, and NOD2/CARD15, respectively. Ligand-receptor interaction leads to the activation of MyD88 dependent/independent pathways that elicit the translocation of nuclear factors such as NF- κ B into the nucleus and subsequent induction of numerous immunoregulatory genes.

The *TLR4* and *NOD2/CARD15* genes are moderately polymorphic in the bovine species (White et al., 2003; Sharma et al., 2006a; Taylor et al., 2006; Wang et al., 2006); variation in the *TLR2* gene has not been characterized. The *TLR2* and *TLR4* genes are strongly expressed during *Staphylococcus aureus* mastitis implicating their involvement in the host response to intramammary infection (Goldammer et al., 2004); *NOD2/CARD15* expression has not been investigated in the bovine species however, polymorphisms in this gene have been associated with susceptibility to Crohn's disease, a chronic inflammatory bowel disease of humans (Quaglietta et al., 2007). Using a DNA pooling technique followed by sequencing of the coding and 5' untranslated regions (Leyva et al., 2006), several SNPs were identified in the Canadian Holstein population for the *TLR4* and *NOD2/CARD15* genes, but none for the *TLR2*

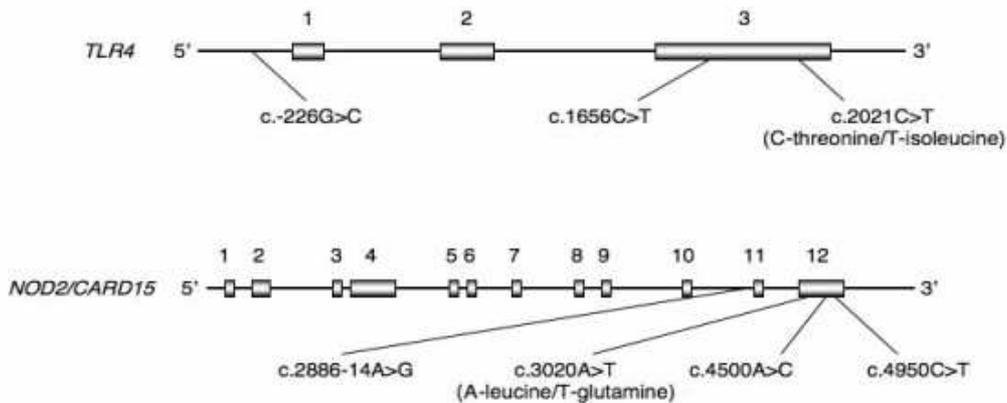


Figure 2. Single nucleotide polymorphisms identified in the bovine *TLR4* and *NOD2/CARD15* genes. The amino acid substitutions are shown in brackets below the non-synonymous SNPs. gene (Figure 2).

SNPs in the *TLR4* gene were associated with milk SCS, among other health and production traits. The C allele of the non-synonymous SNP c.2021C>T for example, was associated with lower milk SCS estimated breeding values (EBVs) ($p < 0.05$) in the Canadian Holstein bull population ($n = 388$ bulls, Sharma et al., 2006a). The C allele of SNP c.-226G>C was also associated with higher milk SCS EBVs ($p < 0.05$) in Holstein cows over three consecutive lactations ($n = 198$ cows, Sharma et al., 2006b). Haplotype reconstruction of the Holstein bull and cow populations was carried out using the SNPs c.-226G>C, c.1656C>T, and c.2021C>T. When all possible haplotypes were compared to the most frequent haplotype, GCC representing 54% and 41% of the bull and cow populations respectively, the CCC haplotype making up 3% of the population was associated with higher milk SCS EBVs in the bull population ($p = 0.04$). The CCT and CTC haplotypes, making up 14% and 26% of the population respectively, were also associated with higher milk SCS EBVs over three lactations in the cow population ($p < 0.05$).

The SNP c.-226G>C was of particular interest because its location upstream from exon 1 raises the possibility that it is within the regulatory region of the *TLR4* gene and may therefore, affect gene expression. *In silico* analysis of a 700 bp region upstream of exon 1 revealed that SNP c.-226G>C was located within potential binding sites for the transcription factors c-Ets-1 (p54), MZF1, and ADR1. Functional analysis of this SNP was carried out by measuring *TLR4* mRNA expression in blood leukocytes from cows with the c.-226G>C genotypes (Figure 3). This SNP was functionally relevant, since cows with the GG genotype had significantly greater *TLR4* expression in response to LPS than cows having the CG and CC genotypes. The functional relevance of this SNP was further validated using a dual-luciferase reporter gene assay (Figure 4). Deletion constructs (-527G, -456C/G, -283G, -199, -99 bp) were prepared from the 5' region of *TLR4* upstream of the ATG start codon in exon 1, cloned into the pGL3 vector containing the firefly luciferase reporter gene, and transformed into DH5a *Escherichia coli* cells. These deletion construct vectors were lipofectamine transfected into bovine mammary epithelial cells (MAC-T cells). Luciferase expression was significantly increased by the -527G and -456G bp constructs, but not by the -283G, -199, -99 bp constructs highlighting the importance of the region between -283 and -527 bp for gene transcription. Comparison of the -456C/G constructs demonstrated that the G allele significantly enhanced gene transcription greater than the C allele, consistent with the *TLR4* gene expression data.

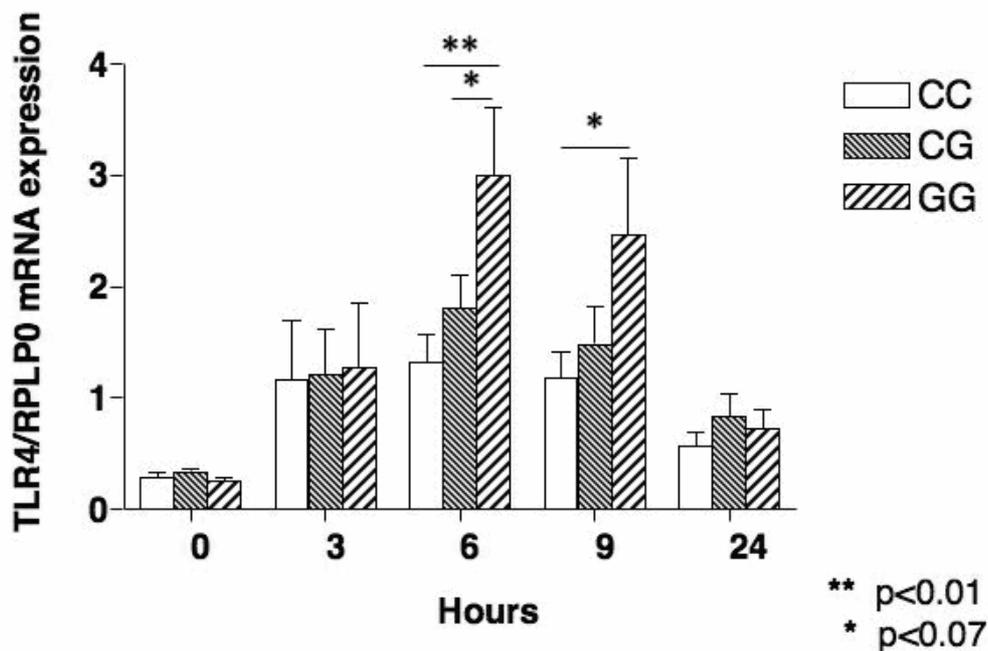


Figure 3. Mean \pm SE blood leukocyte *TLR4* gene expression measured by real-time RT-PCR relative to the RPLP0 house-keeping gene. Whole blood was stimulated with LPS (20 ng/ml) and mRNA extracted over time. Note: n=6 cows per genotype

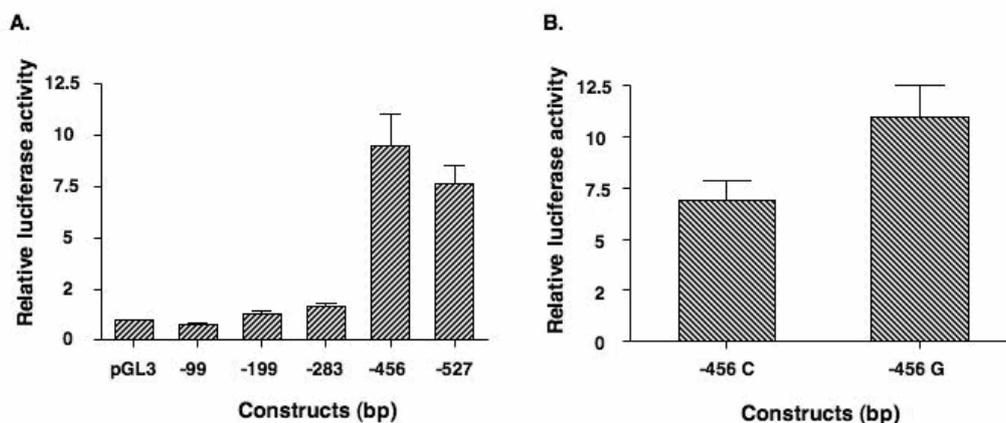


Figure 4. Mean \pm SE relative luciferase gene expression affected by deletion constructs (-527, -456, -283, -199, -99 bp) in the 5' region of the *TLR4* gene upstream of ATG start codon in exon 1 (A), and allelic variants of C/G in the -456 bp deletion construct (B).. Note: These results are representative of 4 independent experiments

A non-synonymous SNP in the *NOD2/CARD15* gene, c.3020A>T, was also significantly associated with EBVs for milk SCS ($p < 0.01$) in the Canadian Holstein bull population ($n = 338$, Pant et al., 2006). This may be a biologically relevant SNP since it is found within the region that encodes the terminal leucine rich repeat (LRR) domain of the NOD2/CARD15 receptor. This LRR domain recognizes muramyl dipeptide (MDP), the minimal bioactive structure of gram-negative bacterial membrane peptidoglycan (PGN). Polymorphisms in this region are believed to alter host recognition of bacterial PGNs leading to inappropriate activation of NF- κ B (Hugot et al., 2001). In this population, bulls carrying the T allele had significantly higher EBVs for milk SCS than bulls with the A allele. Haplotype reconstruction was carried out using two SNPs in exon 12, c.3020A>T and c.4500A>C. When all haplotypes were compared to the most frequent haplotype, AC representing 43% of the population, a trend was observed with haplotype TA; this haplotype represented 26% of the population and was associated with higher milk SCS EBVs ($p = 0.08$). It was interesting to note that of these two most frequently occurring haplotypes, the AC haplotype carried alleles at both loci that are favorable for reducing milk SCS EBVs and increasing production EBVs. This implies that genetic selection based on this haplotype can be used to increase mastitis resistance and production. The functional significance of SNP c.3020A>T is currently under investigation.

Molecules encoded by the highly polymorphic MHC genes are well known to play an indispensable role in induction and regulation of mammalian immune responses and have been implicated with resistance to a variety of diseases (Rothschild et al., 2000;

Stear et al., 2001; Mallard and Wilkie, 2007). In cattle, several studies have documented relationships between bovine MHC (BoLA) class I molecules and resistance or susceptibility to mastitis (Weigel et al., 1990; Mejdell et al., 1994; Aarestrup et al., 1995; Mallard et al., 1995), or adaptive immune responsiveness (Mallard et al., 1995). Other studies have reported associations between *BoLA* class II *DRB* allelic variants with resistance or susceptibility to mastitis (Dietz et al., 1997; Kelm et al., 1997; Starkenburg et al., 1997; Sharif et al., 1998), bovine leukaemia virus (Xu et al., 1993), foot and mouth disease (Garcia-Briones et al., 2000), as well as a variety of other pathogens (reviewed by Stear et al., 2001). To date, *BoLA* associations have rarely been used in commercial settings because of the complex associations with more than one relevant disease or immune response phenotype; however, in the case where one particular disease is highly prevalent in a given environment and causing major health concerns it is possible to utilize *BoLA* associations to reduce disease occurrence. For example, a *BoLA* class II *DR/DQ* haplotype was identified by Maillard et al. (2002) that was highly correlated with resistance or susceptibility to bovine dermatophilosis, and a selective breeding program based on this association was successfully implemented to alter the incidence of this disease (Maillard et al., 2003). Nonetheless, further information is still required to better understand the precise biological mechanisms associated with many of these disease associations before they can be effectively added to the genomic toolbox to improve livestock health and well being.

Recently, data generated by Rupp et al. (2007) on over 300 Canadian Holsteins in the University of Guelph dairy research herds was used to examine associations between expression of *BoLA DRB3.2* allelic variants, immune response, milk somatic cell count (SCC) and clinical mastitis. Cows were evaluated *in vivo* for both antibody-mediated immune response (AMIR) and cell-mediated immune response (CMIR) as the two main components of the adaptive immune system that generally predominate in response to extracellular and intracellular pathogens, respectively. Antibody responses that are dominated by IgG₂ isotype production and interferon- γ are known as type 1 immune responses, driving CMIR; whereas, type 2 responses are dominated by AMIR with IgG₁ or IgA isotype bias, and interleukin-4. Consequently, it was noteworthy, but not unexpected given our current understanding of type 1 and type 2 immune responses (Crawley et al., 2005), that associations between *BoLA DRB3.2* alleles and immune responses tended to be opposing for these two AMIR and CMIR traits. For example, alleles *DRB3.2* *3 and *24 were associated with higher AMIR but lower CMIR; whereas, allele *22 was associated with lower AMIR but higher CMIR. This outcome supports the concept that AMIR and CMIR traits are genetically independent and represent opposing type 1 and type 2 immune responses. Thus, the importance of identifying individuals with the ability to produce both type 1 and 2 responses when general disease resistance is the breeding goal.

In the study by Rupp et al. (2007), *BoLA* allele *DRB3.2**3 was also of particular relevance since it was associated with increased antibody, as well as reduced mastitis and milk SCC. This may reflect a relationship between the ability to produce antibody with a particular isotype and cytokine bias, and enhanced immunity against

intramammary infections caused by extracellular pathogens. Therefore, the *BoLA DRB3.2 *3* allele has been identified as a candidate to further investigate resistance to some types of intramammary infections in Canadian Holstein populations, keeping in mind that it may be associated with lower CMIR and thus higher susceptibility to intracellular pathogens. Discrepancies of this nature are exceptionally difficult to avoid and represent one of the biggest barriers to implementing selective breeding to improve disease resistance based on MHC genotype. Nonetheless, particular *BoLA* alleles can act as reference points for more detailed mechanistic studies and this information can be added to the many new genomics tools currently being investigated to improve animal health and production traits.

Milk somatic cell score quantitative trait loci detection using a SNP chip

The discovery of genetic markers has made it possible to detect regions of the genome that are significantly associated with differences in the expression of a phenotype such as milk SCS, so called QTL. A genetic response can be improved by including the QTL in marker assisted selection, which is a method of selection that makes use of phenotypic, genotypic and pedigree data (e.g., Smith, 1967). In MAS, selection usually does not occur on the QTL directly, unless the genetic marker is the causal mutation, but on the marker that is linked to the QTL through linkage disequilibrium.

In the past, genotyping many markers was expensive and therefore specific experimental designs were developed to reduce the impact of having fewer markers on statistical power. The granddaughter design in dairy cattle for example, makes use of the high sire EBV accuracies due to progeny tests to maximize power while lowering the number of genotyped animals (Weller et al., 1990). However, more recently, high throughput methods have been developed to genotype markers such as SNPs, which have significantly reduced the genotyping costs. It is currently possible to genotype animals for thousands of SNPs with a GeneChip array, and the bovine genome can be covered with a dense SNP map to potentially increase the power of association studies. The QTL detection studies performed to date have found a large number of QTL in dairy cattle for traits of medium to high heritability, such as milk yield and composition traits (Khatkar et al., 2004; Polineni et al., 2006). The advent of high throughput genotyping technology gives hope to finding more QTL for health and fertility traits for which heritability is usually low.

Recently a genome scan was performed using a dense SNP map to detect potential QTL in traits of medium to low heritability, including milk SCS, via variance component linkage analysis (VCLA) (George et al., 2000) and linkage disequilibrium single locus regression (LDRM) (Grapes et al., 2004). The QTL detection was performed using 484 proven Holstein bulls, of which 427 were from 10 grandsire families. Bulls were genotyped for 9919 SNPs using the Affymetrix MegAllele GeneChip Bovine Mapping 10K SNP array (Affymetrix Inc., 2006). Four thousand eight hundred fifty six of the 9919 SNP were located to chromosomes (in base pairs) using the bovine genome sequence (Btau-2.0) at the time of the research, and formed

the basis for the analyses. The SNP cM positions were interpolated from their base-pair positions using a microsatellite framework map available from the National Centre for Biotechnology Information, USA (National Centre for Biotechnology Information, 2006). Bull's EBVs for milk SCS were used as observations and were obtained from the Canadian Dairy Network (CDN), Ontario, from the May 2006 genetic evaluation (Van Doormaal, 2007). Both approaches were effective in detecting potential QTL after accounting for a 5% false discovery rate. The VCLA analysis detected three significant QTL on BTA 14 at positions 22, 53 and 74 cM (likelihood ratio test (LRT)= 11.1, 9.2 and 6.2, respectively) and one QTL on BTA 3 at 41 cM (LRT= 10.8) (Daetwyler et al., 2007). A QTL on BTA 14 close to 22 cM was also reported by Zhang et al. (1998). The LDRM analysis detected SNP associations with milk SCS in twelve BTA. Table 1 shows the significant associations and their agreement with previously reported QTL (Daetwyler et al., 2007).

Gene expression profiling to identify candidate genes associated with mastitis disease resistance and enhanced immune responsiveness

Identification of specific genes and genetic profiles associated with livestock diseases are approaches being investigated to improve animal health. Some studies have examined one or a few candidate genes associated with prevalent cattle diseases; however, genetic interactions and the regulated expression of genes are still poorly understood. cDNA microarrays commonly include hundreds or thousands of known or anonymous genetic elements and have been used as an efficient method to simultaneously determine gene expression profiles in numerous species, including cattle (Burton et al., 2005; Coussens et al., 2002).

Table 1. Significant single nucleotide polymorphism (SNP) associations from linkage disequilibrium single locus regression model analysis for milk somatic cell score

BTA	Location (cM)	Number of SNPs	Maximum absolute t-value
1	131 ¹	4	4.3
2	109 ²	1	3.7
4	32	1	3.9
5	77	2	3.6
5	86	1	3.8
6	52	1	3.0
6	72	1	4.2
6	73	1	2.9
6	74	1	3.4
6	75	1	2.9
6	78	1	2.8
6	81	1	2.8
6	82	1	3.2
6	83	3	3.9
11	119	4	3.5
16	48 ¹	1	3.5
19	47 ²	1	3.2
23	52 ³	2	3.5
25	55	1	3.4
27	28	1	3.3
29	5	1	3.1
29	67	1	3.3

¹In agreement with QTL reported by Rodriguez-Zas et al. (2002)

²In agreement with QTL reported by Bennewitz et al. (2003)

³In agreement with QTL reported by Ashwell et al. (1998)

In some cases, specialized arrays have been developed to study sets of genes with known relevance to the immune system (Alizadeh et al., 1999; Huffman et al., 2006; Sarson et al., 2007). Our group specifically designed a bovine immune-endocrine array that was used to examine the transcriptional mRNA expression of about 200 genes known to be involved in aspects of innate and adaptive immunity (Tao et al., 2004). In one study, genes associated with persistent bovine *Staphylococcus aureus* mastitis were examined in a case-control design (Tao and Mallard, 2004; Tao and Mallard, 2007). Blood-derived mononuclear cells, as well as milk somatic cells were obtained from Holstein dairy cows actively and chronically shedding *S. aureus* and their herd/age/parity/stage of lactation-matched healthy controls. In total, 22 genes were differentially expressed in blood and 16 genes were differentially expressed in milk somatic cells from case versus control samples. The array data were validated by real-time PCR and interpreted in the context of other biological knowledge utilizing gene

pathway and ontology information. Some of these genes, such as interleukin-8, have been previously shown to be involved in other chronic diseases, while others appear to represent novel pathways or genes involved in persistent bovine mastitis (Tao and Mallard, 2007). This is a first step in the goal to improve host resistance to gram-positive bacteria, and to increase our understanding of host response to *S. aureus* mastitis.

Previously an immune response index was developed to classify cows as high, low or average for AMIR and CMIR (Hernández et al., 2003); the concept being that cows high for both AMIR and CMIR may provide a more balanced immune response to diverse pathogens enhancing the potential for improved broad-based disease resistance. By comparing the gene expression patterns of high and low responders it is possible to identify some of the genes involved in regulating these immune response phenotypes. In recent studies, the bovine immune-endocrine microarray was used to evaluate differential gene expression of blood-derived mononuclear cells from cows previously classified as high or low immune responders for either, or both AMIR and CMIR. One year after the original classification, cells from these cows were re-stimulate with the original antigens used to determine the responder phenotype, and mRNA was collected to evaluate differential gene expression (Nino-Soto et al., 2006). Immune response is a quantitative polygenic trait, and as expected the expression of several cell signaling molecules, such as T-cell receptor and MHC genes, differed between cows of high or low responder phenotypes compared to cows with average immune response. Chemokine and cytokine gene expression also varied with the immune response phenotype (Nino-Soto et al., 2006). These studies are part of an ongoing research program to identify genes associated with beneficial immune response phenotypes of livestock that associate with enhanced disease resistance.

Conclusion

A number of different strategies are currently being utilized to identify genes that confer mastitis resistance in dairy cattle. This manuscript has highlighted several of these strategies including: 1) establishing associations between polymorphisms in candidate genes involved in pathogen recognition and mastitis disease resistance phenotypes such as milk SCS and enhanced immune responsiveness, 2) the identification of QTL for milk SCS by genome scan using a dense SNP map, and 3) the use of microarray analysis to identify novel pathways and candidate genes associated with the host immune response. Integrating genomic strategies such as those described above to identify genes involved in mastitis disease resistance appears to be a promising first step in the process of improving resistance to this disease through genetic selection. The biological function of each of these genes however, needs to be sufficiently characterized before they are incorporated into breeding programs to help circumvent any detrimental effects on dairy cow immunity that may occur as a result of long-term selection.

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18.5 INFLUENCE OF COW FACTORS ON THE TEAT CONDITION IN DAIRY COWS

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In the following study, cow factors that might have an impact on severe teat end hyperkeratosis and udder health were assessed. Furthermore, possible relationships between teat condition and udder health were evaluated. The study was conducted on 20 dairy farms in North Germany with an average of 64 lactating cows (German Holstein). From each udder quarter, foremilk samples were collected three times in weekly intervals during the afternoon milking. Before sampling, teat condition was evaluated by teat end scoring. The milk samples were examined cyto bacteriologically. The udder tissue was palpated following milking. From each animal, number and stage of lactation and mastitis category for all udder quarters based on somatic cell count and bacteriological finding were recorded. Overall, 1282 animals with 5064 examined udder quarters participated in this investigation. In 226 udder quarters with a positive bacteriological finding (n=508), udder pathogens could be found at least twice. Therefore, 4.46 % of all udder quarters were considered as infected. With increasing number of lactation, there was a rise in frequency of clinical mastitis, udder infections and unspecific mastitis. With regard to the stage of lactation, macroscopic changes of the udder secretion and unspecific mastitis were observed more often towards the end of lactation. The teat end score on udder quarter basis was primarily influenced by the factors herd and cow. The present status of infection did not have an influence on the teat end score. A statistically significant influence on teat condition was found for the stage of lactation, quarter localization, and as a trend for palpation findings and number of lactation. According to those results, the highest probability of severe teat end hyperkeratosis was found for the second stage of lactation (101-200 d), front quarters, second lactation and marked morphological findings based on udder palpation.

In conclusion, the teat end score on udder quarter basis was primarily influenced by farm and cow factors. An independent effect was also found for age, stage of lactation and quarter localization. Whereas only a weak association seems to exist between teat condition and udder health, the cause relationships of which still need to be elucidated.

18.6 IMPACT OF KETOSIS ON REPRODUCTION AND MILK PRODUCTION OF IMPORTED JERSEY COWS IN INTENSIVE DAIRY FARMS OF THE PERIURBAN AREA OF DAKAR.

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The aim of this study was to assess the effect of ketosis on reproduction and milk production of imported Jersey cows reared in the periurban area of Dakar. 45 pregnant cows have been followed up since the ninth month of pregnancy until 12 weeks postpartum with blood BHB and progesterone assays, body condition score notation and two dairy control at one and two months of lactation. The results showed that at 8 weeks post-partum the cumulated proportions of clinical and subclinical ketosis were respectively 26,66% (n=12) and 37,78 % (n=17). Only 35,56% (n=16) of the 45 cows did not show ketosis during the study. The maximum of subclinical ketosis prevalence has been recorded at 4 week post-partum with 54,7% of high blood BHB levels and the maximum of clinical ketosis was recorded at 6 weeks with 19,1% of cases. It has been noticed that increased blood BHB level during the last month of pregnancy resulted in high risk of abnormal calving. There is a significant difference of the means lengths of ovarian activity resumption after calving between cows with physiological Blood BHB during the 2nd week after calving and the cows with subclinical ketosis ($p<0,05$) and cows with clinical ketosis ($p<0,01$). These lengths were respectively $32\pm 7,5$ days, $45\pm 9,3$ days and $53\pm 1,2$ days post-calving. The clinical and subclinical ketosis resulted in the decrease of respectively 25,6% and 16,5% of the daily milk yield when the cows with physiological BHB have an increment of 18,7% of the milk production between 1 and 2 months post partum. It is noticed that 80% of ketosis cows at 6 week post calving have had a moderate loose (0,5- 1) or a severe lose (>1) of their BCS since the calving date.

Key words: ketosis, Jersey cows, BHB, reproduction, dairy production, Senegal.

18.7 PARASITIC PREVALENCES IN BEEF BOVINES IN TWO FARMS OF TUSCANY

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Pathologies caused by gastrointestinal parasites in farmed bovines can determine serious productivity losses in milk and meat. In the years 2004-2006, coprological analyses were performed in bovines of the Chianina beef breed, raised according to cow-calf line, in two farms in Val Tiberina (Tuscany), during November when animals returned from pasture. In farm Pieve 15 bovines in 2004, 36 in 2005 and 36 in 2006 (mostly cows) were examined. In almost all cases, there was no significant difference among yearly prevalences of animals positive to the various parasites. Mean prevalences were: for *Eimeria* spp (31%), *Fasciola hepatica* (7%), *Dicrocoelium dentriticum* (16%), *Moniezia benedeni* (2%). For gastrointestinal Strongyles spp instead, the mean prevalence in 2004 and 2006 was 18%, while the prevalence was significantly higher in 2005 (72%). In farm Fresciano 13 bovines in 2004, 40 in 2005 and 30 in 2006 were examined. For all parasites significant differences in yearly prevalences were evidenced. The prevalence of *Eimeria* spp (80% in 2004) diminished in the following years (mean 11%). An analogous trend was observed for Strongyles spp (from 77% to 33%) and for *Fasciola hepatica* (from 38% to 10%). For *Paramphistomum* spp, there was instead a significant increase in the years from 69% to 97%. *Dicrocoelium dentriticum* appeared in 2005 (38%) and diminished significantly in 2006 (10%). *Moniezia benedeni* appeared in 2006 (10%). Although in these farms some anthelmintic treatments are carried out, they are probably performed in a defective way, because either prevalences diminished but not enough or they remain constant. In some cases new parasites appeared.

18.8 HAPTOGLOBIN IN SYNOVIAL FLUID OF PIGS WITH ALTERED JOINT SURFACES

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The acute phase protein haptoglobin (Hp) is known to be elevated in synovial fluid of osteoarthritic joints in humans (1). Furthermore Hp mRNA is shown to be increased in tissue homogenates of rat Achilles tendon with experimentally induced arthritis (2). Leg weakness, a syndrome which is often accompanied by degenerative alterations of the articular cartilage, is one of the most serious problems in the pig industry.

The objective of this study was to determine the relationship between Hp and histopathological alterations of joint surfaces in fattening pigs. **Material & Methods:** From 139 fattened pigs (105-110 kg) of a F2 crossing from Pietrain x Duroc and Duroc x Pietrain, blood and synovial fluid from the shoulder, elbow, hip and knee joint of one carcass half were collected after slaughter. The Hp concentration in serum and synovia was analyzed by an ELISA (3), the total protein in synovial fluid was measured with a commercial BCA kit (Sigma). In addition the joint surfaces of the proximal and distal humerus and femur (representing shoulder, elbow, hip and knee) were histopathologically classified into four groups (no alterations, marginal alterations, severe alterations, massive alteration) (4). **Results:** Hp concentrations in porcine synovia ranged from 0.3 µg/mL to 1590 µg/mL (median 172 µg/mL). Serum Hp and Hp in synovia were correlated ($r=0.58$; $p=0.01$). The total protein in synovia from joints with massive alterations was higher compared to joints without alterations ($p=0.003$), but Hp concentrations in synovial fluid from joints with different histopathological scorings were not different. However, significant differences in the concentrations of haptoglobin ($p=0.008$) and protein ($p<0.001$) among the different joints could be determined, whereas the synovial fluid from the hips showed the highest concentrations.

Conclusions: The histopathological alterations of the joints might be attributed to impairments belonging to the past and therefore the point of sampling might be unsuitable to detect relationships between a sensitive acute phase protein and cartilage alterations. However, the elevated total protein in joints with massive alterations does not support this assumption. **References** 1 Willumsen and Fries (1975): *Scand J Rheumatology* 4: 234-240 2 Smeets et al. (2003): *Int. J. Exp. Path.* 84: 69-743 3 Hiss et al. (2003): *Vet Immunol Immunopathol* 96: 73-824 4 Rudolph et al. (2007): *Proceedings of the ADSA-PSA-AMPA-ASAS Joint Annual Meeting 2007 San Antonio, TX*

18.9 ARACHNOMELIA IN FLECKVIEH AS AN EXAMPLE OF MONITORING AND CONTROL OF AN INHERITED DISEASE IN CATTLE

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Problem: Severe genetic diseases in cattle are usually recessively inherited. Therefore, the frequency of affected animals is very low and most of the diseases are not recognized as inherited. However, in some cases, e.g. after inbreeding or in the case of the intensive use of single sires carrying a disease allele, the increase of the frequency of the mutation will lead to a larger number of affected animals. **Aim:** To avoid economic losses, as well as to protect animals from potential suffering, a monitoring system for inherited diseases should be established for the main Bavarian cattle breeds. Furthermore, effective control measures shall be developed and applied.

Material and methods: At the 'Institut für Tierzucht, Bayerische Landesanstalt für Landwirtschaft, Grub' (ITZ) a monitoring system has been established in cooperation with the 'Erzeugerringe für tierische Veredelung in Bayern e.V., Munich' (LKV). A questionnaire was developed that is filled in by the farmer. The information is revised by qualified personnel and collected in a database at the LKV. This system is complemented by a project in cooperation with the 'Tiergesundheitsdienst Bayern, Grub' (TGD). Within this project, malformed calves are diagnosed pathologically. From these calves tissue and DNA samples were collected for molecular genetic analyses.

Results: The effectiveness of the monitoring system and the use of different control measures, from the identification and flagging of carriers to the development of a genetic testing system, could be shown using arachnomelia as an example. At the end of the year 2005, thirteen calves with similar pathological symptoms resembling the 'Syndrom der Arachnomelie und Arthrogrypose', as initially described in Braunvieh cattle, were diagnosed. By pedigree analysis a single founder could be identified and it has been shown that the SAA in Bavarian Fleckvieh is most likely an autosomal recessively inherited disease. Until April 2007, a total of 26 sires could be identified as carriers of the disease. These were excluded from use in artificial insemination. Two sires with only one affected calf could be identified as non-carriers by means of statistical analysis of inbred matings. Genetic mapping of the disease is underway using tissue samples collected in the course of the monitoring program.

Conclusions: Continuous and effective monitoring is a prerequisite for the control of inherited diseases. It could be shown that the Bavarian monitoring system provides an early alert system and the collection of material provides a good starting point for the development of a genetic testing system.

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