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STUDY ON MINERAL METABOLISM OF DAIRY COWS

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1 Introduction

All minerals and their deficiencies affect the production and reproductive function of dairy cattle and can lead to severe sometimes life threatening diseases or at least impaired reproductive performance. Many cows are culled because of poor fertility, uterine disorders, mastitis or milk fever. The proper supply of dairy cows with minerals can prevent such health problems and furthermore improve the reproductive and productive performance. Macro- and micro-minerals affect the reproductive function due to their impact on metabolism, maintenance and growth. Decreased fertility is often the first sign of deficiencies ranging among the border although other clinical signs are not obvious (HURLEY and DOANE, 1989). Inadequate blood levels of macro minerals such as calcium, phosphorus, magnesium or potassium are associated with lower feed intake, poor gastrointestinal motility, decreased productive performance and increased susceptibility to diseases. The minerals play roles in important body functions such as nerve irritability, muscle function or homeostasis and their deficiencies can lead to metabolic disorders such as the “downer cow syndrome”. This disease occurs after parturition and the animals lose the ability to rise to their feet (GOFF, 2004).

Not only macro minerals are important for the health and productivity of lactating dairy cattle. Also trace elements like zinc, manganese, copper and cobalt are needed for protein synthesis, vitamin metabolism, formation of connective tissue and immune function (SICILIANO-JONES et al., 2008). The terms micro-minerals and trace elements are used for minerals or elements which are needed only in small amounts and also supplied at low levels. Although they are needed in small amounts trace elements play a role in many body functions mostly as components of enzymes which are needed for metabolic, immune and reproductive functions (VANEGAS et al., 2004). A proper supply with these elements can affect dairy production and lactation, reproduction and fertility as well as health aspects such as claw integrity or immune function. Furthermore recent studies showed that the bioavailability of mineral supplements is higher if the source is based on organic forms like amino acid complexes compared to inorganic supply. Due to this the retention of trace elements is better for organic supplements and so the effects of the minerals such as increased milk production and milk protein or better claw integrity are more expressed (SICILIANO-JONES et al., 2008).

On the other hand the incidence of such productive and reproductive diseases increased with the years due to genetic selection for higher milk yield and so higher demands for appropriate nutrition. The diets and body stores are not able to cope with the high demands especially in case of high productive stages. Due to these facts metabolic problems can develop which manifest as severe diseases such as milk fever and displaced abomasum as well as minor diseases like lameness and mastitis or reproductive issues such as retained fetal membranes and infertility. Therefore these diseases are called production diseases and caused by the tendency to breed animals with higher and higher production levels (MULLIGAN and DOHERTY, 2008).

2 The general aspects of different minerals – REVIEW

2.1 Macrominerals

2.1.1 Calcium

2.1.1.1 Function

99% of the calcium is bound to the skeleton which supports the muscle function and movement, is important for the body growth and protects important organs. The calcium is needed for the mineralization and ossification of the growing bone.

Calcium also has non-skeletal functions and 1% of the calcium in the body can be used. It is bound to serum proteins or available in acids or as free ions. 50 to 60% of the total calcium in the plasma is ionized which is needed for nerve conduction, the contraction of muscles, cell functions, activation of enzymes or blood clotting (SUTTLE, 2010).

Furthermore calcium is involved in mechanisms during the synthesis of steroids in the ovaries and adrenal glands such as the usage of cholesterol by the mitochondria or the conversion of pregnenolone to progesterone. Also the steroidogenic pathway in the placenta depends on calcium. Even the release of LH from the pituitary gland is impaired due to inadequate Ca levels. The above mentioned functions of calcium can explain the impact of calcium concentration in the body on the reproductive performance of a cow (HURLEY and DOANE, 1989).

2.1.1.2 Metabolism, absorption and homeostasis

The optimal blood calcium concentration ranges from 8.5 to 10mg/dl (GOFF, 2004) and its metabolism is regulated by the uptake from the diet in the small intestines and the movement of calcium into and out of the bones. The absorption from the small intestines is regulated by the parathyroid hormone (PTH) and dihydroxycholecalciferol (1.25-$(OH)_2D_3$). PTH is excreted when the concentration of calcium in the extracellular fluid decreases. This process stimulates the double hydroxylation of vitamin D3 to 1.25-$(OH)_2D_3$ in the kidneys, bone marrow, skin and intestinal mucosa. If the calcium concentration exceeds 1 mmol/l calcitonin is secreted which inhibits the hydroxylation of vitamin D3.

There are two more substances which are important for calcium metabolism – osteocalcin and PTH-related protein (PTHrP). Osteocalcin is important for the bone growth. Short before calving it decreases and stays low for 14 days or longer to reduce the inbuilding into the bone tissue. The PTHrP is expressed and released by the mammary gland. It mimics PTH in the maternal circulation to increase the absorption of calcium (SUTTLE, 2010).

2.1.1.3 Interactions

The availability is reduced by magnesium deficiency which causes a reduced effectiveness of PTH and vitamin D. Dairy cows before parturition should receive around 0,4% of DMI to reduce the risk of milk fever. Adversely, high levels of phosphorus contribute to milk fever and should not exceed 0.3% of DMI (WILDE, 2006).

On the other hand an increased Ca concentration may also impair the reproductive performance as it inhibits the absorption of other minerals like phosphorus, magnesium, zinc, copper and other trace elements and causes a secondary deficiency of these minerals (HURLEY and DOANE, 1989).

2.1.1.4 Sources

Calcium can be found in forages (especially alfalfa), legumes and additives like lime, limestone or calcium-chloride (SUTTLE, 2010).

2.1.2 Chlorine

2.1.2.1 Functions

Chlorine is the most important negatively charged mineral in the body fluids as it maintains osmotic pressure and plays the counterpart to cationic elements like sodium and potassium. Furthermore it can pass the membrane of erythrocytes and due to this it stimulates the movement of other minerals between plasma and red blood cells. As a result of this mechanism CO_2 can be fixed and then liberated in the lung. Chlorine even contributes to formation of hydrochloric acid and activation of enzymes such as α-amylase in the gastric juice (GEORGIEVSKII et al., 1982a).

2.1.2.2 Interactions

The concentration of chlorine in the serum is adjusted to the levels of positive charged minerals like sodium or potassium. This mechanism is needed to keep the electroneutrality of the serum as chlorine has a negative charge. The regulation is especially related to Na levels as sodium and chlorine are both found in the extracellular compartment (CHAN et al., 2005).

2.1.3 Magnesium

2.1.3.1 Functions, homeostasis and absorption

Magnesium is an important intracellular cation and needed as a cofactor for enzymatic activities which influence metabolic reactions. Extracellular it is needed for normal nerve irritability, function of muscles and bone formation. To fulfill these functions a plasma concentration of 1.8 – 2.4mg/dl is needed. The concentration is maintained by gastrointestinal absorption and secretion of Mg (GOFF, 2004) as well as renal excretion (PALLESEN et al., 2008). Magnesium is absorbed in the rumen and reticulum and secreted in the small intestines. There are different factors which influence the absorption such as ruminal Mg concentration and a functioning active transport mechanism which is sodium dependent. The solubility of the mineral in the rumen depends on the dietary content, the ruminal pH and magnesium binders in forages. If the content in the diet is low and the pH over 6.5 the solubility of Mg decreases whereas binders form insoluble salts and complexes with magnesium. Also the transport of Mg through the rumen can be influenced. Sodium is needed for the transport and low levels in the ratio impair the absorption as well as high potassium contents. Also the water content of the diet can alter the transport by increasing the passage through the rumen (GOFF, 2004).

2.1.3.2 Requirements and deprivation

Optimal dietary magnesium concentration ranges between 0.35 and 0.4% (GOFF, 2004). There is a sharp decrease of plasma magnesium concentration short after parturition (RAMOS-NIEVES et al., 2009). This deprivation blocks PTH secretion and reduces tissue sensitivity to PTH leading to altered calcium metabolism. It contributes to hypocalcaemia by this way. Furthermore reduction of blood Mg levels leads to tetany which is also contributed by plasma Ca levels <5mg/dl. The hypomagnesemic tetany can be treated by intravenous application of 50 to 75% of the extracellular magnesium concentration (GOFF, 2004).

2.1.4 Potassium

2.1.4.1 Functions

Also potassium contributes to the maintenance of osmotic pressure and cation-anion equilibrium. It plays a role in cellular metabolic activities, activation of enzymes, nerve and muscle function and excitation, heart function and oxygen transport in erythrocytes. In the forestomachs potassium supports the buffer system as well as the moistureness of the ingesta. Due to this a proper environment for microbial activity is maintained (GEORGIEVSKII et al., 1982a).

2.1.4.2 Interactions

A high potassium level has an adverse effect on calcium homeostasis, metabolism absorption and resorption and is one cause for hypocalcaemia after parturition due to this (RERAT et al., 2009). To prevent hypocalcaemia and milk fever dietary potassium should range around 1% (GOFF, 2004). Furthermore a high content of potassium in the ruminal fluid can decrease the absorption of magnesium and lead to lower plasma magnesium levels (RERAT et al., 2009).

2.1.4.3 Sources

Potassium can be found in forages, grass and grass-legumes in high amounts. Especially the pastures treated with fertilizers based on organic manure contain high potassium concentrations as the high potassium levels of the organic slurry are transferred to the soil. It is not supplemented with other sources and so different forages can lead to different potassium concentrations in the diet (RERAT et al., 2009).

2.1.5 Phosphorus

2.1.5.1 Functions

Phosphorus is an important component of nucleic acids, nucleotides, phospholipids, coenzymes and proteins. As part of the above mentioned substances it is needed for the transfer and utilization of energy (ADP, ATP), phospholipid metabolism as well as phospholipid and cAMP synthesis. These functions affect also the reproduction and fertilization (HURLEY and DOANE, 1989). Furthermore it is essential for buffer systems and bone mineralization (GOFF, 2004).

2.1.5.2 Homeostasis

The homeostasis is controlled by absorption from the gastrointestinal contents, excretion especially with the urine and reabsorption. These mechanisms keep the plasma P levels in balance – a hormonal control is not needed (RAMOS-NIEVES et al., 2009). To keep the homeostasis dietary phosphorus level should be around 0.4% of dry matter (GOFF, 2004).

2.1.5.3 Interactions

Plasma P levels are influenced by dietary Ca levels. Low Ca content in the diet increases the plasma P levels and high calcium levels decrease the phosphorus. During parturition P and Ca levels decrease. First calcium recovers due to the activity of PTH. In a second stage of recovery P and Ca concentrations increase again due to renal and intestinal absorption (RAMOS-NIEVES et al., 2009). On the other hand too high P levels (~6mg/100ml) impair the proper calcium homeostasis. An enzyme which converts 25-hydroxyvitamin D to 1.25-dihydroxyvitamin D is inhibited and so the production of the hormone needed for intestinal calcium transport is blocked (GOFF, 2004).

2.1.5.4 Deficiency

Phosphorus deficiency affects most cell types in the body and so other signs are often apparent before phosphorus induced fertility develops. Nevertheless inadequate concentration of P can be accompanied by altered or irregular estrus, decreased conception rates, higher incidence of cystic follicles, reduced ovarian activity or just generally decreased reproduction (HURLEY and DOANE, 1989). More general signs are unthriftiness, slow growth and decreased productive performance during lactation (PALLESEN et al., 2008).

2.1.6 Sodium

2.1.6.1 Functions and metabolism

Sodium is essential for normal activities of tissues like heart muscle, the role of buffer systems, excitability of nerves and muscles as well as the maintenance of osmotic pressure of fluids. The mineral also supports the activity of the ruminal microflora and produces in form of bicarbonate a buffering system in the forestomachs. This buffer system helps the transport of fatty acids.

Sodium is excreted and reabsorbed in the convoluted tubules of the kidneys controlled by aldosterone. The brain regulates the excretion of sodium, too. Especially in the hypothalamus sodium- or osmo-receptors can be found which react on drops of sodium concentration in the cerebrospinal fluid. In case of lower Na levels in the brain the excretion of the mineral through the kidneys is reduced (GEORGIEVSKII et al., 1982a).

2.1.6.2 Requirements and sources

Many feedstuffs don´t contain enough salt for cows in late gestation. A supplementation of small amounts of salt can help to reach the needed requirements of ~0.12%. On the other hand unlimited access to salt is also not beneficial because it increases the risk of udder edema (GOFF, 2004).

2.2 Micro-minerals and trace elements

2.2.1 Cobalt

2.2.1.1 Functions

Cobalt is essential for the synthesis of cobalamin (vitamin B12) but can also exist as a free form. The free form can be taken up selectively from cell organelles and so the effects of cobalt are not limited to cobalamin synthesis.

The main symptoms of cobalt deficiency are general ones like anemia and un-thriftiness leading to secondary reproductive disorders such as the reduction of conception rate, abnormal estrus, delayed puberty, impaired ovarian function, abortion, weak newborns and general infertility. If the deficiency is not severe the calves are born alive and weak but die after a short time.

The supplementation of deficient herds with cobalt can alleviate the present disorders. Cobalt supply increases the conception rates and the intensity of estrus and decreases the risk of silent and irregular estrus (HURLEY and DOANE, 1989).

2.2.2 Copper

2.2.2.1 Requirements and sources

Optimal plasma and serum concentrations are 9.4µmol/l and 7.5µmol/l. They can be maintained by depletion of the copper stores in the liver (MULLIGAN et al., 2006). During the peri-parturient period plasma concentrations are changed. Also other substances like sulphur, iron, calcium, zinc, molybdenum and strong reducing agents influence the activity of copper. Their excess decreases its availability. Especially

Molybdenum reduces the ability of the liver to store copper whereas cobalt has a positive impact on the functions of copper.

In plasma copper is bound to ceruloplasmin which is secreted from the liver. After absorption from the intestines Cu enters the blood stream and binds to the albumin of amino acids (HURLEY and DOANE, 1987).

2.2.2.2 Functions

Generally copper is a component of several metalloenzymes and has many functions. The most important ones are anti-oxidant activities in form of superoxide dismutase (SOD), respiration of cells, heart function, bone mineralization, metabolism of lipids and carbohydrates, functions of the immune system, development of connective tissue, keratinization and myelinisation of nerve tissue like the spinal cord. Unbound inorganic copper has pro-oxidant effects and can stimulate lipid peroxidation (ANDRIEU, 2008).

Cu containing proteins have enzymatic functions in the body. It influences the activity of superoxide dismutase which produces hydrogen peroxide from superoxide. Hydrogen peroxide is utilized further in three different ways. It can be used in phagocytic lysosomes for oxidative processes or catalase in peroxisomes degrades them or they are reduced by glutathione peroxidase.

A reproduction specific function is the maintenance of the activity of hypophyseal hormones in the blood. Furthermore it supports the work of prostaglandin E2 (PGE2) in the body by enhancing the PGE2-receptor bonding. Copper is released extracellular at the axonal terminal where it regulates the activity of prostaglandin E2 on neurons releasing LH (HURLEY and DOANE, 1989).

Another function of copper is to strengthen the claw horn in form of the enzyme thiol oxidase. This enzyme forms disulfide bonds between keratin filaments. Another enzyme depending on copper is lysyl oxidase which strengthens the suspensory connective tissue within the claw capsule by cross-linking collagen fibers. If the connective tissue around the distal phalanx is overloaded with tension, the corium is compressed and claw lesions can develop (SICILIANO-JONES et al., 2008).

2.2.2.3 Deficiency

Copper deficiency can be caused either due to insufficient supply or due to impaired utilization and result in reduced fertility. As an example cows grazing on copper deficient pastures show signs like infertility and depressed or delayed estrus. Especially in female animals copper deficiency manifests in reproductive problems. The main reproductive disorders caused by inadequate copper status are early embryonic death, depressed ovarian activity, altered estrus, lower conception rates, increased incidence of retained fetal membranes, problems during parturition and congenital rickets.

Copper supplementation in such cases can increase the conception rate from 53 to 67%, if cobalt is supplied to it is enhanced up to 93%. The addition of Cu and Mg to the diet has a positive impact in the fertility (HURLEY and DOANE, 1989).

2.2.2.4 Toxicosis

Copper toxicosis in dairy cattle is chronic process caused by excess dietary copper contents over a longer period of time. The mineral is stored in the liver and can be released in case of decreasing blood concentration to balance it. As the liver Cu concentration depends and reflects the dietary copper level more copper is stored if the feeding level increases. Due to this excess Cu supplementation over a longer period can damage the liver and necrotized the liver cells. Other symptoms are hemolysis of erythrocytes, jaundice and apathy. Very severe cases show signs of hepatic coma and spasms and die in the end (GEORGIEVSKII et al., 1982b).

2.2.3 Iodine

2.2.3.1 Functions and deficiency

Iodine can occur bound to proteins or in the thyroid gland, is required for the synthesis of the thyroid hormone and affects even the fetal thyroid activity. In case of deficiency the fetal development can be stopped at any time leading to early embryonic death, resorption of the fetus, stillbirth and abort as well as weak and goitrous calves. Furthermore it can be accompanied with prolonged gestation and parturition or retained fetal membranes. Protein bound iodine reduction is accompanied by abortion whereas a combination with decreased thyroid iodine is more likely to cause ovarian cysts. If the deficiency is moderately but lasts for a longer period the ovarian function is impaired and the sexual maturity can be delayed. The above described effects can be caused by decreased gonadotropin outputs from the pituitary gland in case of hypothyroidism (HURLEY and DOANE, 1989).

Deficiency of iodine during pregnancy can lead to goiter (MULLIGAN et al., 2006) which is the main form of manifestation. Although they are important reproductive disorders occur only as secondary manifestations. Sometimes reproductive disorders and goiter can occur together. Goiter combined with irregular or suppressed estrus or even infertility indicates a necessary therapy with iodine.

On the other hand increased levels of protein-bound iodine increase the fertility and reproduction and the repletion of iodine concentrations after deficiency restore the sexual functions. Supplementation decreases the services per conception as well as the risks for stillbirths, abortion and retained fetal membranes. Excess of iodine in the level of toxic amounts impairs the reproductive performance, too, leading to abortion or deformities of the newborn calves (HURLEY and DOANE, 1989).

2.2.4 Manganese

2.2.4.1 Functions

General functions of manganese are anti-oxidative actions, growth of bones, metabolism of carbohydrates and lipids, support of reproductive performance and functions of the immune and nervous system (ANDRIEU, 2008). Manganese is needed for the production of proteoglycans which can be found in the synovial fluid, cartilage and loose connective tissue. Galactotransferase and glycosyl transferase are Mn-dependent enzymes and needed for the formation of proteoglycans.

It also has an impact on the synthesis of steroids and muco-polysaccharids or Mn-dependent metallo-enzymes like hydrolases, kinases, decarboylases and transferases. Furthermore Mn is involved in redox-processes and blood formation, tissue respiration, bone formation and growth as well as function of endocrine organs and reproductive issues related to the endocrine functions.

Manganese can also influence the fertility of the cow due to its importance for cholesterol synthesis (SICILIANO-JONES et al., 2008). In case of deficiency the function and synthesis of cholesterol and its precursors is inhibited (HURLEY and DOANE, 1989). Cholesterol is needed for the synthesis of steroids like estrogen, progesterone and testosterone. Also the corpus luteum can possibly be influenced by the content of manganese in the diet and supplements because it contains a high amount of it (SICILIANO-JONES et al., 2008). Mn takes part in the metabolism of the luteal tissue and ovaries which is characterized by a high concentration in these tissues. Even in the pituitary gland the level of manganese is increased whereas cystic ovaries contain less amounts of Mn. In return gonadotropins influence the transport and tissue uptake of the manganese (HURLEY and DOANE, 1989).

2.2.4.2 Deficiency and supplementation

Deficiency in manganese manifests rarely in ruminants but if it occurs the synthesis of sexual hormones and steroids is inhibited. Due to this the reproductive performance is depressed or impaired. There are many symptoms – some of them are (extended) anestrus and irregular estrus, decreased estrus intensity, poor follicular development and delayed ovulation, lower conception and higher abortion rates, smaller ovaries, delayed puberty as well as weak and deformed newborn calves.

Cows have an increased demand for manganese supply during the gestation period and supplementation of them with manganese can increase the conception rates and fertility. Interestingly cows which experienced manganese deficiency already from the beginning of their life didn´t show more symptoms than their twins which were supported adequately with Mn (HURLEY and DOANE, 1989).

2.2.5 Molybdenum

2.2.5.1 Functions and excess

Molybdenum is needed for the adequate function of metalloenzymes like xanthine-, aldehyde- and sulfite-oxidase but the requirements for it are met with the normal diet. Especially cows are intolerant to excessive supply with the diet and show severe signs of molybdenosis such as delayed puberty, anestrus or increased non return rates. The severity of the described disorders depends on the chemical form of Mo that is used. Deficiency on the other hand alters the gastrointestinal microflora.

As molybdenum interferes with the copper metabolism and its utilization from the tissues Mo toxicities and Cu deficiency can´t be distinguished. Due to this the supplementation with copper can alleviate the caused disorders (HURLEY and DOANE, 1989).

2.2.6 Selenium

2.2.6.1 Functions

The main functions concern antioxidative processes which protect many biological functions from oxidative degeneration. Selenium interacts with the activity of glutathione-peroxidase and the reduction cytosolic peroxides. Due to these antioxidative effects it protects the ovaries from oxidative damage and enhances the fertility before ovulation (HURLEY and DOANE, 1989). Furthermore selenium is a part of important selenoproteins and enzymes which are involved in immune and antioxidant defense mechanisms and the conversion of thyroxin (T4), the inactivated thyroid hormone, to triiodthyronine (T3), the activated form. It influences the function of immune cells such as neutrophils which support the uterine health. Due to the above mentioned effects selenium can reduce the risk of retained fetal membranes and lengthened or poor uterine involution (RUTIGLIANO et al., 2008).

2.2.6.2 Deficiency and supplementation

In case of deficiencies the protective functions are decreased and so the defense against infectious diseases is suppressed. Glutathione-peroxidase lowers microbial activity and load. This fact is the reason for an increase of leucocytes and higher risk for microbial diseases in presence of inadequate Se levels. Such diseases, for example metritis, can be prevented by proper supply with selenium and vitamin E. Although the supplementation of Se and vitamin E can prevent deficiency the response to supply differs between the herds. One explanation for the changing responses are several different factors and complex interactions which interfere with reproductive processes and functions in the body.

Selenium supplementation can be especially effective in areas with low plant Selenium contents (< 0.05ppm) as herds grazing in this area are often deficient HURLEY and DOANE, 1989). Even silage forages used in the dry period as main part of the diet contain often not enough selenium (HARRISON et al., 1984). The supply of deficient animals improves the reproductive performance and is beneficial when the inadequate selenium status limits the reproduction. Cows supplied with Se and vitamin E show a lower incidence of retained fetal membranes, metritis, cystic ovaries and a decreased duration of uterine involution in cows with metritis. As selenium accumulates in the placentomes, ovaries and pituitary as well as adrenal glands these tissues have a specific requirement for it (HURLEY and DOANE, 1989).

2.2.7 Zinc

2.2.7.1 Functions

Zinc is a component of more than 200 proteins and enzymes and a part of them influence the functions of reproductive organs and tissues. It activates enzymes which are needed for steroidogenesis. Even the pituitary gland is affected by zinc and due to this also the activity of gonadotropin hormones, FSH and LH. Zinc (like copper) affects the activity of superoxide dismutase and the fate of superoxide and hydrogen peroxide in the body. Due to the above mentioned function the supplementation of zinc can increase the conception rates in dairy cattle (HURLEY and DOANE, 1989).

Zinc plays an important role for the claw integrity. This role is based on enzymes which are zinc dependent, RNA polymerases and nucleotide transferases, alkaline phosphatase, carbonic anhydrase and carboxypeptidase which are needed for the differentiation of keratinocytes. Zinc in form of Zn-finger proteins is needed to form filaments of keratin inside the keratinocytes. Furthermore zinc regulates calmodulin and inositol phosphate and subsequently affects calcium metabolism. Calcium then activates the epidermal transglutaminase which is needed for the linkage of keratin fibers as well as the differentiation of epidermal cells (SICILIAN-JONES et al., 2008).

3 The effect of minerals on the main diseases of dairy cows – REVIEW

Macro and micro minerals clearly play a role in the prevention of different diseases of the peri-parturient and high-yielding dairy cows like hypocalcaemia, mastitis, lameness, retained placenta. All of them influence the subsequent lactation period and fertility. Compared to the macro minerals which mainly influence the acid base status and calcium homeostasis, the trace minerals mainly support the immune state and prevention of diseases. (WILDE, 2006) Often the organic forms of supplementation are better absorbable than the inorganic sources, like the organic forms of zinc (WILDE, 2006).

3.1 Subclinical hypocalcaemia and milk fever

3.1.1 Requirements

The requirements of Ca increase slowly during pregnancy followed by a massive increase after calving. During the period after calving and the peak lactation the cows use the body calcium and lose calcium to keep the production (SUTTLE, 2010). Around 120g or more (~1%of DMI) can avoid nutritional deficits and related problems like milk fever (WILDE, 2006) and if the serum calcium concentration sinks below 2.2 mmol/l post-calving the incidence for displaced abomasum is increased (CHAPINAL et al., 2011).

3.1.2 Calcium deprivation

A calcium deprivation can occur very fast in one or two day after either an acute increase in the requirements which is called metabolic deprivation or a chronic failure to meet the demand. It leads to an increase in PTH and calcitriol which alter the bone metabolism. Other detectable changes are increased levels of plasma hydroxyproline (a marker for bone resorption), PTHrP (enhances bone resorption) and osteocalcin (a marker of bone growth) which can be measured frequently during parturition. Such raised levels of hormones and markers are more expressed in primiparous cows.

During parturition a cow can lose 23g of calcium within 24h in 10kg colostrum but in the bloodstream there are only 3g available. Furthermore the cows often don´t eat for some hours before, during and after parturition and so there is also less calcium ready for absorption. The post-parturient hypocalcaemia can last for 14 days but the healthy cow can readjust to the situation due to increased bone resorption and absorption from the small intestines regulated by the increased hormones (SUTTLE, 2010).

Often the feet intake decreases for 2 to 3 days and the deprivation of DMI reaches 20 to 30%. This decreased DMI leads to a negative energy balance which complicates the situation and can also be a cause for milk fever, retained placenta, displaced abomasum or ketosis (WILDE, 2006).

In milk fever these mechanisms fail to work because the target-organs are insensitive to PTH and calcitriol and the plasma level declines to an intolerable degree (<1.25 mmol/l). Also serum phosphorus and magnesium can decline.

Also grain-based diets or diets based on roughages low in calcium and loss of appetite during a longer time after reaching peak lactation can lead to chronic deprivation. The skeletal depletion reaches a point when milk yield is impaired. Also the genetical background is a reason for deprivation as the cows with the highest milk yield have the highest requirements for calcium (SUTTLE, 2010).

3.1.3 Influences of dietary anion-cation difference (DCAD) on the calcium status

Potassium can be often found in high amounts in the forages used for dry cow rations. This high amounts lead to a high DCAD and also a metabolic alkalosis which interferes with the calcium homeostasis and the above mentioned procedures to increase the calcium absorption and resorption. A diet low in potassium like timothy hay (13 g/kg of DM = Dry Matter) leads to a lower DCAD (195 mEq/kg of DM) than high dietary potassium content (33 g/kg of DM and 514 mEq/kg of DM). Due to the lower DCAD in the diet with the lower potassium magnesium was increased around parturition and also the calcium and magnesium excretion with the urine was higher which can be due to higher calcium metabolism. Also the daily feed intake and so mineral supply and bioavailability was higher for the diet with lower potassium content. Furthermore the phosphorus and calcium plasma concentrations turned to a normal level more rapidly in case of the lower DCAD (RERAT et al., 2009). Especially short after calving calcium is increased compared to higher DCAD diets and phosphorus can be increased before, during and after parturition due to low DCAD diets (RAMOS-NIEVES et al., 2009). Calcium plasma levels can be increased during and short after parturition by lowering the DCAD but not during the dry period (SEIFI et al., 2010).

Consequently, by lowering the potassium content it is possible to decrease the DCAD but not deep enough to create a metabolic acidosis. Another way to lower the DCAD would be to add anionic salts for example sulfate or chloride (RAMOS-NIEVES et al., 2009; RERAT et al., 2009). This method has a greater effect on the anion-cation difference than low potassium levels of the diet but it also decreases the palatability of the diet. (RERAT et al., 2009) Consequently acidifying the diet allows high calcium supply without causing hypocalcaemia. The DCAD should be between -100 and -200 mEq/kg of DM for the transition cow to prevent milk fever after parturition and during high lactation (WILDE, 2006). A DCAD below 30mEq/100g of DM increased the concentration of plasma and ionized calcium without any effect on parturient paresis. Even the incidence of subclinical and clinical hypophosphatemia can be reduced with decreased DCAD in diets. The only negative of lowered DCAD can be lower dry matter intake (DMI) before parturition which increases after parturition (RAMOS-NIEVES et al., 2009).

3.1.4 Subclinical hypocalcaemia

In subclinical hypocalcaemia the total blood calcium level lays around or below 2.0mmol/l (MULLIGAN et al., 2006). 50% of the multiparous cows have Ca concentrations in the blood after calving below the threshold (OETZEL and MILLER, 2012). The incidence for it ranges between 23 and 39%. Also subclinical reduce of plasma Ca concentration can be accompanied by higher risk of mastitis, dystocia, prolapse of the uterus, retained fetal membranes, endometritis, longer uterine involution, delayed ovulation, ketosis, impaired gastrointestinal motility and abomasal displacement. The influence of the gastrointestinal motility can reduce the feed intake (MULLIGAN et al., 2006). As cows suffering from subclinical hypocalcaemia can hardly be identified the best approach to deal with is the prevention (OETZEL and MILLER, 2012).

3.1.5 Milk fever

Milk fever is a disease of high-yielding dairy cows in association with parturition and early lactation, normally within 48h after calving. The clinical signs are listlessness, muscular weakness and twitching, circulatory failure, anorexia and rumen stasis. The second stage shows mainly drowsiness, staring and sternal recumbency followed by lateral recumbency and coma in the final stage. In the affected cows a severe hypocalcaemia is present (<1,0-1,2mmol/l) which can be accompanied with loss of appetite, decreased peripheral blood flow, hypothermia and hypoxia, hyponatremia, rumen hypomotility, abomasal displacement and myocardial lesions. Due to these secondary problems the cows may not respond to treatment with calcium alone. An associated hypomagnesaemia can lead to convulsions or tetany.

3 to 10% of grazing dairy herds is affected with milk fever and the same percentage doesn´t respond to treatment (SUTTLE, 2010). Another study reports 4 to 7 in some herds up to 41% incidence of milk fever (WILDE, 2006). Cows which had milk fever in a previous lactation period are 2.2 times more likely to suffer from it again than cows which never had it before. Increased incidence occurs in case of high evaporation, large variation in air temperature, high rainfall, low grass temperature, assistance at calving, extremely low or high BCS and older cows (age-related decline of calcitriol receptors in intestinal mucosa). Also a high dietary cation-anion difference increases the risk of milk fever as it lowers the amount of calcium in bones which can be used for resorption (SUTTLE, 2010).

3.1.6 Treatment of hypocalcaemia

The most important points for the treatment are the rapid reversal if the hypocalcaemic state of the cow and a new decline in plasma calcium below 1.75mmol/l. For this the supply with calcium absorbed from the gut and mobilized from the bones has to be maximized. An acute state of milk fever is treated with a single intravenous calcium infusion. This infusion consists of 600ml of 40% calcium borogluconate and magnesium hypophosphite. With this infusion 50% of the affected animals can stand up again and 40% more of the cows can recover after one or two more treatments. The later the infusion is applied and the more severe the hypocalcaemia is the more likely the cow will resist to the first treatment (SUTTLE, 2010).

3.1.7 Prevention of hypocalcaemia

The prevention is very important as cows suffering from milk fever have a higher risk for limb injuries, mastitis, ketosis and a depressed milk yield. One way to prevent milk fever are acidic diets (like silages preserved with acids) (SUTTLE, 2010) or diets containing low amounts of calcium (<30g of Ca/day) before (MULLIGAN et al., 2006) or around calving as they increase the sensitivity of bones and kidneys to PTH and so the resorption and absorption of calcium. As a consequence the plasma calcium level is increased before calving. Furthermore anions increase the availability of magnesium which protects the cows from milk fever (SUTTLE, 2010). Block showed that anionic diets reduce the incidence of milk fever compared to cationic ones (BLOCK, 1984).

Another way for prevention is supplemental calcium add especially to calcium poor diets. The calcium can be mixed into the diets or offered as free-access mineral mixtures like limestone (SUTTLE, 2010). One source of Ca is $CaCl_2$ which, despite its beneficial effects, can be caustic to the mucosa of the mouth and esophagus especially when supplementation with large amounts is repeated.

Even oral calcium boli can be given short after calving to decrease milk fever. Especially cows which were lame before calving responded to this way of calcium supplementation. The application of boli prevents further injuries and is beneficial for the overall health of the animals. Also cows producing higher levels of milk yield than the average of the herd (>105% of herd rank) are susceptible to treatment with Ca boli. Their milk yield increased compared to the previous lactation period (OETZEL and MILLER, 2012).

Another approach to the prevention of milk fever is to optimize the supply with other minerals such as magnesium and phosphorus. The optimal concentration of Mg in the diet is 0.4% of the dietary dry matter. The blood concentration should range between 0.8 and 1.3mmol/l. The optimal content of phosphorus in the diet is below 0.3% of the dry matter. Higher concentrations increase the incidence of milk fever by blocking the production of 1.25-dihydroxyvitamin D3 (MULLIGAN et al., 2006).

3.2 Mastitis

3.2.1 Occurrence

In the season 1998/1999 an average of the herds in the UK had an incidence of 25.6% for the presence of a quarter case of mastitis which was increased to 39.1% in 2000/2001. Single herds differed between 7.9 to 80.8% in 2000/2001. Furthermore cows with a previous mastitis had a 1.6 higher risk to experience a new one.

A critical period for the occurrence of a new mammary infection is the dry period in which 60% and more of all infections with Gram-negative bacteria like E. coli can occur. It was proved that more than 50% of coliform infections take place during the dry period. Infections during the dry period become earlier of clinical interest than later infected ones and peak around 14 days after parturition with over 30% of the cases (WILDE, 2006). The peak clinical response of cows at marginal copper concentrations can be reduced by Cu supplementation. Spears and Weiss proved this by experimental E. coli infections which lead to less severe clinical symptoms after addition of copper to the diet (SPEARS and WEISS, 2008).

Also in case of selenium deficiency the mammary glands are more susceptible to new infections. This contributes to the immune and protective functions of Se in the body. Selenium affects especially the ability of the polymorph-nuclear neutrophils to kill invading bacteria and other pathogens. In case of deficiency this ability is reduced and the risk of mastitis as well as the duration of infection is increased (SMITH et al., 1984).

3.2.2 Effect on fertility

Intra-mammary infections caused by Gram-negative bacteria have a negative effect on fertility. A clinical mastitis following such infections can lead to increased days in service as well as open days and services per conception. If the clinical mastitis occurs

3 weeks after the first insemination the fertility can be reduced by 50% and more (WILDE, 2006).

3.2.3 Prevention and treatment

Zinc is an essential trace element which plays an important role in more than 300 enzymes in metabolism and therefore is involved in many different healing processes. Its absorption from the rumen declines with a greater number of parturitions. Zinc is effective against the prevention of mastitis.

The main effects are the enhanced antioxidant functions and the support of the keratinization of teat canals due to zinc supply. Zinc is a part of metalloenzymes which play an important role in the catalysis, structure and regulation of the keratinization of the teat canal. The keratin lining the teat canals represent a physical and chemical barrier against bacteria and nearly 40% of it is removed during the milking process. Consequently a steady renew of the keratin layer is required and important for the udder health. Another important role of zinc is its part in the Copper/Zinc superoxide dismutase (SOD). The SOD prevents lipid peroxidation and so supports the immune function.

Zinc can be supplemented as zinc oxide or zinc sulphate in order to reduce the incidence of milk fever as well as the somatic cell count (SCC). Both forms of supplementation have the same bioavailability in cattle but recent studies showed that organic forms of zinc supplementation are absorbed and retained better by the animals than inorganic ones. Furthermore the replacement of inorganic sources with organic zinc supplements can reduce the SCC short after parturition, lower the infected quarters of the udder, decrease the new infection rate of mastitis and increase the milk yield. The greater the percentage of organic zinc is, the more expressed are the above described effects (WILDE, 2006).

Another trace element which plays an important role in the prevention of mastitis is selenium. Due to its immune system supporting function it can decrease the duration of clinical symptoms by >40% and the duration of infection by >25%. Selenium deficiency increases the time in which clinical signs are seen. These effects are not valid for infections with Streptococcus agalactiae or Staphylococcus aureus (SMITH et al., 1984).

3.3 Lameness

3.3.1 Occurrence

The incidence for lameness increased during the last 20 years and varies between 2.6% and 39.5% among the different herds with an average of 18.9% in the season 2000/2001. Important reasons for skin ulcers are digital (41%), inter-digital (38%) and sole ulcers (21%). Sole ulcers mainly occur at the junction of sole and heel at the outer claw. Another disease causing lameness is the separation of the white line due to tensile forces. These forces originate from the great amount of body weight the white line area bears (WILDE, 2006). Another study named white line separations and sole hemorrhages (85%) as the most common claw lesions. These lesions are a manifestation of microcirculatory disturbances in the corium and result in degeneration at the junction of dermis and epidermis of the claw (SICILIANO-JONES et al., 2008).

Furthermore processes during calving weaken the connective tissue supporting the claw which increases the possibility for lameness. Deficiencies around parturition reduce the claw keratinization leading to poor horn quality and a higher risk for lesions. The lesion can be caused already in the dry period but seen during early lactation as the horn growth takes 4 to 8 weeks (WILDE, 2006).

3.3.2 Connection between lameness and reproduction

Former studies that lameness can be accompanied by longer calving to first service intervals and increased intervals between calving and conception, especially in case of sole ulcers and white line lesions. Furthermore lameness is accompanied by delayed ovarian function short after parturition. Sometimes the delay of ovarian function can be 3.5 times higher for lame cows. Also longer calving intervals and extra services per conception can be observed for lame cows (WILDE, 2006).

3.3.3 Prevention

Zinc is also in duty to prevent lameness during lactation by improvement of the keratinization of the claws. Although the disease occurs during lactation the wounds can be caused already before parturition. A zinc deficiency can lead to parakeratosis, hyperkeratosis and deformities of the hoof epidermis. Lame cows have lower zinc concentrations in the horn than healthy ones and the hard keratin of the claw wall contains more zinc than the softer heel keratin.

Zinc plays an important role during keratinization by regulating and activating the production of keratin protein (WILDE, 2006).

Also the way of supplementation is important for the prevention of hoof lesions and lameness. Organic zinc supplements like zinc bound to amino acids and peptides showed better microscopic horn qualities and traction resistance than inorganic supplementation due to better absorption and utilization of the organic forms. Addition of organic zinc to inorganic supply can reduce the cases of heel erosions, foot rot, inter-digital dermatitis, laminitis, sole ulcers and white line separations (WILDE, 2006; SICILIANO-JONES et al., 2008).

3.4 Retained placenta

3.4.1 Occurrence and further risks

Retained placenta means an inadequate separation of the fetal part from the maternal part of the placenta which can be caused by different nutritional, environmental, pathological and physiological factors Around 25% of the treated diseases of dairy cows are related to genital infections and the risk for this route of infection is enhanced from 10 to 54% in case of retained fetal membranes (JULIEN et al., 1976). The uterine infections occur mostly in the post-partum period and can cause metritis. The risk of the cows to suffer from them can be reduced with selenium supplementation (HARRISON et al., 1984).

The average incidence of retained placenta in dairy cattle ranges around 4% (WILDE; 2006) and it occurs more often in primiparous than multiparous cows (EGER et al., 1985). It is often accompanied by longer calving intervals, higher risks of metritis and increased days to first service as well as services per conception. Factors which are often associated with retained fetal membranes (RFM) include dystocia, twinning, stillbirth, a negative energy balance/ketosis and hypocalcaemia (WILDE, 2006).

3.4.2 Prevention

Two important factors in the prevention of retained placenta are the prevention of hypocalcaemia and the adequate supplementation with selenium. A good way to support dairy cows with selenium is the offer of selenium yeast. Selenium is needed for the work of the Se dependent glutathione peroxidase (GSH-Px) which reduces the incidence of RFM. If the plasma levels of GSH-Px are low the risk for RFM increases.

Selenium can be offered as selenomethionine and selenocysteine (mainly in feedstuffs) or as selenite (in supplements). Selenomethionine and selenocysteine are amino acids and so an organic form of selenium supply. They can be found in selenium yeast and are retained better in tissues and blood than inorganic supplements. Inorganic selenium supply can prevent deficiencies whereas selenium yeast can enhance selenium status and its associated immune functions during critical periods like parturition (WILDE, 2006). Especially in herds deficient in selenium the supply with it can reduce the incidence of retained placenta (JULIEN et al., 1976).

Beside the supplementation with Se also adequate levels of vitamin A and E as well of calcium and copper should be assured to prevent RFM (HURLEY and DOANE, 1989). A combined supplementation with organic forms of cobalt, copper, manganese and zinc can decrease the problems following RFM and reduce the days to first estrus, luteal activity and corpus luteum (CAMPBELL et al., 1999). Also boli consisting of iodine, selenium and cobalt reduce the occurrence of retained placenta significantly (COOK and GREEN, 2007).

4 The effects of mineral supplementation on the main productive and reproductive parameters – REVIEW

Supplementation of dairy cows with minerals like copper, zinc, manganese or selenium can improve the conception and pregnancy rates, lower the needed services per conception and shorten the days to first estrus, service and conception, especially when the supplementation is done with organic sources (WILDE, 2006; SICILIANO-JONES et al., 2008). Supplementation with organic trace elements can even increase milk production and the content of milk solids (RABIEE et al., 2010).

The supplementation with macro- and micro-minerals can influence the productive and reproductive performance of dairy cattle. But also diseases caused or supported by mineral deficiencies like retained placenta or mastitis (reported above) have an impact on reproduction. Occurrence of RFM correlates with lower conception rates, increased open days and days to first service or reduction of conception rate at first service. Mastitis can be accompanied by more days to first service, open days and services per conception as well as a higher incidence of abortion (VANEGAS et al., 2004).

4.1 Minerals and their role in milk production

Potassium and sodium have a sparing effect on each other and so if one of them is high in the diet DMI, 4% fat corrected milk and milk fat are high if the other mineral is low in the ration. In case of high dietary Na content increased milk yield and 4% fat corrected milk were also influenced by high Ca levels and greater DMI. Dry matter intake, milk yield and 4% fat corrected milk yield are highest at 0.58% of sodium and 0.40% of magnesium in the diet (SANCHEZ et al., 1994b) whereas the maximum of milk fat percentage is reached at 0.6% sodium, 1.34% potassium and 0.69% chlorine. Even the DCAD influences the milk yield. 3.5% fat corrected milk yield is highest between +30 and +50 (SANCHEZ et al., 1994a). On the other hand a tertiary mixture of 33% $NaHCO_3$, 33% NaCl and 33% KCl decreases milk protein content (SANCHEZ et al., 1997).

Trace elements such as Zn, Mn, Cu and Co can improve the milk production and the yields of milk protein and solids especially when they are supplemented in organic forms like amino acid complexes (NOCEK et al, 2006; SICILIANO-JONES et al., 2008; HACKBART et al., 2010). When the duration is extended – for example over 2 lactations – the supportive effects on milk production are more expressed (HACKBART et al., 2010).

4.2 The influence of trace elements on reproductive performance

Trace element deficiencies can be accompanied by reproductive problems such as RFM, abortion or weak calf syndrome. Even the way of micro mineral supplementation can influence the reproductive performance (MULLIGAN et al., 2006). The absorption of dietary supplements can be influenced by other nutrients compared to parenteral supplementations. Parenteral supply of combined trace elements has different effects according to the mineral content in the ration. In deficient herds improvements in the performance such as increased calving rates or reduced days to first service and open days can follow a parenteral treatment Herds fed at or above the requirements don´t show improvements (VANEGAS et al., 2004).

Deficient selenium status is thought to impair the neutrophil functions and can influence the uterine health and increase uterine problems due to this (RUTIGLIANO et al., 2008). If the Se plasma concentration is at least 0.06µg/ml at calving the occurrence of cystic ovaries is minimized and injections applied pre-partum can lower the incidence of ovarian cysts from 50 to 19% (HARRISON at al., 1984). Selenium deficiency combined with inadequate iodine concentrations lead to increased incidence of retained placenta, milk fever or discharge from the vulva (MULLIGAN et al., 2006) whereas parenteral supply with selenium and vitamin E results in higher fertilization and pregnancy rates, decrease in open days and reduced occurrence of ovarian cysts and RFM (VANEGAS et al., 2004). Selenium can also be supplied in form of selenium-yeast, sodium selenate and sodium selenite. The supply with yeast results in higher blood Se concentrations than in case of supplementation with inorganic sources, most probably due to the absorption of selenium containing amino acids such as methionine and cysteine. If there is enough organic selenium (0.5mg/kg feedstuff) available in the diet there is no difference in the reaction of the cows to organic or inorganic sources (RUTIGLIANO et al., 2008).

Copper deficiency is related to early embryonic death, embryonic resorption, increased incidence of RFM, necrotized fetal membranes and weak or silent heats whereas higher levels decreased the open days, days to first service and services per conception (SICILIANO-JONES et al., 2008). Furthermore the amount of copper which reduces the clinical deficiency signs are maybe not enough to ensure an optimal immune function (MULLIGAN et al., 2006). On the other hand Cu supplementation to deficient cows before and during the breeding season is able to increase early conception rates (MUEHLENBEIN et al., 2001). Also decreased zinc levels lead to reduced fertility and abnormal estrus, abortion as well as changes of the activity of the myometrium leading to longer durations of labor. Addition of 800 mg of zinc 6 weeks before parturition is able to reduce the days to first estrus and service in the coming lactation (SICILIANO-JONES et al., 2008).

On the other hand dietary levels of trace minerals which are higher than the recommendations have adverse effects on the reproductive performance in first calf heifers, especially in case of combined mineral supplements (OLSON et al., 1999; VANEGAS et al., 2004). If the supply starts 60 days before the breeding season also cows show a reduction in reproductive performance. Consequently the supply of dairy cows with trace animals above their requirements is either of low value or maybe has a negative effect on reproductive performance (VANEGAS et al., 2004).

4.3 Calcium deficiency and its influence on (re)production

Calcium is an important component of the milk and mobilized by the body in high amounts for milk production. An adequate supplementation of the dry and lactating cow with it can enhance the milk production, dry matter intake and 4% fat-corrected milk (NOCEK at al., 1983).

Also the energy balance can influence the fertility and milk yield. As calcium is important for the muscle contraction hypocalcaemia can cause a negative energy balance (NEB) by lowering the abomasal motility and contraction strength and hence leading to a decreased dry matter intake. The NEB further causes fat mobilization resulting in fatty liver syndrome and ketosis as well as even more suppressed appetite due to ketone bodies. Not also fertility and milk yield can be influenced by calcium deficiency also prolonged calving, impaired involution and retained placenta are caused by it as the uterine muscle tone is decreased (WILDE, 2006).

Also subclinical hypocalcaemia can influence reproduction. In case of calcium deficiency ovarian function is decreased leading to smaller follicles at first ovulation and a lower progesterone level which is connected with the function and existence of the corpus luteum. Even the involution time can be elongated by subclinical hypocalcaemia (KAMGARPOUR et al., 1999).

4.4 The influence of sodium on milk production

The addition of sodium bicarbonate based to the diet is able to increase the dry matter intake, milk production (KILMER et al., 1981; ROGERS et al., 1985) and ruminal NH_3 concentration faster and improves the acid-base balance during the early post-weaning period. During this time the ration changes abruptly from fiber-rich in the dry period to high concentrate contents after parturition. The cow needs to adapt to this changes which have a negative impact on the above mentioned factors. Sodium bicarbonate accelerates the adaption and improves DMI, production and ruminal ammonia concentrations. The increased ruminal NH_3 improves the nitrogen retention and the solubility of ruminal protein. All together sodium bicarbonate increases the milk yield by altering the nitrogen metabolism as well as increasing the uptake and utilization of nutrients (KILMER et al., 1981). High concentrate diets lower the duration of mastication and so the salivary flow rates as well as the $NaHCO_3$ content in the rumen. $NaHCO_3$ supplementation replaces the lack in the ruminal fluid and has a buffering effect, too. Due to the above described effects the milk fat content can be increased if it is low (BLOCK, 1994).

5 Materials and Methods

5.1 General information about the farm and animals

The farm is large scale dairy cattle farm and situated in the middle of Hungary. Around 1000 Holstein-Frisian dairy cows are kept there in loose keeping systems. Around 80% of them are milked and the rest is in the dry period. The animals are grouped according to their stage within the productive cycle and the level of milk production within this stage. A cow in lactation can give for example 20, 30 or 40kg milk. The average milk production per cow is more than 9000kg for a 305 day long standard lactation period. The cows receive a total mixed ration (TMR) according to their stage and production level. The TMR consists of corn and alfalfa silage, meadow and alfalfa hay as well as different cereals. The cereals are first mixed to a compound feed with the mineral and vitamin supplementations and then the whole mixture is added to the TMR.

5.2 Sample taking

Blood and urine were taken from dairy cows belonging to 6 different groups which are close up cows 14 days pre-partum, fresh cows which are 1 to 7 days in milking (DIM), primiparous cows 8 to 30 DIM, multiparous cows 8 to 30 DIM, primiparous cows 60 to 100 DIM and multiparous cows 60 to 100 DIM. For the hair samples only the close up cows, the fresh cows, the multiparous cows 8 to 30 DMI and the primiparous cows 60 to 100 DMI were used. All 6 groups were evaluated according to the Body Condition Scoring (BCS) system, too. In all 6 groups 5 cows were evaluated.

Blood samples were taken at the Vena epigastrica superficialis (milkvein) with an open collecting system. The blood was collected with Na-heparine containing tubes. After the sampling it is necessary to turn over the tubes to avoid blood clotting.

The urinary samples were collected directly from the urinary bladder of the cows. The catheterization was done with metal catheters through the urethra and directly into the bladder. The urine was collected into tubes.

Hair samples can only be taken from pigmented hair. In case of Frisian-Holstein cows these hairs are black. The samples were taken at the lateral side of the thorax along the ribs. If the animals were only white in this area it was impossible to take the samples. The samples were taken with Aescoulap hair collecting equipment. In case of missing data for the hair samples the animals were white and so the sample could not be obtained (see Table 3). As hair stores the minerals for a longer period it reflects not the actual mineral status but the state of 2 to 3 months ago.

5.3 Laboratory analysis of urinary and blood parameters

Zinc and copper concentrations in the blood plasma are examined with the atomic absorption test by Price W. J. (1977) whereas blood levels of glutathione-peroxidase are measured with a method discovered by Sedlal and Linsay (1986) which analyses the activity of GSH-Px in red blood cells.

Calcium, inorganic phosphorus and magnesium concentrations are analyzed for blood as well as the urine. Calcium levels can be measured with three different methods. The first is the spectrophotometry by Gitelman (1967), the second is the complecometry according to Sajo (1973) and the third method is the atomic absorption test by Price again. Phosphorus concentration is examined by spectrophotometry with molybden blue which was investigated by Velösy and Szabo (1971). Magnesium state of the animals can either be examined by spectrophotometry according to Mann and Yoe (1956) or like inorganic phosphorus or like zinc and copper.

The method of choice to measure sodium and potassium in the urine is the atomic absorption test by Price which can be also used for the measurement of zinc and copper in blood as well as the investigation of calcium and magnesium levels in the urine and blood.

6 Results and discussion

6.1 Results and discussion of Body Condition Scoring (BCS)

Table 1 shows the BCS of all 30 cows divided into the above mentioned 6 groups with 5 cows inside each group. Most of the animals have a BCS within the norms (2.5 to 3.5). Only 1 cow in the group of multiparous cows 60 to 100 DIM is over-conditioned with a BCS of 4. Furthermore the table shows a slight decrease of the BCS with increased lactational performance until the peak lactation is reached after 60 to 100 DIM. This decrease can be due to the higher energy demand for milk production. They need more energy than they can take up with their feed. Due to this they use up their body reserves, the body condition gets worse and the BCS sinks.

If the BCS is increases in the pre-calving period the fat mobilization increases which can lead to fat accumulation in the liver. On the other hand the feed intake decreases linearly. These effects can be associated with fatty liver syndrome, calving problems, RFM and displaced abomasum. Furthermore dairy cows which have a BCS of 4 or higher are more likely to experience milk fever. On the other hand a BCS of 2.5 or more during early lactation period can avoid reproductive problems and improves the breeding success (MULLIGAN et al., 2006).

6.2 Results and discussion of blood sample analysis

The results are presented in the Table 1.

6.2.1 Calcium

Blood calcium concentration (Figure 1) was within the normal range (2.1-3.0 mmol/l) for all six groups. The average blood Ca level for the fresh cow group was the lowest but a decrease in calcium is normal after parturition due to the Ca demand for milk production.

Deviation of calcium concentration in the blood can be due too high DCAD values during the dry off and close up period which impair the proper starting of Ca absorption and resorption processes pre-calving. Due to this the animals are not able to adapt to the increased calcium demand fast enough which can lead to subclinical hypocalcaemia and milk fever (RERAT et al., 2009; SUTTLE, 2010). The hypocalcaemic state increases the risk for other diseases like mastitis and ketosis and decreases the milk production as well (SUTTLE, 2010).

6.2.2 Phosphor

The blood anorganic phospate concentration (Figure 2) lay within the required borders between 1.6 to 2.3mmol/l throughout all six groups. Only in the close up group 2 cows have levels below the lower reference value. In the other 5 groups the anorganic phosphate concentrations are either within the norm or a bit higher than 2.3 mmol/l. A decreased phosphor state of the animals would lead predominantly to general signs like unthriftiness and decreased lactational performance due to the appearance of the mineral in all cell types. Reproductive problems such as delayed estrus and general reproductive failure occur only secondary to the general symptoms of phosphorus deficiency (HURLEY and DOANE, 1989; PALLESEN et al., 2008).

6.2.3 Magnesium

Nearly all average values of blood magnesium levels (Figure 3) of the different groups are the same as the higher reference value (0.8-1.2 mmol/l) or higher. The value of the multiparous cows 8-30 DIM was a bit lower and the level was the lowest for the fresh cows. Also these two groups had blood concentrations of magnesium within the values. The decrease of Mg level after parturition was reported by Ramos-Nieves, too (RAMOS-NIEVES et al., 2009). In case of magnesium deficiency PTH secretion and so calcium metabolism is altered. This alteration leads to decreased Ca concentrations in the blood. Another result of Mg deprivation is tetany (GOFF, 2004).

6.2.4 Copper

All copper concentrations (Figure 4) in the blood were elevated (>18.9µmol/l) especially for the groups with the primiparous cows. Copper is an integral port superoxide dismutase (SOD) which influenced antioxidative functions (ANDRIEU, 2008). Also other proteins contain copper which regulate enzymatic functions such as the reduction of superoxide. Another function of the mineral is to maintain hypophyseal hormone levels which influence the reproductive performance like LH. This is one reason for the influence of copper on the reproductive performance of the animals. For example adequate Cu levels can prevent the occurrence of RFM (HURLEY and DOANE, 1989). Furthermore copper strengthens the claw horn and supports its integrity (SICILIANO-JONES et al., 2008). Copper deprivation causes decreased fertility, infertility, delayed estrus or early embryonic death (HURLEY and DOANE, 1989). The blood Cu levels in this special case indicate a bit increased values. Such concentrations don´t cause acute problems but for long terms they can cause chronic copper toxicosis. The toxicosis can lead to severe generalized problems like hemolysis and can terminate with death (GEORGIEVSKII et al., 1982b). A more detailed description of chronic copper toxicosis is written in the evaluation of copper concentrations of the hair.

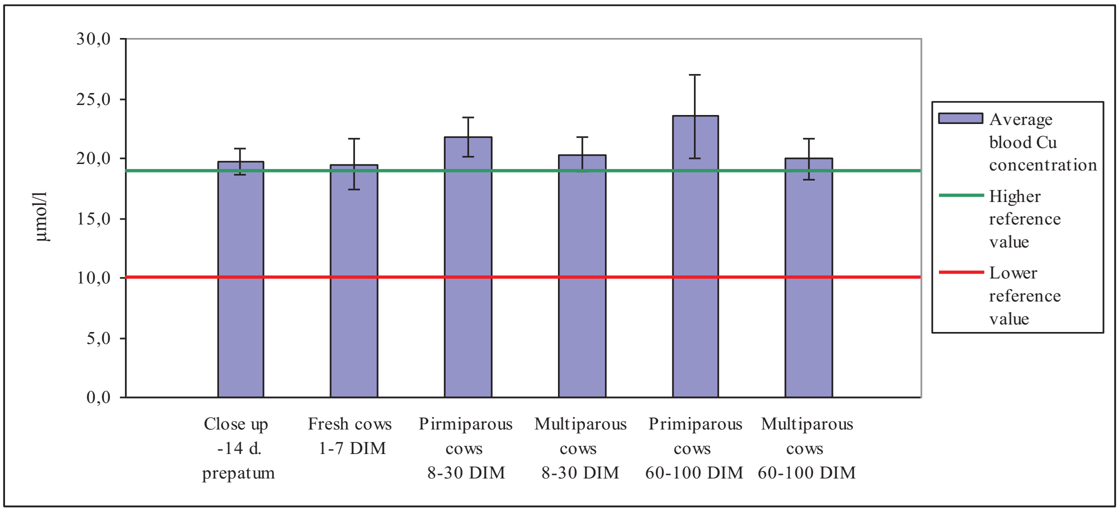

Figure 4: Average blood Cu concentration of the different groups

6.2.5 Zinc

All zinc values of the blood analysis (Figure 5) ranged between the normal values of 10.0-30.6µmol/l. Also zinc is a part of the SOD and other enzymes. Due to this it supports the keratinization of the teat canals and claw horn. Furthermore it helps to prevent mastitis and accelerates the healing after an intramammary infection WILDE, 2006). Zinc can influence the reproductive performance, too. As an example zinc supplementation increases the conception rate at first service (HURLEY and DOANE, 1989).

6.2.6 Glutatione-peroxidase and Selenium

Glutathione-peroxidase (GSH-Px) is dependent on selenium and reflects the blood Se concentrations due to this (WILDE, 2006). As shown in Figure 6 it is analyzed only for the close up cows, the multiparous cows 8-30 DIM and the primiparous cows 60-100 DIM. The values of GSH-Px in the blood are nearly the same for all 3 evaluated groups and within the reference values (20-30U/g Hb). Selenium and GSH-Px play an important role in antioxidative processes (HURLEY and DOANE, 1989) and immune functions. Due to this the mineral influences the reproductive performance and prevents diseases. Supplementation with GSH-Px prevents mastitis (SMITH et al., 1984) and reduces the occurrence of retained placenta, increases conception rates and decreases the days to first estrus (WILDE, 2006). In case of deficient states the risk of infectious diseases caused by microbes such as metritis is increased (HURLEY and DOANE, 1989) whereas the reproductive performance is decreased (WILDE, 2006).

6.3 Results and discussion of urine sample analysis

The results are presented in the Table 2.

6.3.1 Calcium

The normal range of calcium urinary concentration is 0.1 to 1.5mmol/l. On this farm the excretion is increased around 3 times for the cows in the close up period and still a bit higher for the fresh cows. The primiparous cows have an average level of 1.6 mmol/l which is nearly the same as the higher border of the normal range. The concentrations of the last three groups range within the normal values. The general tendency of calcium excretion with the urine is decreasing until peak lactation (Figure 7). After calving until peak lactation there is a steady and massive increase of calcium demand for milk production. This demand is covered by increased absorption from the intestines and mobilization from body reserves such as the bones (SUTTLE, 2010). The use of Ca for lactation could be a reason for the decreasing urinary concentrations from calving to peak lactation after 60 to 100 DIM. This thesis is in contrast with Suttle who reports only little amounts of calcium excreted in the urine which are independent of the actual state of the animal. The mineral is excreted mainly with the feces (SUTTLE, 2010).

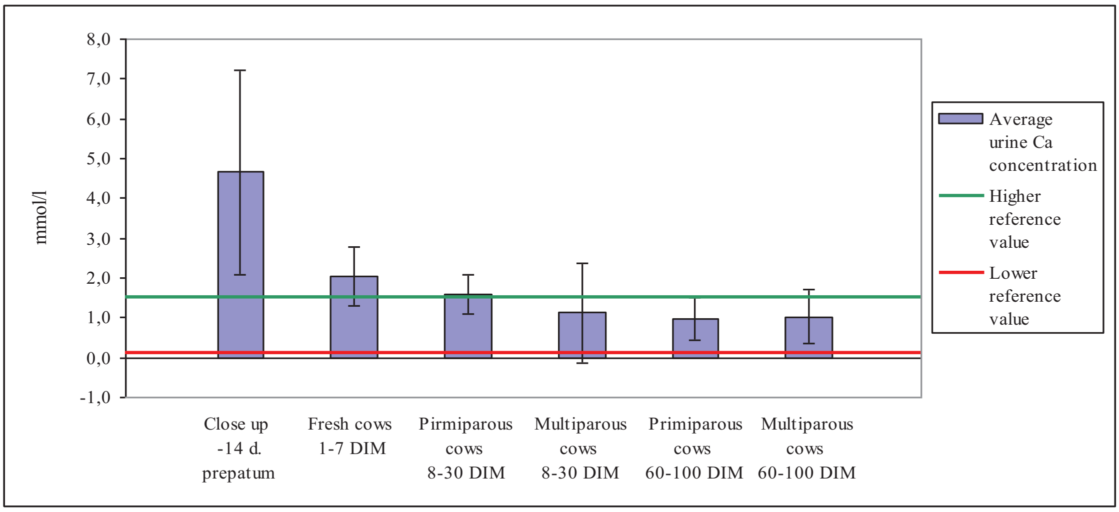

Figure 7: Average urine Ca concentration of the different groups

6.3.2 Phosphorus

Figure 8 shows the average phosphorus levels analyzed for the 6 groups. All of them lay within or near the reference values between 0.3 and 5.2 mmol/l. The values for the fresh and close up cows are 1.3 and 1.4 mmol/l and the concentration of both multiparous groups is 0.9 mmol/l. The analyzed level for both primiparous groups is higher than for the other 4 groups but still in the normal range or at the higher reference value. The average value after 8-30 DIM is 5.3 mmol/l due to 3 cows in this group with too high results (8.6, 6.3 and 5.5 mmol/l) and after 60-100 DIM it is 3.2 mmo/l due to a cow with a phosphor urine concentration of 11.0 mmol/l. Phosphorus homeostasis is balanced by absorption from the diet, renal excretion and tubular reabsorption in the kidneys (RAMOS-NIEVES et al., 2009). The above described results may indicate a probably too high P concentration in the diet of the primiparous cows 8-30 days in milking or increased P exretion as a cosequence of acid load. Dietary phosphorus levels around 0.4% of dry matter maintain the homeostasis. Too high blood levels have a negative impact on the calcium homeostasis (GOFF, 2004) whereas deficiency can lead to general problems and reproductive failure due to the occurrence in nucleic acids, phospholipids, enzymes and the role of the mineral in energy utilization (HURLEY and DOANE, 1989).

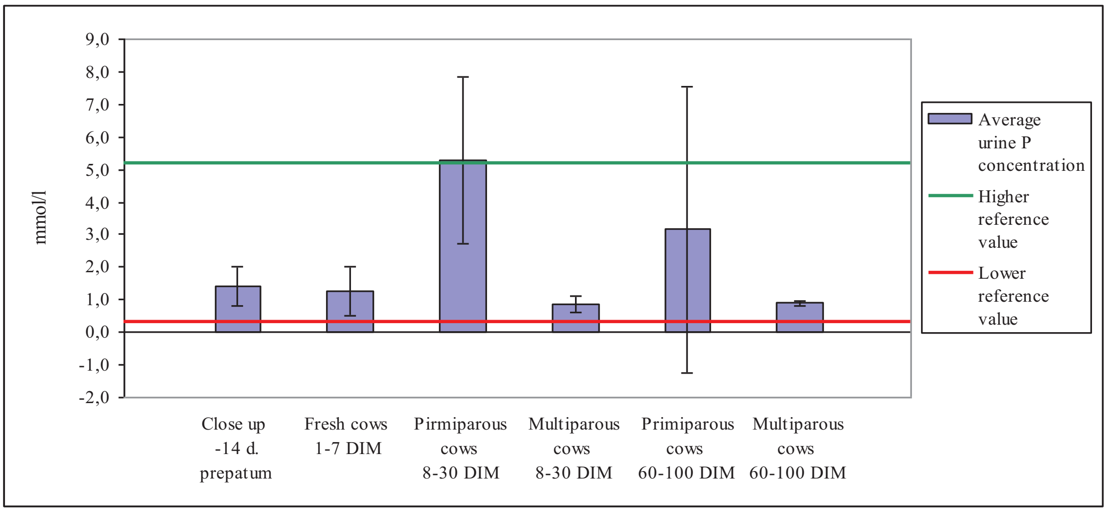

Figure 8: Average urine P concentration of the different groups

6.3.3 Magnesium

The magnesium concentrations in the urine should range between 6.2 and 16.5 mmol/l. Although the average levels of the fresh cows, multiparous cows 8-30 DIM and all cows 60-100 DIM are within this range there are great individual differences. The lowest value is 2 mmol/l and the highest 24 mmol/l. The other two groups have concentrations above the higher reference value. The average level for primiparous cows 8-30 DIM is 19.2 mmol/l with individual concentrations up to 32 mmol/l. The peak value with 45mmol/l is reached by an animal in the group with the close up cows which is also the group with the highest average value (28 mmol/l). The average levels are represented in Figure 9.

Magnesium is absorbed in the rumen und reticulum, transported through the rumen by a sodium-dependent mechanism, secreted in the small intestines (GOFF, 2004) and excreted over the urine (PALLESEN et al., 2008). The absorption can be influenced by different factors. Low Mg solubility in the rumen due to Mg-binders, low dietary content and high potassium levels decreases the rate of absorption (GOFF, 2004). As urine is the main route of excretion (SUTTLE, 2010) and the blood Mg concentrations of all 6 groups' average around the higher reference value for blood magnesium (Figure 3) the urine Mg levels are most likely to reflect the dietary concentration of the mineral. Urinary magnesium increases with increasing dietary Mg content and if the Mg absorption is decreased due to high potassium levels magnesium excretion decreases, too (GOFF, 2004). Due to the above described facts the high values of the close up cows and the primiparous cows 8-30 DIM are most likely caused by too high Mg contents in the diet. The high individual range between 2mmol/l and 45mmol/l can be due to individual interactions with other minerals such as potassium. To avoid such situations a dietary magnesium concentration between 0.35 and 0.4% of dry matter should be ensured (GOFF, 2004).

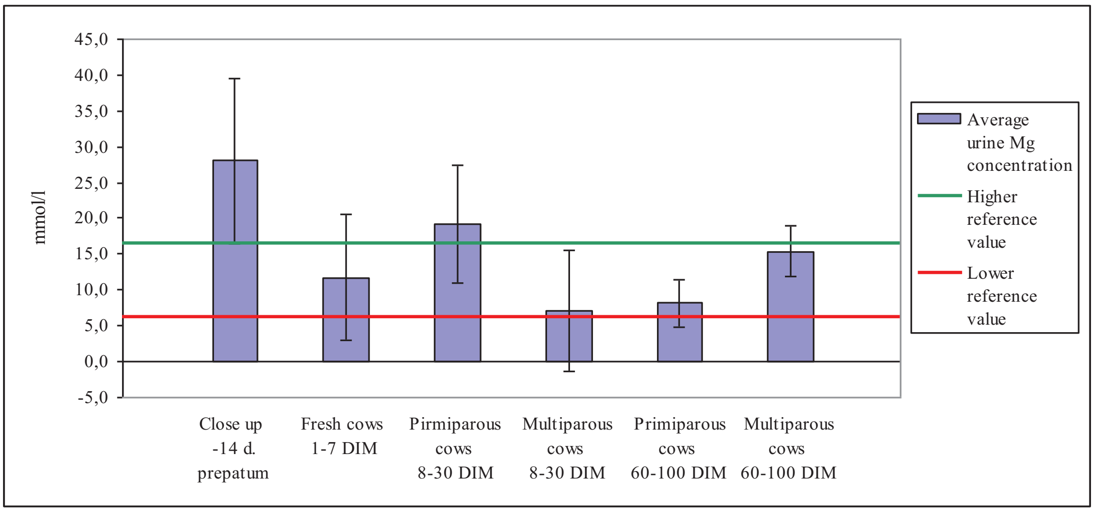

Figure 9: Average urine Mg concentration of the different groups

6.3.4 Sodium

None of the average sodium concentrations lay within the range (20-80 mmol/l). The close up cow group has levels below 20 mmol/l with 14mmol/l for the average of the group and individual levels as low as 2mmol/l. The average and many individual concentrations of the other 5 groups lay above the higher reference with single levels up to 164 mmol/l (Figure 10). Sodium is absorbed from the gastrointestinal tract, for example the small intestines, mainly as NaCl and transported by an active mechanism. The main sources in the rumen are NaCl in the diet and Na contents in the saliva which is swallowed into the rumen. The amount of sodium in the saliva depends on the concentration in the diet.

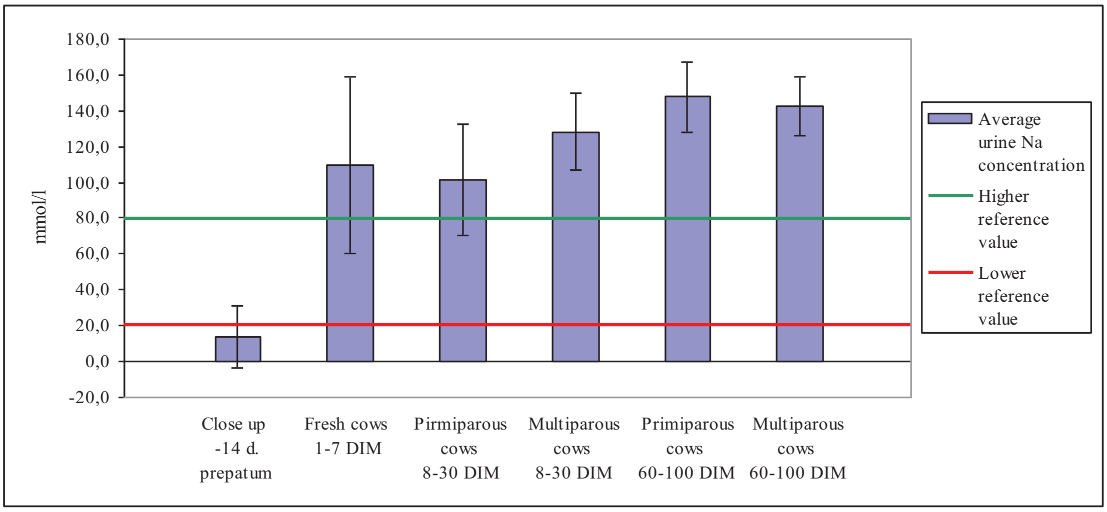

Figure 10: Average urine Na concentration of the different groups

The excretion of sodium is mainly regulated by filtration and reabsorption in the kidneys. This process depends on the dietary water and Na content on the one hand and on the regulatory activities of the brain on the other hand. In case of low sodium levels in the blood the excretion of sodium is decreased and the reabsorption increased. Due to the reabsorption either potassium or hydrogen ions are excreted as exchange (GEORGIEVSKII et al., 1982a). The high sodium concentrations in the urine of all groups except the close up cows group can be related to high dietary contents. The extremely low level of the cows 14 days before parturition can be due to deficient dietary concentrations. As the diets of the other cows are more likely to contain too high sodium diets the low values can also be related to the relatively high excretory levels of potassium in this group (Figure 11). As described above high sodium excretion lowers the potassium levels in the urine and the opposite. To avoid too high plasma and urine sodium concentration the dietary Na content should be around 0.12% of the dry matter (GOFF, 2004).

6.3.5 Potassium

The required 140 to 320 mmol/l of potassium in the urine are only reached by the cows before and after parturition as well as the primiparous cows 8 to 30 days in milking. The cows in the other groups have average and individual concentrations below the requirements (Figure 11). Potassium is needed for important body functions like osmotic pressure, cation-anion equilibrium or buffer systems in the forestomachs (GEORGIEVSKII et al., 1982a). Furthermore it influences the absorption and plasma concentrations of other minerals such as calcium and magnesium as well as their transport within the rumen (RERAT et al., 2009). Another interaction exists between sodium and potassium. Higher dietary potassium levels increase the K concentrations in the body and blood and subsequently lower the Na levels. Furthermore both minerals are mainly eliminated via the kidneys and antagonize each other also during this process. If one of the two minerals is reabsorbed the other is excreted and the opposite. On the other hand urinary potassium levels depend on the dietary K content and reflect it. The higher the content is the more potassium is excreted to keep the balance (GEORGIEVSKII et al., 1982a).

Due to these interactions the low potassium concentrations in the urine of the groups with cow 8 to 30 and 60 to 100 days in milk can be caused either by low dietary potassium or high urinary sodium as presented in Figure 10. The other two groups have urinary K levels within the range but the value for the close up group is relatively high compared to this others. Due to this the dietary potassium and sodium contents of all groups should be checked. Dietary potassium should range around 1% of DM to prevent diseases such as milk fever (GOFF, 2004).

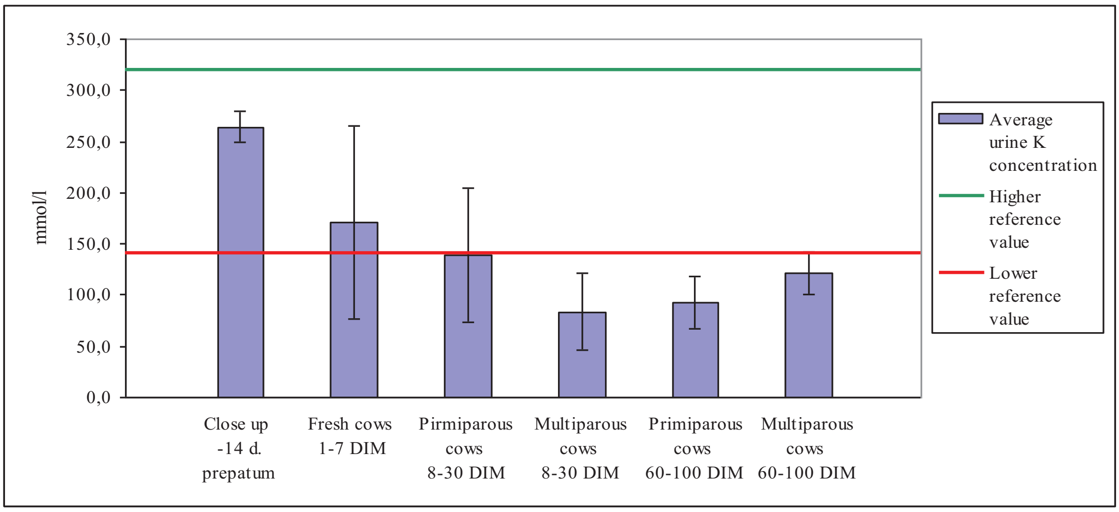

Figure 11: Average urine K concentration of the different groups

6.4 Results and discussion of hair sample analysis

The results are presenred in Table 3.

Hair sample analysis reflects not the actual mineral concentrations of the cows but the mineral state 2 or 3 months ago. The samples are taken from pigmented areas of the lateral thorax. In this the concentrations of manganese, copper and zinc in the hair are analyzed. In case of the lack of pigmented areas at the thorax samples can´t be obtained. Due to this fact values are missing for the primiparous cows 8-30 DIM and the multiparous cows 60-100 DIM. In case of the close up cows 14 days prior to parturition results exist only for one cow.

6.4.1 Zinc

The results of the zinc analysis of the hair are presented in Figure 14. All cows have concentrations above the required 100 mg/kg. The group with the lowest value is the one with the primiparous cows 60-100 DIM with 127.2 mg/kg on average. In the close up cow group only one cow is evaluated and its hair Zn concentration is 154 mg/kg which is also the highest individual value. The individual results range between 116 and 154 mg/kg. As the highest increase is 1 ½ times the reference value the concentrations fit the requirements. During the last 2 to 3 months period the zinc feeding management was appropriate. The blood Zn levels fit the requirements, too (Figure 5). So the zinc supplementation within the last 2 to 3 months was managed well and no alterations in the zinc supplementation are needed. As zinc is part of the superoxide-dismutase and plays a role in immune functions, healing processes and the keratinization of the canal and claw horn (WILDE, 2006) it is important to keep this good supplementation status.

6.4.2 Manganese

Manganese concentrations in the hair should be at least 6 mg/kg or more to ensure an appropriate dietary Mn content in the last 2 to 3 months. The average levels of all 4 evaluated groups don´t reach this threshold and only 3 individual results are as high as 6 mg/kg or even higher (6.6 and 8.0 mg/kg). The hair samples of two cows have the lowest concentration with 2.0mg/kg. One of these two cows is in the group of multiparous cows 8-30 DIM and one belongs to the cows 60-100 DIM. The highest average group value is 5.0mg/kg for the fresh cows and the lowest average group results is measured for the close up cows which is represented by only one cow with 3.0 mg/kg (Figure 12).

The general deprivation can indicate a deficient supply with manganese at that time. Manganese is needed for cholesterol synthesis (HULEY and DOANE, 1989), synthesis of steroids, metallo-enzymes, redox-processes, growth, functions of endocrine organs (SICILIANO-JONES et al., 2008) and anto-oxidative processes (ANDRIEU, 2008). Due to the influence on endocrine organs manganese has a positive impact on reproductive functions and fertility. Furthermore it can be found in high concentrations in reproductive organs and tissues such as the corpus luteum (SICILIANO-JONES et al., 2008), ovaries and the pituitary gland. On the other hand it is contained in low amounts in cystic ovaries.

Cows with deficient manganese states can experience reproductive problems. Such symptoms of reproductive failure are delayed estrus and ovulation, lower conception rates, increased abortion rates, small ovaries, delayed puberty (HURLEY and DOANE, 1989), increased incidence of retained placenta and delayed first luteal activity (CAMPBELL et al., 1999). To ensure the maintenance of appropriate manganese concentrations as well as reproductive functions and a good reproductive performance a dietary Mn level of 30mg/kg or more is needed (GEOGIEVSKII et al., 1982a).

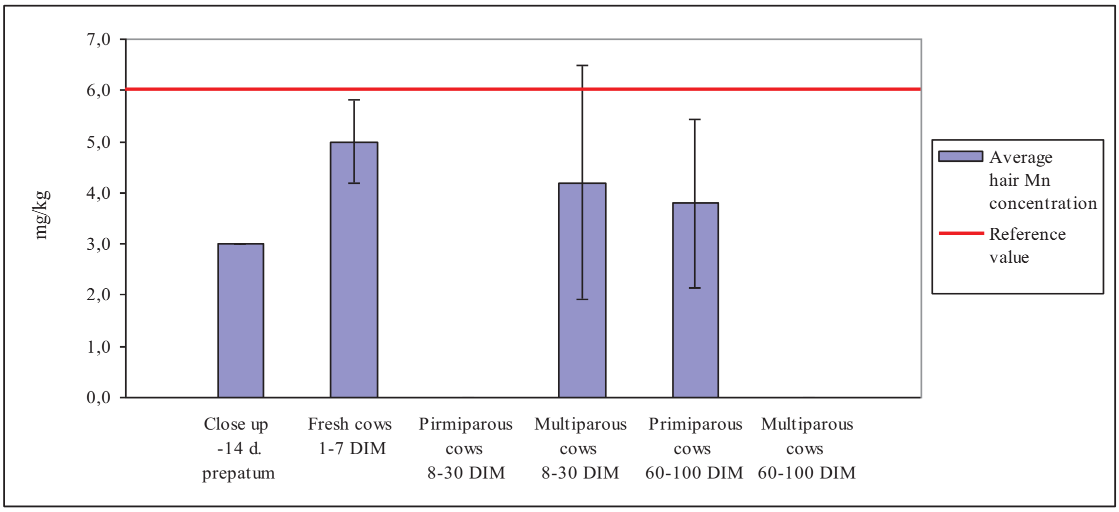

Figure 12: Average hair Mn concentration of the different groups

6.4.3 Copper

The evaluation of the copper status in the hair samples shows (Figure 13) the highest divergence between the reference value (6 mg/kg) and the actual average and individual concentrations. All 4 average values of the groups as well as all individual levels are at least 3 times higher than 6 mg/kg. The average value for the close up cow is 27.0 mg/kg (3 times higher), for the fresh cows 33.3 mg/kg (5 times higher), for the multiparous cows 8-30 DIM 33.8mg/kg (5 times higher) and for the primiparous cows 60-100 DIM 18.0 (3 times higher). Even the lowest individual copper concentration in the hair is with 13.0 mg/kg 2 times higher than the reference. The highest level is measured for a multiparous cow 8-30 days in milking with 38.0 mg/kg. This value is more than 6 times elevated compared to the requirement. Such high concentrations of copper indicate an accumulation of the mineral in the body in the previous 2 to 3 months.

This accumulation can be caused by too high dietary Cu content fed continuously to the cows because the concentrations of the mineral in organs such as the brain, spleen and liver depend on the feeding levels. Also the slightly too high blood Cu levels can indicate an increased dietary feeding level of the mineral (Figure 4). As the blood levels are only minimally elevated there is no incidence of the occurrence of acute problems. Due to the possibility of copper to accumulate in some organs like the spleen, the liver and the hairs a continuous increased supplementation can lead to chronic problems. Especially the liver has the possibility to store and deliver the mineral to keep the homeostasis in balance and is affected by high feeding levels. The most common problem arising from such a supplementation management is the chronic copper toxicosis (GEORGIEVSKII et al., 1982b).

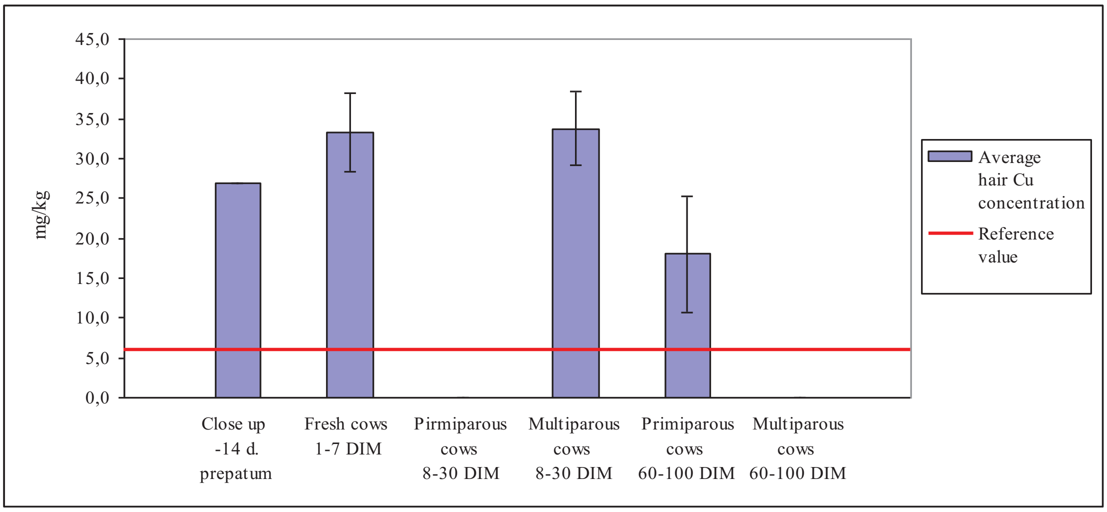

Figure 13: Average hair Cu concentration of the different groups

A high amount of copper in the plasma is bound to ceruloplasmin which has a Cu content of 0.34%. 5-10% of the mineral are bound to albumins and β-globulins. This 5-10% forms the fraction of labile copper which is mainly stored in the liver. Due to the deposition of labile copper compounds in the liver the Cu concentrations in this organ indicate if the dietary copper supplementation is adequate or not. In case of high dietary Cu content more copper is stored in the liver and in case of chronic phases of too high dietary copper levels chronic copper toxicosis can occur. Also increasing the doses of copper abruptly with any possibility of adaptation can cause intoxication with the mineral but this form of copper intoxication is more acute.

Chronic poisoning necrotizes the liver cells and causes other problems such as methaemoglobinaemia, bilirubinaemia and hemolysis of red blood cells. Some clinical signs are jaundice, thirst, no appetite, apathy, accelerated breathing and stronger heartbeat. In case of very severe cases hepatic coma, dyspnea and spasms can be observed which result in death (GEORGIEVSKII et al., 1982b). To avoid a chronic overfeeding with copper the supplementation should not be more than 2 grams of copper sulphate per animal and day (FEKETE, 2008).

7 Conclusions

Minerals fulfill many important functions in the cow´s body and their appropriate supplementation with the feed decreases the risk of the occurrence of metabolic and infectious diseases. Furthermore balanced mineral concentrations in the blood support the milk production and reproductive performance.

In this study the average excretory potassium levels fit only for the close up group and the fresh cows. For the other 4 groups the levels are too low. On the contrary the sodium for all groups except the close up cows is too high. As these two minerals interact with each other on excretory level the high urinary excretion of one mineral can implicit a low urinary concentration of the other. On the other hand both minerals are mainly excreted over the urine and their filtration and reabsorption is influenced by the dietary supplementation of the minerals. To ensure appropriate feeding levels sodium and potassium concentrations in the diet should be 0.12 and 1% of the dry matter.

Other important results are the deficient manganese concentrations in the hair samples. They indicate a deficient supplementation of the mineral 2 to 3 months before the sampling. As manganese influences the reproductive performance and its deficiency can lead to reproductive failure the dietary content should be at least 30 mg/kg to avoid any reproductive problems.

The most important metabolic problem is caused by the increased copper levels in the blood and hair samples. The blood levels are only slightly elevated but the concentrations in the hair are up to 5 times higher than the reference value. These results indicate the possibility of a chronic copper toxicosis which can cause hemolysis of the erythrocytes and jaundice. In case of severe cases hepatic coma can be followed by death. To avoid the chronic poisoning only 2 g of copper sulphate should be administered to each cow per day, that means approximately 10 mg/kg Cu concentration of the daily ration of cows on dry matter basis.

Summary

Macrominerals as well as trace elements take part in important processes in the body and have regulative functions. Sodium, potassium and chlorine keep the cation-anion equilibrium in in balance, calcium builds up the bones and is needed for milk production, phosphate can be found in all cell types and is in duty to transfer energy and magnesium is needed for nerve and muscle irritability. Their deficiencies can lead to severe diseases like milk fever or tetany. On the other hand an excess concentration can harm the animals, too. Too high potassium levels impair the calcium mobilization and contribute to milk fever due to this. Microminerals such as selenium, zinc, copper and manganese support the immune functions, anti-oxidative processes, keratinization of the teat canal and claw horn and reproductive performance. Deprivation of them increases the risk of reproductive problems, infectious diseases like mastitis and bad claw integrity. On the opposite excess amount dietary copper supplemented over a long period can lead to chronic copper toxicosis.

In this study blood, urine and hair samples were taken from Holstein-Frisian cows in a large scale farm in the middle of Hungary. The cows were divided into 6 groups – 1) close up cows 14 days before parturition, 2) fresh cows up to 7 days after parturition, 3) primiparous cows 8-30 days in milking (DIM), 4) multiparous cows 8-30 DIM, 5) primiparous cows 60-100 DIM and 6) multiparous cows 60-100 DIM. Results of blood and urine analysis were available for all 6 groups whereas hair samples were only obtained for one cow in the close up group and the groups with the fresh cows, the multiparous cows 8-30 DIM and the primiparous cows 60-100 DIM.

The two most important results were deficient manganese concentrations in the hair samples and excess copper levels after hair and blood analysis. Manganese deficiency contributes to reproductive failure as it accumulates in reproductive organs and tissues. Increased copper levels indicate too high dietary copper content. As the average hair analysis values of the groups are increased up to 5 times and hair samples present the mineral state 2 to 3 months before the sampling the results indicate excessive Cu supplementation over a longer period. This dietary Cu management can lead to chronic copper toxicosis which can result in death in severe cases. To avoid the above described problems dietary manganese content should be 30mg/kg and copper dietary content should be 10mg/kg on dry matter basis per animal and day.

Key words: Minerals, trace elements, dairy cows, lactation, reproduction

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Appendices

Appendix 1: Used Abbreviations

1.25-$(OH)_2D_3$	Dihydroxycholecalciferol
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BCS	Body condition score
cAMP	Cyclic adenosine monophosphate
DCAD	Dietary cation-anion difference
DIM	Days in milking
DM	Dry matter
DMI	Dry matter intake
EDTA	Ethylendiamintetraacetat
GSH-Px	Glutathione-peroxidase
LH	Luteinizing hormone
NEB	Negative energy balance
PTH	Parathyroid hormone
PTHrP	PTH related protein
RNA	Ribonucleic acid
SCC	Somatic cell count
SOD	Superoxide-dismutase
RFM	Retained fetal membranes
TMR	Total mixed ratio

Appendix 2: Tables and Figures

Table 1: Results of the blood sample analysis

Groups	ID	BCS	Ca mmol/l	Anorg. P mmol/l	Mg mmol/l	Cu µmol/l	Zn µmol/l	GSH-Px U/g Hb
Reference value		2,5-3,5	2,1-3,0	1,6-2,3	0,8-1,2	10,0-18,9	10,0-30,6	20-30
Close up -14 d. prepatum	7980	3,5	2,6	2,0	1,2	21,1	16,0	23,0
	4561	3,5	2,5	1,3	1,1	18,8	14,0	21,0
	6779	3,5	2,6	1,0	1,2	19,9	18,0	22,0
	8213	3,0	2,6	1,9	1,1	20,3	16,0	23,0
	4466	3,0	2,4	1,7	1,2	18,5	15,0	20,0
Average		**3,3**	**2,5**	**1,6**	**1,2**	**19,7**	**15,8**	**21,8**
Standard deviation		**0,3**	**0,1**	**0,4**	**0,1**	**1,1**	**1,5**	**1,3**
Fresh cows 1-7 DIM	4869	3,0	2,0	2,2	1,0	18,0	14,0	
	5731	2,5	2,4	1,8	1,1	22,0	18,0	
	5897	3,0	1,9	1,7	1,1	17,1	15,0	
	7294	3,0	2,3	2,1	0,9	19,0	16,0	
	8112	3,5	2,3	1,9	1,2	21,3	16,0	
Average		**3,0**	**2,2**	**1,9**	**1,1**	**19,5**	**15,8**	
Standard deviation		**0,4**	**0,2**	**0,2**	**0,1**	**2,1**	**1,5**	
Pirmi-parous cows 8-30 DIM	8179	3,0	2,3	2,3	1,2	19,1	17,0	
	7977	2,5	2,4	2,5	1,5	22,1	16,0	
	8195	2,5	2,3	2,5	1,1	21,8	16,0	
	8226	3,0	2,3	2,3	1,3	22,6	19,0	
	7938	2,5	2,5	2,3	1,4	23,4	18,0	
Average		**2,7**	**2,4**	**2,4**	**1,3**	**21,8**	**17,2**	
Standard deviation		**0,3**	**0,1**	**0,1**	**0,2**	**1,6**	**1,3**	
Multi-parous cows 8-30 DIM	4992	3,0	2,4	2,2	1,1	20,6	17,0	20,0
	7280	2,5	2,4	1,8	1,1	22,4	15,0	22,0
	5535	3,5	2,3	1,6	1,2	20,8	17,0	22,0
	7848	3,0	2,3	2,5	1,3	18,9	19,0	22,0
	7299	2,5	2,4	2,1	0,9	19,0	16,0	22,0
Average		**2,9**	**2,4**	**2,0**	**1,1**	**20,3**	**16,8**	**21,6**
Standard deviation		**0,4**	**0,1**	**0,4**	**0,1**	**1,4**	**1,5**	**0,9**
Primi-parous cows 60-100 DIM	8099	3,0	2,4	1,9	1,3	28,9	14,0	23,0
	7965	2,5	2,5	2,6	1,3	22,3	18,0	21,0
	7863	2,5	2,5	2,2	1,3	19,8	19,0	22,0
	7787	2,0	2,5	2,3	1,3	21,8	19,0	20,0
	8142	2,5	2,3	1,9	1,1	24,8	18,0	23,0
Average		**2,5**	**2,4**	**2,2**	**1,3**	**23,5**	**17,6**	**21,8**
Standard deviation		**0,4**	**0,1**	**0,3**	**0,1**	**3,5**	**2,1**	**1,3**
Multi-parous cows 60-100 DIM	5685	2,0	2,4	1,9	1,3	20,6	18,0	
	4674	4,0	2,4	1,9	1,3	20,8	14,0	
	6742	2,5	2,4	2,4	1,1	18,5	16,0	
	6131	2,0	2,3	2,3	1,3	17,8	17,0	
	6338	3,0	2,4	2,3	1,0	22,0	14,0	
Average		**2,7**	**2,4**	**2,2**	**1,2**	**19,9**	**15,8**	
Standard deviation		**0,8**	**0,0**	**0,2**	**0,1**	**1,7**	**1,8**	

DIM … Days in milking GSH-Px … Glutathione-peroxidase

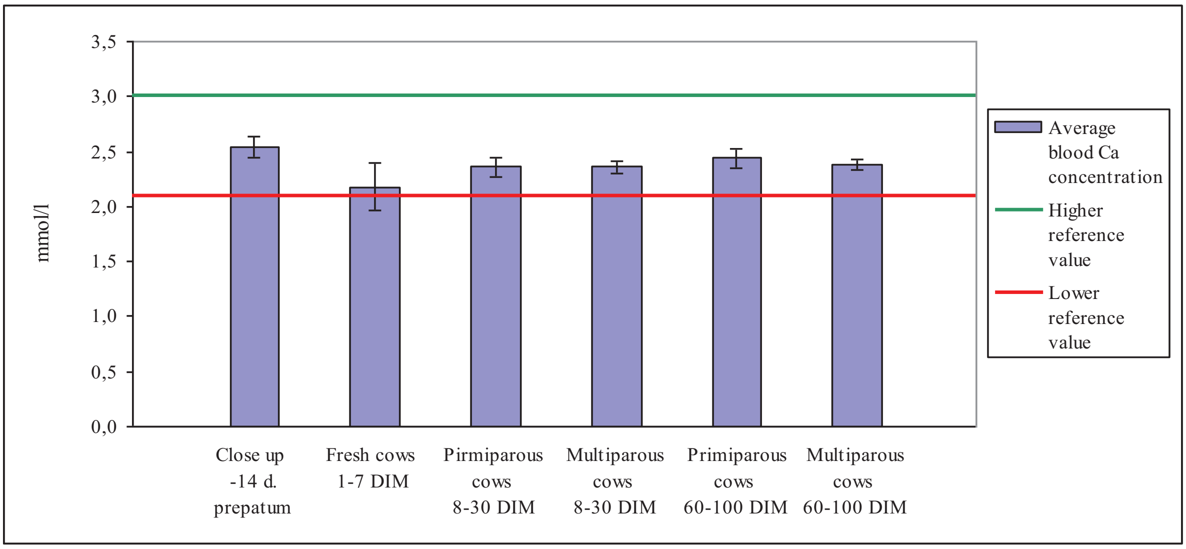

Figure 1: Average blood Ca concentration of the different groups

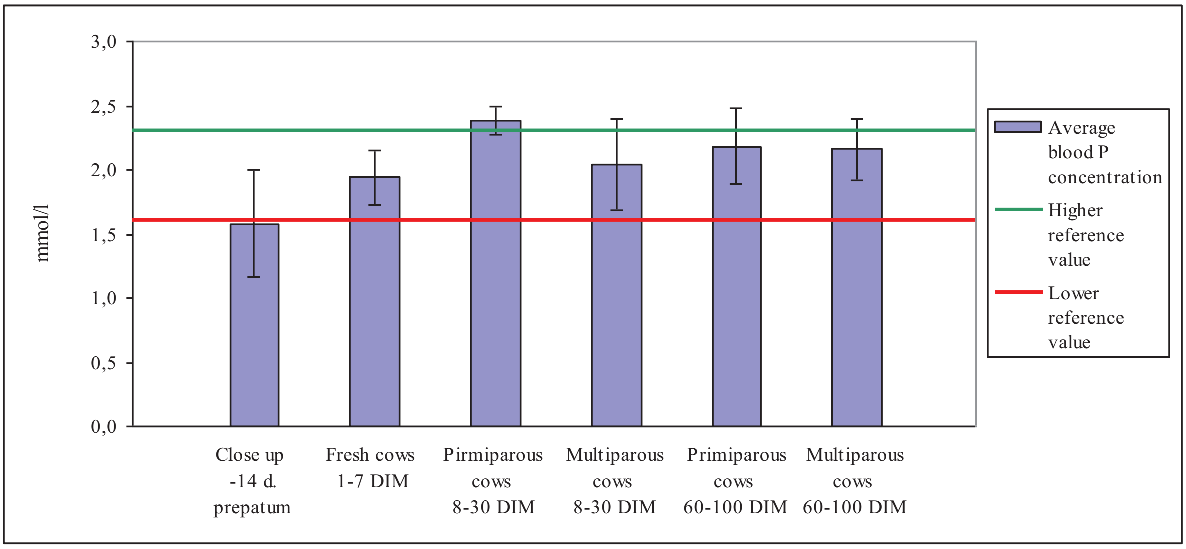

Figure 2: Average blood anorganic P concentration of the different groups

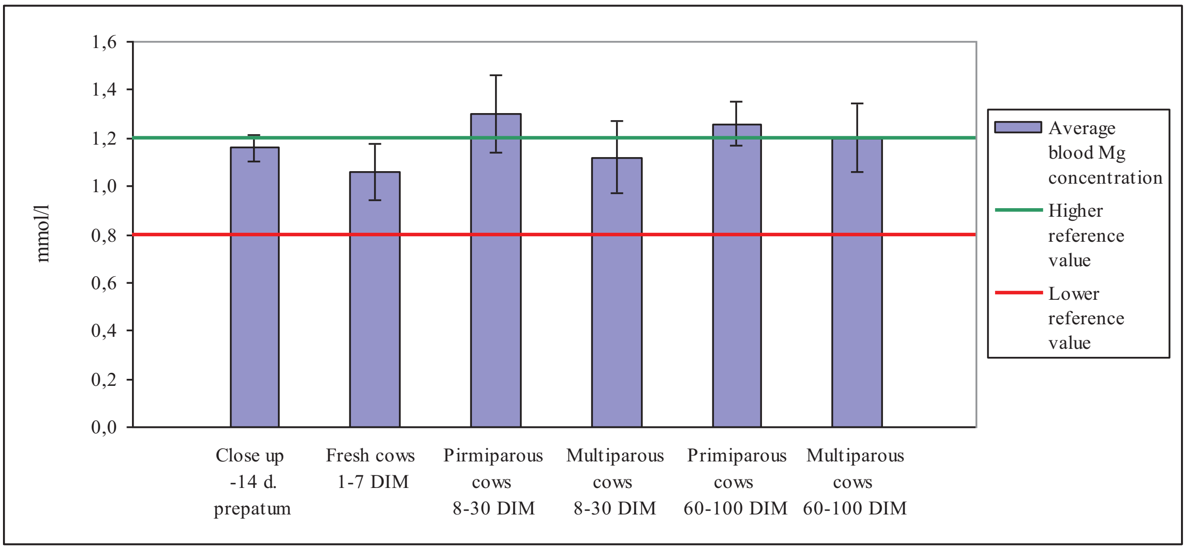

Figure 3: Average blood Mg concentration of the different groups

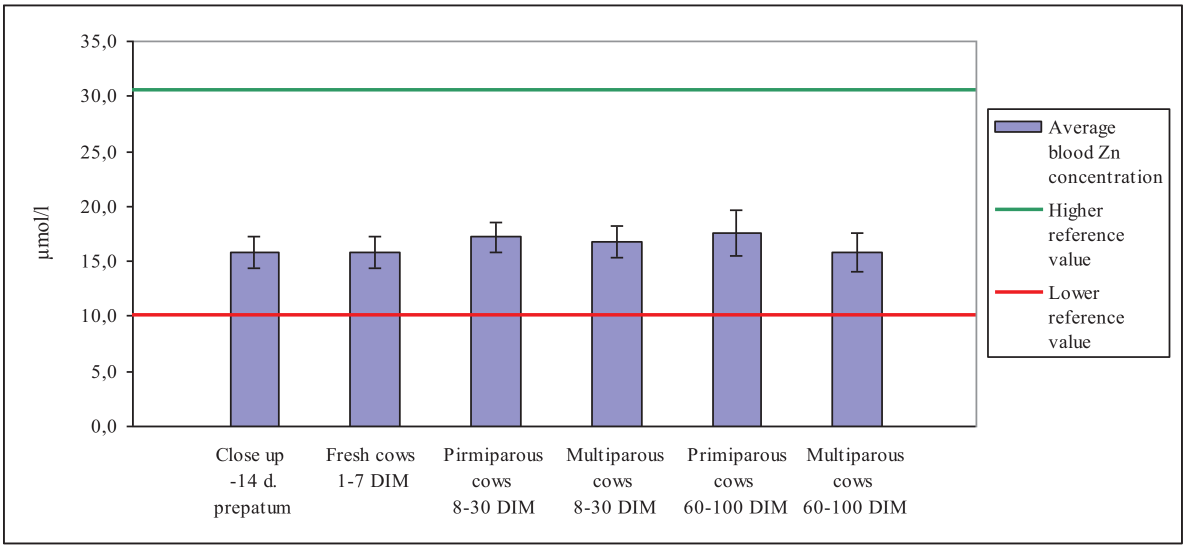

Figure 5: Average blood Zn concentration of the different groups

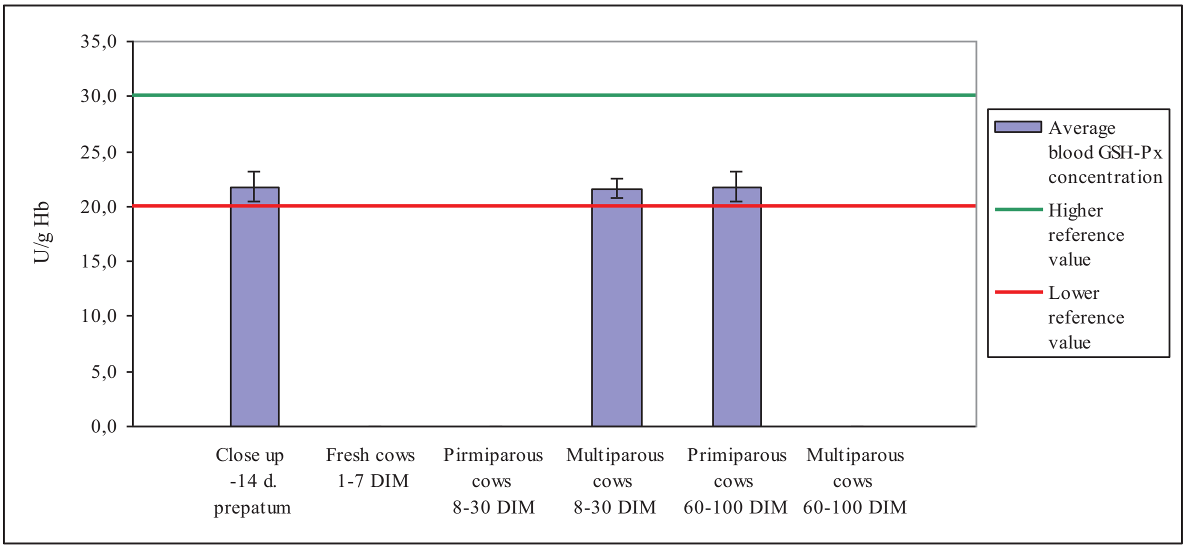

Figure 6: Average blood GSH-Px concentration of the different groups

Table 2: Results of the urine sample analysis

Groups	ID	Ca mmol/l	P mmol/l	Mg mmol/l	Na mmol/l	K mmol/l
Reference value		0,1-1,5	0,3-5,2	6,2-16,5	20-80	140-320
Close up -14 d. prepatum	7980	8,9	1,3	45,0	2	251
	4561	3,8	1,2	24,0	40	274
	6779	5,1	1,0	32,0	23	282
	8213	3,0	1,1	25,0	2	247
	4466	2,5	2,5	14,0	2	268
Average		**4,7**	**1,4**	**28,0**	**14**	**264**
Standard deviation		**2,6**	**0,6**	**11,5**	**17**	**15**
Fresh cows 1-7 DIM	4869	2,6	2,4	3,0	143	100
	5731	1,1	1,1	24,0	42	308
	5897					
	7294	2,7	0,8	11,0	149	123
	8112	1,8	0,8	9,0	105	153
Average		**2,1**	**1,3**	**11,8**	**110**	**171**
Standard deviation		**0,8**	**0,8**	**8,8**	**49**	**94**
Pirmi-parous cows 8-30 DIM	8179	1,9	4,3	32,0	72	218
	7977	0,9	8,6	20,0	77	90
	8195	1,5	6,3	12,0	100	89
	8226	2,0	1,7	20,0	110	203
	7938	1,5	5,5	12,0	149	95
Average		**1,6**	**5,3**	**19,2**	**102**	**139**
Standard deviation		**0,5**	**2,5**	**8,2**	**31**	**66**
Multi-parous cows 8-30 DIM	4992	3,0	0,9	6,0	144	80
	7280	0,5	0,8	4,0	158	62
	5535	0,6	1,2	2,0	114	75
	7848	0,4	0,9	22,0	120	148
	7299	0,4	0,5	2,0	106	54
Average		**1,1**	**0,9**	**7,2**	**128**	**84**
Standard deviation		**1,3**	**0,3**	**8,4**	**22**	**37**
Primi-parous cows 60-100 DIM	8099	0,6	11,0	9,0	114	110
	7965	0,4	1,9	3,0	156	55
	7863	1,1	1,3	8,0	150	122
	7787	2,0	0,9	12,0	164	88
	8142	0,9	0,7	9,0	155	92
Average		**1,0**	**3,2**	**8,2**	**148**	**93**
Standard deviation		**0,5**	**4,4**	**3,3**	**19,5**	**25,5**
Multi-parous cows 60-100 DIM	5685	1,9	0,9	15,0	150	102
	4674	1,0	0,9	19,0	150	108
	6742	0,3	0,8	15,0	153	142
	6131	0,8	0,9	18,0	147	110
	6338	0,3	1,0	10,0	113	145
Average		**1,0**	**0,9**	**15,4**	**143**	**121**
Standard deviation		**0,7**	**0,1**	**3,5**	**17**	**20**

DIM … days in milking

Table 3: Results of the hair sample analysis

Groups	ID	Mn mg/kg	Cu mg/kg	Zn mg/kg
Reference value		> 6.0	> 6.0	> 100
Close up -14 d. prepatum	7980	3,0	27,0	154,0
	4561			
	6779			
	8213			
	4466			
Average		**3,0**	**27,0**	**154,0**
Standard deviation				
Fresh cows 1-7 DIM	4869			
	5731	4,0	37,0	153,0
	5897	5,0	26,0	121,0
	7294	6,0	34,0	125,0
	8112	5,0	36,0	128,0
Average		**5,0**	**33,3**	**131,8**
Standard deviation		**0,8**	**5,0**	**14,5**
Multi-parous cows 8-30 DIM	4992	4,0	27,0	123,0
	7280	8,0	36,0	139,0
	5535	2,0	31,0	131,0
	7848	3,0	38,0	134,0
	7299	4,0	37,0	149,0
Average		**4,2**	**33,8**	**135,2**
Standard deviation		**2,3**	**4,7**	**9,7**
Primi-parous cows 60-100 DIM	8099	6,0	31,0	135,0
	7965	5,0	16,0	116,0
	7863	2,0	13,0	128,0
	7787	3,0	15,0	129,0
	8142	3,0	15,0	128,0
Average		**3,8**	**18,0**	**127,2**
Standard deviation		**1,6**	**7,3**	**6,9**

DIM … days in milking

Figure 12: Average hair Mn concentration of the different groups

Figure 13: Average hair Cu concentration of the different groups

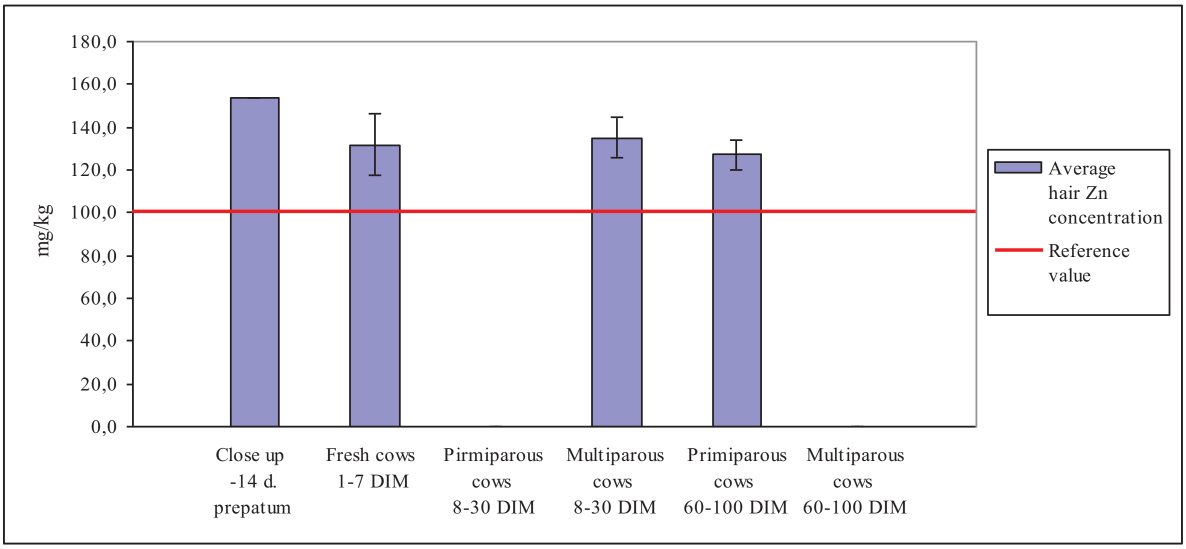

Figure 14: Average hair Zn concentration of the different groups

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Contact information (e-mail): barbara@koeglberger.com

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