CURRENT VIEW OF THE SIGNIFICANCE OF ZINC FOR RUMINANTS: A REVIEW

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Abstract

The study compiles knowledge about the significance of zinc for ruminants, the causes and effects of insufficient zinc, sources of zinc and its concentration in milk in connection with the human population, analyses the situation of zinc deficiency in cattle in the Czech Republic and addresses inorganic and organic forms of supplementing.

Key words: zinc sources, zinc requirement, zinc content in blood plasma, hypozincaemia, milk, inorganic and organic forms of zinc

INTRODUCTION

Intensification of production and increase of the milk yield of cows requires not only full coverage of the requirements for proteins and energy, but also an appropriate balancing of the mineral elements and vitamins with regard to physical form and interaction between the components of the feed provided (Strusińska et al., 2003). Provision of mineral-vitamin supplements has the effect of increasing the concentration of vitamins and macro and micro elements in the colostrum and milk (Strusińska, Iwańska, 1994) as well as influencing the production and quality of milk (Kinal et al., 2004a). In the case of a Zn deficit in the feed ration, there is a reduced level also in milk, which has an unfavourable effect on the development of young ones (Lamond, Périgoud, 1973 in Vrzgula et al., 1990).

State of feeding with zinc in the Czech Republic and significance of zinc for the organism of ruminants

Within the conditions of the Czech Republic we encounter zinc deficiency disease primarily in shortfed calves and bullocks, and less frequently in dairy cows (Illek, 1990). The results of recently conducted monitoring of the zinc content in cow's milk in 84 stocks in 16 districts of the Czech Republic (Trávníček et al., 2004) showed only approximately 13 % subnormative values and an average concentration of 4.66 \pm 0.59 mg.l⁻¹, and toxic limits were thus not exceeded. A similar value was determined in Spain: 4.41 mg.l⁻¹ (Rodriguez et al., 2001 in Dobrzański et al., 2005). In Poland, within the Silesian macro region there was an average of $3,307 \pm 1,108 \ \mu g \ Zn.l^{-1}$ of milk in cows at full lactation (Dobrzański et al., 2005). Very low values were recorded e.g. in the milk of cows in Croatia - only $0.51 \pm 0.16 \text{ mg Zn.kg}^{-1}$ (Sikirić et al., 2003) and in California, where concentrations were close to the analytical detection limit (McCaughey et al., 2005).

Zinc influences several biological functions, encompassing DNA synthesis, cell division and gene expression (Prasad, 1991), and ranks amongst nutrients which share in bone formation on a cellular level (Roughead and Kunkel, 1991), as a component of alkaline phosphatase (ALP) it plays a role in ossification. Suchý et al. (1998) mention its integration into photochemical processes of vision. Its positive effect on the condition of epidermis and epithelium, skin and hooves (Kruczyńska, 2004) may be placed within the context of its role in keratinisation of mucous membranes, skin and skin derivatives (Suchý et al., 1998). According to research studies, zinc intake is important for the regular function of the immune system (Conway, 1988), is necessary for cell-mediated immunity (Prasad, 1991), and an insufficient amount of zinc, as observed by Szczepanik and Wilkołek (2004) was the cause of immunosuppression in trychophytosis. Present studies substantiate the favourable effect of organically bound zinc in increasing the level of immunoglobulins in the colostrums and in blood serum (Kinal, 2005a; Kinal et al., 2004b), on increasing glucose and proteins in blood serum (Strusińska et al., 2003), and on the production (Kinal et al., 2004a; Kinal et al., 2005; Šimek et al., 1995 in Šiške, 1997) and reproduction properties of cattle (Uchida et al., 2001; Campbell et al., 1999). Zinc plays a role in the formation of insulin (Kruczyńska, 2004) and is necessary for the activity of several enzymes in biological systems (Prasad, 1991; Vrzgula et al., 1990), e.g. alcohol dehydrogenase, alkaline phosphatase, aldolase, lactate dehydrogenase, RNA and DNA polymerases, reverse transcriptase, carboxypeptidase A, B, G (Bencko et al., 1995) and superoxide dismutase (SOD) (Yo, 1994 in Markiewicz et al., 2005), which has antidioxant effects - catalyses the conversion of superoxide anions to oxygen and hydrogen peroxide (Noor et al., 2002 in Blander et al., 2003).

Manifestations of deficiency and excess of zinc in ruminants

In animals a Zn deficiency may be manifested in changes in taste perception, accompanied by damage to the tongue epithelium, as well as disorder of keratin synthesis, limited growth of limb bones and sight disorders (Anke et al., 1994). The mechanism of growth retardation in the case of zinc deficiency can be seen in loss of appetite, imperfect use of nutrients from feedstuffs and in disorders of the protein and energy metabolism (Illek, 1990). A specific disorder resulting from zinc deficiency is parakeratosis – a disorder of the epidermal layer of the skin occurring in calves, sheep, goats and piglets. In calves it manifests itself in characteristic shedding of the coat around the eyes ("glasses"), on the head, neck and limbs (Suchý et al., 1998). Similar findings in free living ruminants were observed by Abdou (2005). Excess intake of Zn is relatively rare in farm animals. It occurs for example in piglets treated with medicaments with high zinc content. An excess of zinc reduces the digestibility of phosphorus, causes anaemia and digestive disorders. Poisoning is conditioned primarily by the antagonistic relationship of zinc to iron and copper (Suchý et al., 1998). Noakes et al. (2001) note that excessive intake of Zinc additives may lead to disorders of the essential fatty acid metabolism, which influences synthesis of prostaglandin.

Relationship of zinc to reproduction, milk efficiency and quality parameters of milk

The influence of Zn on reproductive functions resides in its effect on hormones (impacting on excretion of gonadotrophins, androgens and prostaglandins, sharing in prolactin release, playing a role in contraction of myometrium during birth, influencing the motility of sperm and their ability to penetrate the egg) (Suchý et al., 1998) and in its role as an antidioxant (Fettman, 1991 in Noakes et al., 2001).

The effects of Zn deficiency relate to both male and female individuals. The higher Zn requirement in males in comparison with females is connected to the considerable quantity thereof in prostate secretion. In the case of diets with insufficient Zn, retarded development of testicles and impaired spermatogenesis has been observed (Anke et al., 1994), as well as poor sperm quality (Swenson and Johnson), reduced libido sexualis, in females impaired synthesis and secretion of FSH and LH, abnormal ovary development, disorders of the oestrous cycle (Bedwal and Bahaguna, 1994), difficulties in becoming gravid as a result of impaired ovulation, reduced number of fertile eggs, as well as low birth weight, extended gravidity period (Anke et al., 1994), complications during birth and frequent occurrence of miscarriages (Bedwal and Bahaguna, 1994). Plasma zinc may be a useful biomarker for risk of spontaneous miscarriage, and decline in plasma zinc may be caused by PGF2alpha (Graham et al., 1995). Zinc significantly reduces the effect of retardation of intrauterine development (Simmer et al., 1991), and intrauterine deficit thereof retards development of testicles and may delay the onset of sexual maturity. These manifestations of deficiency also relate to humans (Anke et al., 1994). Feeding of organically bound Zn together with Cu, Co and Mn to dairy cows significantly reduces the insemination interval (Campbell et al., 1999) and the length of the service period (Uchida et al., 2001). Organically bound zinc favourably influences milk production (Kinal, 2005a; Kinal et al., 2005) and reduces the number of somatic cells in milk thanks to improved keratin formation in the teat channel (Šimek et at., 1995 in Šiške, 1997). In contrast with the aforementioned authors, Uchida et al. (2001) did not determine the impact of the organic form of Zn on milk efficiency and the number of somatic sells. Zn supplementation significantly (P < 0.01) increased the

Zn concentration in the cream but not in the skim milk fraction, supporting the hypothesis that the effect of Zn may be associated with an effect in the milk fat globule membrane (Hermansen et al., 1995). In raw cow's milk a correlative dependency was determined between the Zn content and the content of milk proteins (r_{yy} 0.30) and urea in milk (r_{yy} 0.44) (Trávníček et al., 2004).

Inorganic versus organic zinc supplements and other factors influencing the requirement for zinc in animals Zinc occurs most frequently in the forms of ZnCl₂, $ZnSO_4 \cdot 7 H_2O$ or ZnO, and may also occur in the form of organic salts (chemical binding of metallic cation with the remainder of organic acid). Studies conducted in the 1970s enabled the compilation of a biotechnological method of synthesis of substances - binding of microelements with amino acids, polypeptides, proteins or polysaccharides (Słupczyńska and Kinal, 2003a). Considerable interest in the use of chelate or organic trace elements in the diet of ruminants was supported by contributions for improved growth, reproductions and health in ruminants fed by an organic form of trace elements (Spears, 1996). Use of amino acid chelates and Bioplexes of zinc for the production of mineral additives for ruminants is also substantiated by their favourable physical-chemical properties in comparison with sulphates and oxides (Słupczyńska and Kinal, 2003b). The effectiveness of organic forms resides in their higher activity and biological availability (Hemken et al., 1996 in Kinal et al., 2004a), digestibility of Zn from bonds with proteinates, yeast cells, lactates etc., and is several times higher in comparison with inorganic compounds. Use of Zn from zinc sulphate indicates 15–40 % (Suchý et al., 1998). Higher absorption was observed in zinc sulphate than in zinc oxide (Kinal, 2000). Higher absorption and utilisation of zinc in its organic forms is also confirmed by Kinal (2005b) in lambs, whilst against this Spears (1989 in Spears, 1996) mentions equal absorption of Zn from the form of Znmethionine and zinc oxide. Upon use of the organic form, statistically significant differences of the Zn content in the serum of calves (Kinal et al., 2004a) and piglets (Novotný et al., 2005) were observed. Whitaker et al. (1997) did not determine differences in the number of somatic cells in the milk of cows supplemented with an addition of the inorganic form of Zn + Zn-proteinate and dairy cows fed only with the inorganic form of Zn. A positive impact of organically bound Zn was also found in poultry (Jankowski et al., 2003).

Resorption of zinc is further dependent on the quantity thereof in the feed (in the case of a reduced quantity of zinc resorption is increased and conversely is reduced in the case of a high quantity of zinc), on age, and interaction with other elements: Ca, Fe, Cu, Cd, Mn, Se (Suchý et al., 1998), Mg, As, Pb, Hg and Al. Low Zn content in earth and feeds, high intake of phosphates, deficiency of carbohydrates, vitamin A and amino acids in feed conditions the occurrence of hypozincaemia (Vrzgula and Sokol, 1987). Absorption of zinc is also reduced by an excess or deficiency of proteins in feed (Vrzgula et al., 1990). Lower absorption of zinc was also observed after oral feeding of large doses of zinc and in the presence of interfering factors in diet, such as phytates, whereas absorption of zinc is increased in the case of low body weight and reduced content of zinc in the organism (Beneš in Neuberg, 1978 in Bencko et al., 1995). Of all the dietary additives, phytic acids, ordinarily found in plants, have the most apparent impact on the degree of utility of zinc. This however probably concerns only hexaphosphate and pentaphosphate derivatives of inositol, forming insoluble and thus unusable calciumzinc-phytate complexes, which shows that the degree of utility of zinc from a diet containing phytate depends also on the presence of calcium (Kvasničková, 1998). In balancing the Zn requirement it is necessary to devote attention also to the content of Fe and Cu (Grela and Pastuszak, 2004). Increasing the Zn dose to the level of 1,000–3,000 mg.kg⁻¹ of feed led to a decline in the concentration of Cu in blood serum (Hill et al., 2001 in Grela and Pastuszak, 2004) and in connection with a reduced concentration of ceruloplasmin to lower use of Fe.

Resorption of zinc is an active process, a part of zinc is retained in intestinal mucous, from where it is slowly released. In the intestine this binds to protein and upon consumption of ATP is carried over the basal membrane. Absorbed zinc binds to albumin and is distributed via the liver to further tissues (Suchý et al., 1998). In experimental animals absorption of zinc is stated within a scope from 10 to 90 %. In healthy persons, 58-77 % absorption was determined upon provision of low doses of ⁶⁵ZnCl₂, in patients with acrodermatitis enterohepatica only 16–42 % absorption of the given dose was determined (Lombeck et al., 1975 in Bencko et al., 1995). Elimination of Zn takes place mostly through defecation (Vrzgula et al., 1990), e.g. in mice 50 % of the given dose was found in defecations during the course of one week following intravenous dosing of 0.3 μ g ZnCl₂, secretion in urine was lower (< 5 %) (Sheline et al., 1943 in Bencko et al., 1995). In dairy cows Kroupová (2002) states Zn content in defecations within the range of 39.3–196.4 mg.kg⁻¹ of dry matter. Excessive intake of calcium plays a role in increasing Zn secretion through defecation (Kroupová et al., 2001).

Contemporary researches have engaged with the impact of the yeast culture *Saccharomyces cerevisiae*¹⁰²⁶ on the availability of mineral substances for milk synthesis. The effect depends on the dynamics of degradation of mineral Bioplexes in the rumen and the mineral status of dairy cows (Iwańska et al., 1999). The addition of yeast cells stimulated an increase in the Zn level in milk (Strusińska et al., 2004). The addition of yeast cells and chelates of mineral elements increases their availability for rumen micro-organisms, and thus conditions the growth of transport of micro-elements into tissues or blood (Strusińska et al., 2003).

Sources of zinc for animals, evaluation parameters of degree of supply of zinc to animals and its occurrence in the organism

Natural sources of zinc for farm animals are primarily bran, grains and fodder yeasts (Suchý et al., 1998). The average zinc concentration in feeds is 36 mg.kg⁻¹ of dry matter (Minson, 1990 in Underwood and Suttle, 2001). Bujanowicz-Haraś et al. (2004) determined an average of 33.60 mg Zn.kg⁻¹ of dry matter in standard feed doses in central and eastern Poland. A liquid additive of mineral elements (Nutra-Lix, Billings, MT), which contains inter alia 330 mg Zn.kg⁻¹ (Earley et al., 1999) is also currently commercially available for cattle.

Upon an evaluation of zinc deficit, measurement of the zinc concentration in blood plasma is taken as the basis (Kvasničková, 1998). In a human this is approximately 15 µmol Zn.l⁻¹ (Jackson, 1989 in Tapiero and Tew, 2003) in blood plasma, a referential physiological range of 10.7–16.8 µmol.1⁻¹ is stated (Masopust, 1982). The normal scope of Zn in the plasma of dairy cows is 10.5–17 µmol/l (Calamari et al., 1989 in Masoero et al., 1998). Strusińska and Iwańska (1994) publish the value of 15.7 µmol Zn.1⁻¹ in the blood plasma of cows 60 days post partum. The Zn requirement for cattle is 50 mg.kg⁻¹ (min. 45, max. 250) of dry matter of feed dose, for dairy cows in lactation this is 850–1,200 mg Zn.day⁻¹ (upon consumption of 17-24 kg per dry matter) and for nonlactating dairy cows 600 mg/day (upon consumption of 12 kg per dry matter) (Kruczyńska, 2004). In clinically healthy dairy cows in full lactation and age of 4–12 years an amount of $2,575 \pm 644 \,\mu g \, Zn.1^{-1}$ of whole blood was determined (Dobrzański et al., 2005), Kruczyńská (1992) observed that the level of zinc in blood serum of cows is on average 13.0-48.7 µmol.l⁻¹ and in calves 10.7–39.0 µmol.l⁻¹.

Zinc concentrations in blood serum or plasma are the most widely used indicator, but low values are usually an early change and a measure of deficiency, lacking certainty and sensitivity as a diagnostic criterion. However, a recent study reported a reduction in growth before any fall in plasma zinc in heifers (Engle et al., 1997 in Underwood and Suttle, 2001).

There is a considerable quantity of Zn in cow's milk in comparison e.g. with the Cu content. According to Illek et al. (2000) milk contains 4–5 mg Zn.1-1. The concentration of Zn in milk is higher than the concentration of Se (Hatano et al., 1985 in Alaejos and Romero, 1995). Campillo et al. (1998) determined $3 \pm 0.2 \,\mu g \, Zn.ml^{-1}$ in cow's milk and 23.5 \pm 0,3 $\mu g.g^{\text{-1}}$ in dried milk. The Zn content in milk can be influenced by nutrition, whilst the content of certain elements (Ca, P) in milk is constant and is not reduced even in the case of a severe deficiency of these elements, because dairy cows release these elements into milk from the skeleton (Illek, 1998). According to McCaughey et al. (2005), the mineral composition of milk does not depend on diet. Lehti (1990) determined that age has no significant influence on the Zn quantity in milk.

The most zinc in the organism is found in muscle tissue, the liver, bones and mammary glands (Suchý et al., 1998). In milk Zn is bound primarily to colloidal calcium phosphate of the casein micelle (Silva et al., 2001). In the blood zinc is 75 % bound in plasma (primarily to proteins), 22 % in erythrocytes 3 % in leukocytes (Sollman, 1957 in Bencko et al., 1995). The concentration of zinc in plasma is approximately 15 μ mol.l⁻¹ of which 84 % is bound to albumin, 15 % to a2–macroglobulin and 1 % to amino acids (Jackson,

1989 in Tapiero and Tew, 2003). After intravenous dosing ⁶⁵Zn quickly disappears from blood and appears in a high concentration in the pancreas, liver, spleen, kidneys, and only in low concentrations in the muscles and brain (Rubini et al., 1961, Spencer et al., 1965 in Bencko et al., 1995). Vrzgula et al. (1990) state similar findings, describing the creation of reserves in the liver, spleen and pancreas which can be mobilised. Following Zn supplementation an increased Zn content was observed in bone tissue in bulls (Illek, 1990) and other animals, e.g. piglets (Novotný et al., 2005).

The effect of zinc supplementing, zinc content in products of animal origin and determination of the zinc requirement in people

Experiments conducted on calves, heifers and bullocks in feeding found a positive impact of zinc supplementing on growth and also on certain biochemical parameters of the blood. Long-term supplementing of Zn and Co to feed for bullocks in feeding had a fundamental impact on the intensity of growth of animals (16 % higher weight gain), the content of haemoglobin and the number of erythrocytes, and in the short term also the levels of proteinaemia, glycaemia and zincaemia. The content of vitamin B_{12} in blood plasma and Co and Zn in the liver tissue of animals was significantly influenced (Illek, 1990).

Foodstuffs of animal origin contain a larger quantity of zinc in comparison with foodstuffs of a vegetable origin (Illek, 2000). Milk is a source of very well available zinc for human nutrition. The absorption rate of Zn from milk is stated at approximately 61 % (Bell et al. 1987). Cheeses and yoghurts have a higher Zn content than raw milk (Park, 2000). For persons in the Czech Republic, Zn in milk covers from 18.5 % (breastfeeding women) to 29.6 % (children aged 1-10) and 59.2 % (children aged under 1) of the daily requirement (Trávníček et al., 2004). A higher Fe supplement in infant formulas may lead to reduced zinc absorption from these products (Craig et al., 1984). In human milk there is approximately 1/3 to 3/4 less Zn than in cow's milk: $1.12 \pm 0.05 \ \mu g \ Zn.ml^{-1}$ (Campillo et al., 1998), 2.93 ± 2.11 mg.l⁻¹ (Pilecki et al., 1999), approximately 3mg Zn.1-1 (Schlettwien-Gsell and Mommsen-Straub, 1970 in Bencko et al., 1995), whilst bioavailability of certain mineral elements from human milk is however higher than from cow's milk (Emmett and Rogers, 1997). In order to understand the problem of the zinc metabolism within the human organism techniques have been developed enabling measurement of even small changes in the dosage of the zinc stable isotope in biological samples, the use of which was applied in study of the role of the intestines in maintaining zinc homeostasis, and which are particularly valuable for the study of regulating of homeostasis of Zn in children and women during the sexual cycle (Krebs et al., 1995).

The concentration of Zn and its bioavailability probably determines the content of milk proteins in human milk in a significant manner (Farida and Srikumar, 2000), and the content thereof significantly reduces the length of the duration of lactation (Dai and Tang, 1991). 6,040 \pm 3,590 µg Zn.l⁻¹ was determined in the colostrums of women, and in mature milk approximately 8-times

less: $760 \pm 600 \ \mu g.l^{-1}$ (Rossipal and Krachler, 1998). Benemariya et al. (1995) recorded a reduction of the Zn level in human milk during lactation from an average of $3.8 \pm 0.5 \ \mu g.ml^{-1}$ to the value of $0.75 \pm 0.02 \ \mu g.ml^{-1}$ (after 10 months). Significant changes in zinc concentration in human milk during the course of lactation are also stated by Dorea (2000). In the first days post partum the zinc concentration drops sharply, and later slowly declines, and in the 3rd month reaches a relatively stable level. Despite the increasing consumption of milk by infants the overall zinc intake is reduced as a result of the reduction of its concentration in milk. Although human milk sufficiently covers the nutritional requirements of children for growth, dermatitis from zinc deficit is described in approximately the 3rd month (more frequently in prematurely born infants). The susceptibility of infants to dermatitis conditioned by a zinc deficiency is probably connected to the metabolism of the infant's bodily reserves. Supplementing mothers with zinc with the aim of enriching the mother's milk was not effective, but it seems that abnormalities of the zinc metabolism correct themselves during gestation and lactation. The occurrence of dermatitis due to Zn deficiency in prematurely born infants aged 2-4 months is also mentioned by Zimmermann et al. (1982 in Yu, 1999).

Methods of determining zinc

Determining zinc in biological material was previously conducted spectophotometrically, polarographically and spectrographically. Today priority is given to the atomic absorption spectrophotometric method (Bardoděj et al., 1980 in Bencko et al., 1995). The high sensitivity of this method enables determination of low concentrations of zinc in samples from the environment and also in biological materials (tissues, blood, plasma, hairs, urine). Other methods which can be used at present to determine zinc are neutron activation analysis (Anonymous, 1989 in Bencko et al., 1995) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Silva et al., 2001; Domínguez et al., 2004a). The tested ion chromatographic method was suitable for determining Zn (Buldini et al., 2002). In recent years Inductively Coupled Plasma-Mass Spectometery (ICP-MS) has become the dominant method for trace and ultra-trace analysis, which at present has a considerably lower detection limit for the majority of elements than ordinary analytical methods, and is characterised by high measurement speed, low sample consumption, high productivity and the possibility of determining the isotope composition of elements (Bendl, 1997). The DF-ICP-MS (DF-double focusing), following microwave assisted digestion, is used for concurrent analyses of mineral elements in milk samples. DF-ICP-MS instruments are able to overcome inter-atom interference (mutual interaction), which does not resolve the use of the ICP-MS quadropole (Martino et al., 2001).

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