MUTUAL RELATIONS BETWEEN COPPER AND ZINC IN BLOOD PLASMA IN SOME SELECTED CATEGORIES OF CATTLE AND SHEEP

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Abstract

The aim of the study was to determine mutual relations between copper and zinc in blood plasma of cows, heifers, ewes and lambs from operations in mountainous and submontane regions of the Šumava. Blood analysis showed that mean blood plasma concentrations of both micro-elements in cattle decreased over the monitoring period to the lower threshold of reference values (Cu to 12.08 μmol.l^{-1} and Zn to 12.58 μmol.l^{-1}). In sheep, mean blood plasma zinc concentrations were at the lower limit of reference values or slightly above it (9.56 – 12.16 μmol.l^{-1}).
Statistical relations showed that of the two elements, copper probably has the dominant position. In sheep, this may be due to the fact that they are generally more sensitive to higher copper intakes in feed rations than other farm animals. Low blood plasma concentrations of copper and zinc in the animals studied were probably caused by the undersupply of the two elements in feed rations due to their deficiency in the region monitored. Farmers were recommended to use suitable feed supplement products.

Key words: micro-elements, cooper, zinc, blood plasma, cattle, sheep, Šumava mountains

INTRODUCTION

Recent years have seen a constant growth in demands on the quality of animal products, and besides main feed components such as proteins, glycides, fats and vitamins, the importance of essential elements whose adequate intake influences the condition and health of animals is gradually more appreciated. Their proper use presupposes a thorough knowledge of the theory of functions and effects of microelements on the living organism. In practical terms, it means timely recognition of the problems caused by the over-or undersupply of one or more of the essential elements, because mineral substances have many important functions in the organism (Vrzgula *et al.*, 1990). At present, ten elements are usually given as elements that are essential for the maintenance of vital functions in higher animal species. They are iron, iodine, copper, zinc, manganese, cobalt, molybdenum, selenium, chromium and tin (Bencko *et al.*, 1995).

Copper plays an important role mainly in haematopoiesis of both animals and man (O′Dell, 1976), although its relation to it and to iron metabolism has not been completely elucidated (Bencko *et al.*, 1995). Blood haemoglobin volumes depend on many external factors. The most important ones include nutrition and the hygienic conditions of the animal husbandry system. The amount of haemoglobin in blood is directly dependent on the presence of its initial building components in the diet, and on the organism's ability to absorb those components. They are, first and foremost, proteins with amino acids (glycine and histidine), Fe, Cu, Co, vitamin B_{12}, and other substances necessary for the synthesis and renewal of haemoglobin in the organism. Copper deficiency in the organism reduces haemoglobin production (Shenck and Kolb, 1991). Blood haemoglobin concentrations also depend on the animal's age, weight, sex, yield, nutrition, health and also on the altitude above sea level (or, rather, on the atmospheric pressure) and health status (Sova *et al.*, 1981). Copper is also a co-factor in several oxide reductase enzyme systems, has an important function in osteogenesis, maintenance of myelin in nerves, and in hair pigmentation (Reece, 1998). Copper deficiency is manifested by diarrhoeas, low weight gains, lightly-coloured hair, swollen and painful joints, fragility of limb bones, partial paralysis manifested by a defective posture in calves, infertility, anaemia and weakened immunity system (Maas, 2002). Copper deficiency has particularly negative effects on fertility in females, impairs sexual development, promotes the occurrence of the silent oestrus, irregular oestrus and resorption sterility (McDewell, 1992). Symptoms of copper deficiency include, besides light-coloured hair that is most prominent in animals exposed to sunlight, light-colour spots around the eyes (the so-called copper glasses) and the ears that are very noticeable at first sight (Boyles, 2002). The highest degree of copper deficiency is manifested by distrophic changes on the myocardium, which may cause sudden death after physical exertion. This happens most frequently in spring when animals are taken out for grazing, when dairy cows are taken to dairy parlours or during transport (Illek *et al.*, 1999). Copper needs depend on the animal species, type of management, type of feed rations, production levels, degree of pregnancy, health status and, last but not least, on genetic dispositions (Vrzgula *et al.*, 1990). Reference thresholds for cattle range from 12 μmol.l^{-1} (Slanina *et al.*, 1992) to 31.95 μmol.l^{-1} (Ulrich von Bock and Polach, 1994), and for sheep from 14.01 to 31.01 μmol.l^{-1} (Ulrich von Bock and Polach, 1994).

Zinc is a component or a co-factor of several enzyme systems, including peptidases and carbonate dehydratase necessary for the development of bones and feathers (Reece, 1998). Zinc ions are DNA and RNA synthesis activators (Schenck and Kolb, 1991). Like copper, it occurs practically in all plant and animal tissues, but usually at higher concentrations. Zinc reference limits for cattle are in the range from 10.7 μmol.l^{-1} (Ulrich von Bock and Polach, 1994) to 26.0 μmol.l^{-1} (Slanina *et al.*, 1992), and in the range from 10.7 to 19.88 μmol.l^{-1} for sheep (Ulrich von Bock and Polach, 1994). Zinc deficiency triggers a number of biochemical changes in the organism, e.g. growth and sexual maturation delays, dermatitis, atrophy of testicles, anorexia and weight loss (Benecko *et al.*, 1995). Other symptoms may include imperfect development of hair or feathers, fleece moulting, horny thickened skin, parakeratosis in pigs. Zinc deficiency impairs immunity system functions (impairment of the T- and B-cell function), and it may significantly influence the prenatal development of the brain (Kvasničková, 1998).

Livestock reproduction is also closely related to zinc concentrations Zinc deficiency in females suppresses all stages of their sex cycle (Vrzgula *et al.*, 1990). Metabolic studies have in many cases demonstrated that an analytical determination of diet concentrations of whatever element was not in itself able to provide enough information of that element's usefulness for the organism. Both in the environment and the internal ambient of living organisms, elements enter into interaction with each other and with other organic substances at all times (Lener and Bíbr, 1985).

Interactions between copper and zinc take the form of competition for protein binding sites. In these cases, competition occurs on low-molecular proteins that bind respective metals. Biological effects of one element may also be influenced by modifying the supply of another element. These elements (or their compounds) may, however, be active at different locations along the metabolic chain and need not necessarily interact directly (Pařízek, 1975). A surplus of Mo and Zn, for instance, inhibits the use and storage of Cu in the organism. High Ca and phytate concentrations cause Zn unbinding, Zn surpluses disrupt Cu metabolism and may cause anaemia (Reece, 1998).

Vrzgula *et al*. (1990) pointed out that achieving an adequate supply of trace elements for farm animals is a difficult task because their demand depends on many factors. The most important are the chemical composition of the soil, its fertilization and exploitation. The deficiency and/or surplus of elements in the soil will influence their concentrations in plant feeds, and consequently their intake by animals, and the metabolism. In his blood sample analysis experiments, Henriksen (1999) diagnosed copper deficiency caused by very high molybdenum and sulphur concentrations in drinking water and on pastures. If, on the contrary, we aim at decreasing a high copper intake from plants, pastures should be limed to increase molybdenum concentrations in plants, enhance the endogenous antagonism between the two elements and lower copper intakes by the animals (Suttle, 1995 and 2002).

Insufficient supply of trace elements in some locations is reflected in their lower blood plasma levels, which may also be the result of insufficient supply of mineral feed mixes (Kroupová, 2002).

Copper tolerance is varied and species-specific (Ulrich von Bock and Polach, 1994). Acute copper intoxications of utility animals are relatively rare. In chronic intoxications, copper accumulates in the liver and skeletal muscles (Kolář, 1999). Of all farm animals, sheep are the most sensitive to high intake levels of copper in feed rations (Church and Pond, 1988).

Intoxication in sheep may even occur at diet concentrations below 25 mg.kg^{-1} Cu, which means that dairy cows will tolerate at least four times more dietary copper than sheep (Šimek, 1995). Chronic copper intoxication in sheep is most frequently manifested when, after a long-term process of surplus copper accumulation in the liver, a large quantity of the copper is washed out of the liver to blood circulation, with ensuing anaemia as a result of the stress situation the animals had been subject to (Berger, 2002; Martin, 1998).

The most frequent symptoms of hypercupremia are anorexia, increased thirstiness and depression accompanied with haemoglobinemia, anaemia, icterus and methemoglobinemia. A majority of animals will die in two days of the first manifestations of toxic effects of copper (Merck Veterinary Manual, 1979). Rogers (2001) mentioned that according to EU legislation a sheep feed ration must not contain more than 17 mg Cu.kg^{-1} dry matter. The assumed maximum daily intake of dry matter is 2 kg per ewe. The maximum daily dietary intake permitted by the EU is around 34 mg Cu per ewe (proportionally less for light-coloured sheep).

Zinc tolerance is generally very high. Without any major problems, animals will tolerate up to 1000 mg.kg^{-1} Zn in feed rations. The tolerance of ruminants is somewhat lower (500 – 1000 mg.kg^{-1}). A chronic intake of high zinc doses leads to hypocupremia (Kvasničková, 1998).

MATERIAL AND METHODS

In the project reported here, mean concentrations of two essential elements, i.e. copper and zinc, in blood plasma of cattle and sheep were statistically evaluated and compared. The data evaluated were obtained in a long-term research project implemented between 1999 and 2001. The experiments involved dairy cows and heifers from eight different locations, and ewes and lambs from two locations in the mountainous and submontane regions of the Šumava. Rearing conditions were different in each location (altitude above sea level, type of herd management, feed rations, breeds). Blood was collected from the *vena jugularis* of selected animals prior to their transition to the summer and winter feed rations. A total of 388 bovine and 92 ovine blood samples were examined.

Copper and zinc concentrations in blood plasma were determined by the flame atomic absorption spectrophotometry in the laboratory of the Department of Livestock Anatomy and Physiology (JU, České Budějovice). Weighed arithmetic means, standard deviations, correlation coefficients and t-tests were calculated using Statistica Cz 6.0 software. Degrees of statistical dependence for biological sciences according to correlation coefficient values were determined according to Čermáková and Střeleček (1995) ($0.3 > /r_{xy}/ \rightarrow$ low degree of statistical dependence; $0.3 \leq /r_{xy}/ < 0.5 \rightarrow$ moderate degree of statistical dependence). The results were also expressed in tabular and diagrammatic forms.

RESULTS AND DISCUSSION

In 1999, mean copper and zinc concentrations (18.62 μmol.l^{-1} and 21.74 μmol.l^{-1} respectively) in dairy cows and heifers were within the range of reference values reported by the authors. The differences between the means were statistically highly significant ($P < 0.001$), and the degree of statistical dependence $r_{xy} = 0.001$.

The next year (2000), mean concentrations of copper and especially of zinc in blood plasma of the animals monitored dropped to 14.87 μmol.l^{-1} and 12.75 μmol.l^{-1}, respectively, i.e. to a level close to the lower threshold of reference values reported. In that year, differences between the means of the two elements were statistically highly significant ($P < 0.001$), with a low degree of statistical dependence (r_{xy} = 0,157*).

Mean blood plasma copper and zinc concentrations in dairy cows and heifers gradually decreased over the entire period of monitoring (1999 to 2001) to reach a level just above the lower threshold of reference values according to Slanina *et al.* (1992) in the case of copper (12.08 μmol.l^{-1}) and slightly above the threshold reference values according to Ulrich von Bock and Polach (1994) in the case of zinc (12.58 μmol.l^{-1}) - Tab. 1 and Fig. 1.

Tab. 1 ..: Average concentrations of copper and zinc in the blood plasma of cows and heifers

Period	*n*	Cu [mmol.l^{-1}]		Zn [mmol.l^{-1}]		*t-test*	r_{xy}
		$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$		
1999	106	18,62	5,05	21,74	5,54	***	0,001
2000	185	14,87	4,65	12,75	6,17	***	0,157*
2001	97	12,08	4,22	12,58	3,38	–	-0,099
spring	187	14,19	4,84	13,90	6,04	–	0,096
autumn	201	16,12	5,41	16,34	7,15	–	0,390*
1999 - 2001	388	15,19	5,23	15,13	6,79	–	0,293*

n = number of blood taking

*(P < 0,001)****

*(P < 0,01)***

*(P< 0,05)**

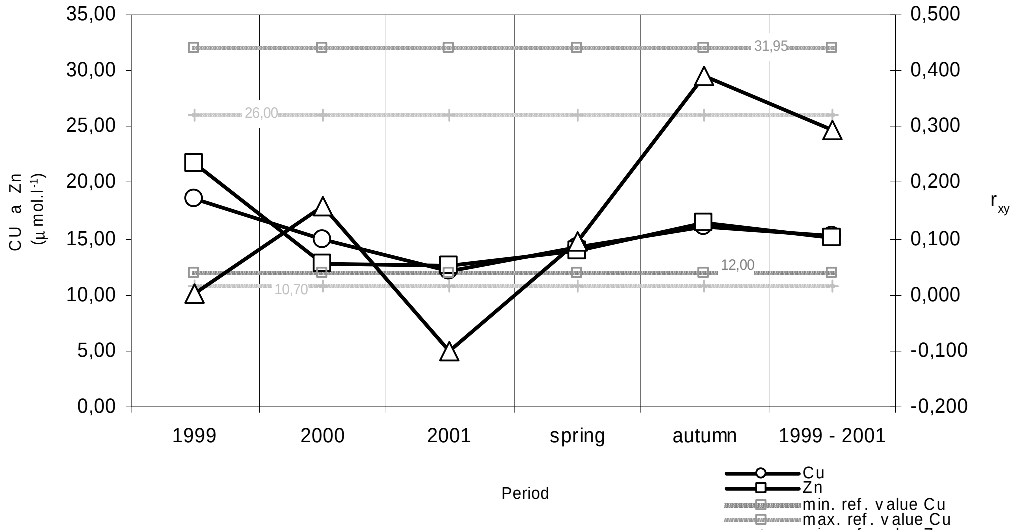

Figure 1.
Aaverage concentration of copper and zinc in the blood plasma of the cows and the heifer

In 2001, a negative correlation coefficient in the degree of statistical dependence was found (r_{xy} = -0.099).
In spring, during the transfer from winter to summer feed rations, copper and zinc blood plasma concentrations were lower (14.19 μmol.l^{-1} 13.90 μmol.l^{-1}, respectively) than in autumn (Cu 16.12 μmol.l^{-1} and Zn 16.34 μmol.l^{-1}). In spring, the degree of statistical dependence r_{xy} was 0.096, while in autumn a moderate degree of statistical dependence was found (r_{xy} = 0.390*). It follows from Fig.1 that almost equal copper and zinc concentrations in blood plasma and within reference values according to Slanina *et al*. (1992) and Ulrich von Bock and Polach (1994) are demonstrably associated with low or moderate statistical dependence between the two elements. This is demonstrated by the correlation coefficient value found in the autumn period (r_{xy} = 0.390*) and the correlation coefficient value (r_{xy} = 0.293*) calculated for the entire period of monitoring (1999 to 2001).
Changes in mean copper and zinc concentrations in blood plasma of ewes and lambs over the monitoring period are given in Figure 2 and Table 2. In 1999, the mean zinc concentration (9.56 μmol.l^{-1}) was below the lower threshold of reference values according to Ulrich von Bock and Polach (1994), while copper concentration (15.52 μmol.l^{-1}) was slightly above that threshold.

Tab. 2. : Average concentration of copper and zinc in the blood plasma of the ewes and the lambd

Period	*n*	Cu [mmol.l^{-1}]		Zn [mmol.l^{-1}]		*t-test*	r_{xy}
		$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		
1999	15	15,53	1,95	9,56	1,45	***	-0,033
2000	55	18,96	7,03	11,77	3,50	***	0,427*
2001	22	14,09	2,86	11,27	3,50	*	-0,308
spring	52	19,42	6,98	12,16	3,94	***	0,261
autumn	40	14,36	2,61	10,18	1,78	***	0,005
1999 - 2001	92	17,25	6,07	11,31	3,33	***	0,324*

n = number of blood taking

*(P < 0,001)****
*(P< 0,01)***
*(P < 0,05)**

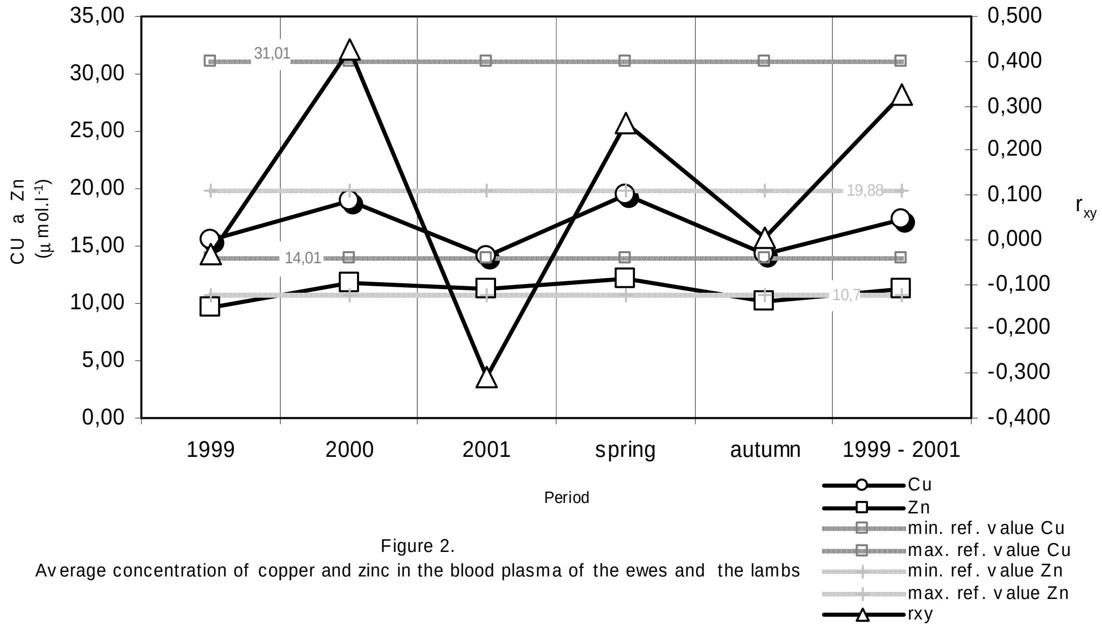

Figure 2.
Average concentration of copper and zinc in the blood plasma of the ewes and the lambs

Differences between the means of the two elements in that year were statistically highly significant ($P < 0.001$), and the value of correlation coefficient was negative ($r_{xy} = -0.033$). The situation in 2001 was similar: concentration levels of both the elements were at the lower threshold of reference values (Cu 14.09 $\mu mol.l^{-1}$ and Zn 11.27 $\mu mol.l^{-1}$), the statistical dependence was negative ($r_{xy} = -0.308$) and the differences between the means of the two elements were statistically significant ($P < 0.05$).

In the autumn, mean copper and zinc concentrations (14.36 $\mu mol.l^{-1}$ and 10.18 $\mu mol.l^{-1}$ respectively) in blood plasma of the animals monitored were at the lower threshold of reference values, the differences between their means were statistically highly significant and the correlation coefficient value was $r_{xy} = 0.005$. In 2000, positive moderate statistical dependence was found (r_{xy} = 0,427*), differences between the means of the two elements were statistically highly significant ($P < 0.001$) and their concentrations were above the lower threshold of reference values (Cu 18.96 $\mu mol.l^{-1}$, Zn 11.77 $\mu mol.l^{-1}$).

In spring, blood plasma copper concentrations were high above the lower threshold of reference values (19.42 $\mu mol.l^{-1}$), and zinc concentration (12.16 $\mu mol.l^{-1}$) was also above its threshold value. Differences between the means of the two elements in that season were statistically highly significant ($P < 0.001$), with a low degree of statistical dependence ($r_{xy} = 0.261$). Similar results were obtained when concentrations of the two elements over the entire period of monitoring were compared, i.e. copper and zinc concentrations were above the lower threshold of reference values (Cu 17.25 $\mu mol.l^{-1}$, Zn 11.31 $\mu mol.l^{-1}$), the differences between the means of the two elements were statistically highly significant ($P < 0.001$) and the correlation coefficient value was r_{xy} = 0.324*.

Lower copper and zinc concentrations in blood plasma of the animals monitored were probably caused by the undersupply of the two elements in feed rations, which, in turn, was due to the deficiency of those elements in the soil and thus also in plants grown there and used in feeding. A relatively low supply of copper, zinc and some other trace elements in the Šumava region has already been reported by, e.g., Šoch *et al.* (2002) and Kroupová (2002).

Because zinc and copper are in a mutually antagonistic relationship (Sova *et al.*, 1981), and, for instance, an oversupply of zinc may disrupt copper metabolism and thus cause anaemia (Reece, 1998), their balanced supplementations is of paramount importance (Sova *et al.*, 1981). The degree of statistical dependence between the two elements is expressed by the value of the correlation coefficient. Negative correlation coefficients were found in both sheep and cattle when the mean copper concentrations in blood plasma of the animals was below zinc concentrations, and also in cases when concentrations of both of the elements were at the lower threshold of reference values according to Ulrich von Bock and Polach (1994) and Slanina *et al.* (1992).

In cattle, moderate statistical dependence was found when copper and zinc concentrations were balanced and above the lower threshold of reference values. In sheep, moderate statistical dependence was found when copper concentration in blood plasma was higher than zinc concentration and above the lower threshold of reference values. Blood plasma zinc concentration, on the other hand, was at the lower threshold of reference values. The results seem to indicate that of the two elements, copper probably has the dominant position. In sheep, this may also be due to the fact that they are generally more sensitive to copper than other livestock (Ulrich von Bock and Polach, 1994).

Because concentrations of the two elements in blood plasma of livestock in the regions monitored showed a decreasing trend, comments to farmers included the recommendation to use various types of supplementation. Trace elements can be administered to livestock (cattle and sheep) in several ways. According to Rogers and Gartley (2000), the most frequently used methods include effective oral supplements of trace elements, regular spreading of trace elements on pastures, oral administration of trace elements dissolved in water (e.g. for grazing animals), veterinary medicine preparations, soil dressing with mineral supplements and foliar dressing. At present, particular emphasis is placed on the administration of mineral substances bound to molecules of organic substances (the so-called chalates), because their utility value is markedly higher that that of their inorganic counterparts (Illek *et al*., 1999).

CONCLUSION

An analysis of blood samples showed that mean copper and zinc concentrations in blood plasma of dairy cows and heifers from mountainous and submontane regions of the Šumava dropped during the monitoring period (1999 – 2001) to the lower threshold of reference values mentioned by the authors quoted. Blood plasma copper concentrations of the livestock monitored decreased to 12.08 μmol.l^{-1}, and zinc concentrations were slightly above the lower threshold of its reference values (12.58 μmol.l^{-1}). In 2001, a negative correlation coefficient in the degree of statistical dependence was found (r_{xy} = -0.099). Lower mean concentrations of the two elements were also found in the spring transition to summer feed rations (Cu 14.19 μmol.l^{-1}, Zn 13.90 μmol.l^{-1}). At that period, a negative correlation coefficient in the degree of statistical dependence was found (r_{xy} = 0.096).

Ewes and lambs from locations in the mountainous and submontane regions in the Šumava did not exhibit any decreasing trends over the monitoring period, but their blood plasma zinc concentrations were at the lower threshold of its reference values or slightly above it (9.56 – 12.16 μmol.l^{-1}). Differences between the means of the two elements in all periods monitored were statistically highly significant ($P < 0.001$), with the exception of the year 2001 ($P < 0.05$). A moderate degree of statistical dependence between copper and zinc was found in the year 2000 (r_{xy} = 0.427*) and also in results for the entire monitoring period (r_{xy} = 0.324*). Negative correlation coefficient values were found in both sheep and cattle when mean copper concentrations in blood plasma of the animals was below zinc concentrations, and also in cases when concentrations of both of the elements were at the lower threshold of reference values of the authors quoted. In cattle, moderate statistical dependence was found when copper and zinc concentrations were almost identical and above the lower threshold of reference values.

In sheep, moderate statistical dependence was found when blood plasma copper concentrations were higher than zinc concentrations and above the lower threshold of reference values, blood plasma zinc concentrations, on the other hand, were at the lower threshold of reference values. It therefore seems that of the two elements, copper probably has the dominant position. In sheep, this may also be due to the fact that sheep are generally more sensitive to higher copper intakes in feed rations than other farm animals. To deal with copper and zinc deficiencies in the animals monitored, farmers were recommended to use suitable feed supplements.

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