

## RELATIONSHIP BETWEEN COPPER AND ZINC ON SELECTED HAEMATOLOGICAL PARAMETERS IN BEEF AND DAIRY CATTLE

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### ABSTRACT

The aim of this study was to evaluate the content of copper (Cu) and zinc (Zn) in blood plasma, faeces and a diet of cattle (cows and heifers) from selected herds and find correlations between these minerals and haemoglobin (Hb), haematocrit (Ht) and leukocytes count (Lc). There was analysing 199 blood and faeces samples of cattle (72 of beef cattle and 127 of dairy cattle) during year 2004. The concentrations of Cu ( $14,50 \pm 3,25 \mu\text{mol.l}^{-1}$ ) and Zn ( $17,99 \pm 4,05 \mu\text{mol.l}^{-1}$ ) in blood plasma of dairy cattle were higher than in beef cattle (Cu  $11,2 \pm 3,87 \mu\text{mol.l}^{-1}$ ; Zn  $13,01 \pm 3,86 \mu\text{mol.l}^{-1}$ ). The lack of Cu and Zn in beef cattle could be due to deficiency of both elements in a diet that was covered only from 86 % by the Cu and 89 % by the Zn. There were found linear positive dependencies between Hb, Ht and Cu and Zn ( $r_{xy}$  = from 0,201\* to 0,335\*) in dairy cows and ( $r_{xy}$  = from 0,520\* to 0,688\*) in beef cattle. The negative significant linear dependency was found between Zn and Lc ( $r_{xy}$  = -0,320\*).

**Keywords:** cattle, copper, haematological parameters, zinc

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### INTRODUCTION

Copper and zinc are essential for several metabolic processes and play a particular role in proliferation and correct functioning of cells and tissues (Ekici et al., 2004). Overload or deficiency of these elements can lead to metabolic disorders and some other diseases. Minerals are found in specific mutual relations with physiological and biochemical processes (Mioč et al., 2000). Yokus et al. (2004) indicated that Cu concentrations in serum vary not with season but with physiological variations (for example pregnancy), and the concentrations of Zn could neither depend on the season of the year nor the physiologic status.

It is well known that copper influences erythropoiesis and also biosynthesis of haem and haemoglobin. Copper administration can cause significant increase in haemoglobin and serum copper levels (Sahin et al., 2001). The biological effect of copper on pregnant dairy cows with hypocupraemia, sideropenia, and hypochromic anaemia was manifested by the improvement in serum Cu

levels, as well as those of Fe, erythrocytes, haemoglobin, and haematocrit (Bíreš et al., 2000). Most of the zinc in the bloodstream (80 %) is present in the erythrocytes as carbonic anhydrase and copper-zinc superoxide dismutase. There are indirect effects of zinc deficiency on erythrocyte membrane composition and stability (Underwood and Suttle, 2001). The lack of zinc results in the decrease of haematocrits. Furthermore, copper and zinc play a crucial role in the immune system (Illek et al., 1999; Minatel and Carfagnini, 2000; Spears, 2000). Changes in total leukocytes accounts were investigated in sheep on a diet containing pollutants from copper and zinc works. The marginal or low zinc and copper contents in pasture, soil or animal feed may have been predisposing factors for the observed deficiencies and might have been responsible for many diseases (Tokarnia et al., 1999; Ahmed et al., 2002).

The aim of this work was to study the influence of copper and zinc on selected haematological parameters in beef and dairy cattle. The first objective was to find out mean concentration of copper, zinc and selected haematological parameters (haemoglobin, haematocrit

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**Table 1: Breed conditions**

Herd	Cattle	Housing	Breed	Altitude	Diet	Mineral fodder additives
A	Beef	Yearly on pasture	C*	910	Pasture and hay	-
B			AA	675		LIZ-PP (Trewit, s.r.o.)
C	Dairy	Free-stall	C + H	550	TMR	UNI-K-Shelter (Eko-MIL, s.r.o.)
D			C + H	400		Turmix S3 (LO) var. B (Tekro, s.r.o.)

AA = Aberdeen Angus; C = Czech fleckvieh (\*mothers); C + H = result of crossbreeding of Czech fleckvieh (dual-purpose cattle) with Holstein breed; Altitude = metres above sea level; TMR = Total mixed ration.

and leukocytes count) in blood plasma. The next aim was to study correlation dependencies between both elements and haematological parameters.

## MATERIAL AND METHODS

### Animals and breed conditions

Four farms were chosen with breeds Aberdeen Angus, Czech fleckvieh (\*mothers) and Holstein cattle from different areas of South Bohemia region in the early 2004. Beef and dairy cows were included in to the experiment. The breed conditions are mentioned in a Table 1.

Contents of Cu and Zn ( $\text{mg}\cdot\text{kg}^{-1}$ ) in mineral fodder additives: LIZ-PP (700;0), UNI-K-Shelter (900; 6400), Turmix S3 (640; 3200).

### Measurements and laboratory analyses

Selected animals ( $n = 12$  to 24) from single herds were examined in spring and autumn season during the year 2004. Blood samples were collected from *vena jugularis* (coated with heparin) and faeces from rectum of the same animals. Altogether, 199 blood samples and faeces of cattle ( $n = 72$  beef cattle;  $n = 127$  dairy cattle) were taken and analysed. Further, samples of animal diet were taken (collected at the time of blood taken, in all farms). Blood was kept cold and analysed until 24 hours. The haematological parameters were determined as follows: leukocytes count (Lc) were determined using

a dilution method and Bürker chamber, the content of haemoglobin (Hb) was estimated photometrically at 540 nm using a spectrometer UV/VIS Unicam 5625. The haematocrit value was determined by capillary microhaematocrit method. The concentration of copper and zinc in blood plasma, faeces and in dry matter of a diet was analysed by flame atomic absorption method using an AA Spectrometer Unicam 969. All the analyses were carried out at the departmental laboratory of Anatomy and Physiology of Farm Animals, Faculty of Agriculture, University of South Bohemia in České Budějovice.

### Statistical analyses

The results were processed by acceptable statistical methods using a program QC.Expert<sup>TM</sup> 2.5<sup>PRO</sup>: Arithmetic mean ( $\bar{x}$ ), Standard deviation ( $s_x$ ), Spearman's Correlation coefficient ( $r_{xy}$ ,  $P < 0.05$ ) and t-test ( $P < 0.05$ ;  $P < 0.001$ ) were performed.

## RESULTS AND DISCUSSION

The comparison of Cu and Zn contents in diet is given in Table 2. The reference values (RfV) of copper (Cu) in blood plasma according to Kováč et al. (2003) are in the range 12.6 – 18.9  $\mu\text{mol}\cdot\text{l}^{-1}$ . The concentrations of Cu in BP in beef cattle were  $11.2 \pm 3.87 \mu\text{mol}\cdot\text{l}^{-1}$  (Table 3), means that it is the lower limit of RfV. Further, deficit of Cu was found in a diet of beef cattle (9.2  $\text{mg}\cdot\text{kg}^{-1}$  dry matter "DM" of the diet) (Table 2), because

**Table 2: The mean values of copper and zinc in diet**

Cattle	n	$\text{mg}\cdot\text{kg}^{-1}$ DM				$\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$					
		Cu		Zn		Cu			Zn		
		$\bar{x}$	Req	$\bar{x}$	Req	$\bar{x}$	Norm	Covering (%)	$\bar{x}$	Norm	Covering (%)
Dairy cattle	8	12.5	10.0	75.4	60.0	190	181	105	128 9	912	141
Beef cattle	7	9.2		53.6		136	159	86	712	797	89

Cu = copper; DM = dry matter; Covering (%) = covering of the norm; Zn = zinc; Req = requirement; n = number of samples

the requirement of Cu in DM of the diet is 10 mg.kg<sup>-1</sup> (Petrikovič and Sommer, 2002). The animals received 136 mg.kg<sup>-1</sup>.d<sup>-1</sup> and a day norm was 159 mg.kg<sup>-1</sup>. Whence it follows that the day norm has been covered only from 86 % (Table 2). It is well known that the soil in some areas of the Czech Republic is deficient in copper (Kroupová, 2002). That is the reason why the diet of beef cattle, which was consisted of a pasture and hay only, should have been affected by this deficit. The lack of Cu has been manifested oneself by lower excretion of Cu in to faeces (17, 97 ± 10.17 mg.kg<sup>-1</sup> DM) as compared to dairy cattle (P<0.001) (Table 3). The mean concentrations of Cu in BP in terms of RfV in dairy cows were: 14.50 ± 3.25 µmol.l<sup>-1</sup> (Table 3). The diet of these animals contained 12.5 mg.kg<sup>-1</sup> Cu in DM of the diet and that is in agreement with 190 mg.kg<sup>-1</sup>.d<sup>-1</sup> at norm 181 mg.kg<sup>-1</sup>.d<sup>-1</sup> and consequently to covering of the day norm out of 105 % (Table 2). Dairy cattle showed following mean concentration of Cu in faeces: 27.88 ± 47.88 mg.kg<sup>-1</sup> DM (Table 3), which is more than in beef cattle (P< 0.001). The available literature does not cite the RfV for Cu in faeces, however Kováč et al. (2003) reported 5.06 ± 1.5 mg Cu.kg<sup>-1</sup> DM in Holstein cows. Statistically significant difference (P<0.001) in mean content of Cu in BP between dairy and beef cattle has been determined. However, Illek et al. (1999) demonstrated copper deficiency in highly productive dairy cows and its low concentration in bulk and concentrated feeds and in total mixed rations.

The content of Zn in a diet of beef cattle was lower (53.6 mg.kg<sup>-1</sup> DM of a diet) than their requirement (60 mg.kg<sup>-1</sup> DM of a diet) according to Petrikovič and

Sommer (2002). Daily requirement of Zn in beef cattle was covered only out of 89 %, because animals had just 712 mg Zn.kg<sup>-1</sup>.d<sup>-1</sup>, whereas a norm was 797 mg Zn.kg<sup>-1</sup>.d<sup>-1</sup>. Low mean concentration of Zn in faeces (113.02 ± 64.78 mg Zn.kg<sup>-1</sup> DM) of beef cattle (P< 0.001) was found, the case was the same for Cu as well (Table 3).

The mean concentration of Zn in BP of beef cows was in a range 13.01 ± 3.86 µmol.l<sup>-1</sup>, which is on a lower limit of RfV or slightly below it. Values of Zn in BP of dairy cattle were 17.99 ± 4.05 µmol.l<sup>-1</sup> in monitoring period. Zn content was 75.4 mg.kg<sup>-1</sup> DM of the dairy cattle diet, which amounts to 1289 mg Zn.kg<sup>-1</sup>.d<sup>-1</sup>m in place of the course of standard 912 mg Zn.kg<sup>-1</sup>.d<sup>-1</sup> which covers 141 % of the standard norm (Table 2). In faeces of these animals higher concentration of Zn was found (163.5 ± 111.9 mg.kg<sup>-1</sup> DM) as compared to beef cattle (P<0.001). Kováč et al. (2003) mentioned the content of Zn in faeces of Holstein cows within the range 55.1 ± 12.1 mg Zn.kg<sup>-1</sup> DM, however, the RfV are not stated in available scientific literature. From the results it can be assumed that the beef cattle diet was poorly supplemented with Zn as well as with Cu. Differences between average values of Zn content in BP of dairy and beef cattle were highly significant at P< 0.001.

The concentrations of Hb and Ht in beef cattle were at an average 110.9±16.89 g.l<sup>-1</sup> and 0.32 ± 0.06 l.l<sup>-1</sup> respectively, during the entire monitoring period. The values of Hb and Ht in blood of dairy cows were recorded as 115.74 ± 21.41 g.l<sup>-1</sup> and of 0.31 ± 0.05 l.l<sup>-1</sup> respectively. Differences in Hb between dairy and beef cattle were significant at P<0.05 (Table 4).

**Table 3:** The mean values of copper and zinc in blood plasma and faeces

Cattle	n	Blood plasma (µmol.l <sup>-1</sup> )				Faeces (mg.kg <sup>-1</sup> DM)			
		Cu		Zn		Cu		Zn	
		$\bar{x}$	s $\bar{x}$	$\bar{x}$	s $\bar{x}$	$\bar{x}$	s $\bar{x}$	$\bar{x}$	s $\bar{x}$
Dairy cattle	127	14.50	3.25	17.99	4.05	27.88	47.88	163.50	111.94
Beef cattle	72	11.20**	3.87	13.01**	3.86	17.97**	10.17	113.02**	64.78

Cu = copper, x = arithmetic mean, DM = dry matter, n = zinc, s $\bar{x}$  = standard deviation, \*\* P < 0.001

**Table 4:** The mean values of haematological parameters

Cattle	n	Haemoglobin (g.l <sup>-1</sup> )		Haematocrit (l.l <sup>-1</sup> )		Leukocytes count (G.l <sup>-1</sup> )	
		$\bar{x}$	s $\bar{x}$	$\bar{x}$	s $\bar{x}$	$\bar{x}$	s $\bar{x}$
		Dairy cattle	127	115.74	21.41	0.31	0.05
Beef cattle	72	110.90*	16.89	0.32	0.06	7.94	4.08

x = arithmetic mean, s $\bar{x}$  = standard deviation, \* P < 0.05

**Table 5: The correlation between copper and zinc and haematological parameters**

Cattle	n	$r_{xy}$					
		Hb:Cu	Hb:Zn	Ht:Cu	Ht:Zn	Lc:Cu	Lc:Zn
Dairy cattle	127	0.201*	0.313*	0.234*	0.335*	-0.174	0.034
Beef cattle	72	0.622*	0.520*	0.606*	0.688*	-0.334	-0.320*

Hb = haemoglobin    Lc = leukocytes count    Zn = zinc    \* P < 0.05  
Ht = haematocrit     $r_{xy}$  = correlation coefficient    Cu = copper

The next aim of this paper was to concretise relations between Cu:Hb, Cu:Ht, Zn:Hb, Zn:Ht, Cu:Lc a Zn:Lc in BP of cattle (Table 5). Just between those couples should exist direct linear dependence, on the basis of findings from scientific literature about their interactions (Bíreš et al., 1999), expressed as positive coefficient of correlation ( $r_{xy}$ ). In dairy cattle, low or mild degrees of statistical dependence ( $r_{xy}$  = from 0,201 to 0,335) was assumed in monitored couples (Table 5). In beef cattle, where the average concentrations of Cu and Zn in BP and in the diet were on a lower limit of RfV (Table 5), in all the tested couples a mean degree of linear relationship have been found ( $r_{xy}$  = from 0.520 to 0.688). Between Cu and Zn and Lc no direct linear dependences have been found (Table 5), a similar conclusion was also drawn by Randhawa et al. (1999), who did not note any significant change in Lc with Cu deficiency. It has managed to prove that the low concentrations of both trace elements could have negative influence on animal haemopoiesis and thereby on their health and production. In accordance with introduced findings of cited authors it is possible to note that direct linear dependences between Hb, Hk and Cu and Zn in beef and dairy cattle be found.

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