

## Differences in the occurrence of selenium, copper and zinc deficiencies in dairy cows, calves, heifers and bulls

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**ABSTRACT:** This study was conducted to evaluate the mineral status of various bovine categories reared on the same farm, and to monitor the quality of their mineral nutrition in this way. Blood samples were collected on 20 farms in various regions of the Czech Republic to diagnose the selenium (Se), copper (Cu) and zinc (Zn) status of dairy cows, calves, heifers and bulls. Blood samples were collected from 5 dairy cows and their calves, 5 heifers and, whenever possible, also from 5 bullocks (10 farms). To assess the Cu and Zn status of the cattle, blood serum concentrations of the elements were determined by flame AAS. The Se status assessment was based on the glutathione peroxidase (GSH-Px) activity in whole blood. Dairy cows and calves showed the higher GSH-Px activity in comparison with heifers and bulls ( $P < 0.001$ ). Overall mean GSH-Px activity in the blood of dairy cows, calves, heifers and bulls was  $720.47 \pm 174.47 \mu\text{kat/l}$ ,  $688.34 \pm 204.12 \mu\text{kat/l}$ ,  $555.69 \pm 318.36 \mu\text{kat/l}$  and  $516.17 \pm 214.70 \mu\text{kat/l}$ , respectively. Se deficiency was diagnosed in 23% of the examined dairy cows (20% herds), 31% calves (25% herds), 51% heifers (50% herds) and in 58% of bulls (50% herds). Dairy cows showed lower Zn and higher Cu concentrations in comparison with the other categories ( $P < 0.001$ ). Mean Zn concentrations in blood serum of dairy cows, calves, heifers and bulls were  $12.21 \pm 3.19 \mu\text{mol/l}$ ,  $18.91 \pm 5.78 \mu\text{mol/l}$ ,  $17.80 \pm 2.76 \mu\text{mol/l}$  and  $16.69 \pm 3.08 \mu\text{mol/l}$ , respectively. Zn deficiency was diagnosed in 41% of the examined dairy cows (45% herds) and 13% of bulls (10% herds). None of the calf or heifer herds was classified as Zn deficient when Zn deficiency was found in only 9% of calves and 1% of the examined heifers. Mean Cu concentrations in blood serum of dairy cows, calves, heifers and bulls were  $13.62 \pm 2.62 \mu\text{mol/l}$ ,  $10.18 \pm 3.22 \mu\text{mol/l}$ ,  $10.96 \pm 2.52 \mu\text{mol/l}$  and  $11.18 \pm 2.40 \mu\text{mol/l}$ , respectively. Cu deficiency was diagnosed in 28% of the examined dairy cows (20% herds), 70% of calves (80% herds), 65% of heifers (75% herds) and 70% of bulls (60% herds). Deficiency of at least one of the microelements monitored was diagnosed on all investigated farms.

**Keywords:** microelements; mineral nutrition; diagnostics; blood; cattle

The need for an adequate supply of microelements follows from their important role in relation to the health of animals. Microelements have a number of structural, catalytic and regulatory functions in the organism, and they also play a major role in the immune system (Underwood and Suttle, 1999). In view of many biological functions the microelements have and because they are transferred through the placenta to the foetus, being also trans-

ferred to colostrum and milk, sufficient saturation of pregnant animals with microelements is very important to meet the needs of their young. Any deficiency during the intrauterine stage of the foetus development or in subsequent rearing stages may negatively influence the animals' health and thus also their production and reproduction (Abdelrahman and Kincaid, 1993; Swecker et al., 1995; Pavlata et al., 2003, 2004a,b; Gierus et al., 2003).

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project FRVS No. 317/2003 and Research Project No. 161700002).

In ruminants, the first factor in the supply of microelements is the geographical location, i.e. where they are reared because the location – through forages and feeds grown there – primarily determines the mineral status of the animals. Higher natural levels of microelements are usually found in concentrates compared with forages. As a rule, mineral feed supplements constitute a necessary source of microelements. The form in which microelements are administered is also important: some are inorganic, others are organically bound. The latter are characterized by better availability, enhanced biological effect and a lower risk of environmental contamination (Nockels et al., 1993; Rabiansky et al., 1998; Lee et al., 1999; Pavlata et al., 2001a; Kuricová et al., 2003).

Hitherto results of investigations into microelement deficiencies in cattle suggest that the situation on Czech dairy farms is far from being satisfactory, and data on other bovine categories are scarce

(Koutnik et al., 1996; Pavlata et al., 2000, 2001b, 2002, 2004b). Furthermore, because mineral element deficiencies were found in the human population in this country (Kvicala et al., 1995, 2003; Kvicala and Jiranek, 1999), information on microelement levels in farm animals may be important not only for the health of these animals but also for human medicine because these animals are used as foodstuffs (Koutnik and Ingr, 1998). However, no evaluations of the incidence of metabolic diseases are made either independently or in the framework of analyses of the reasons for emergency slaughters or for condemnation of carcasses (Kozak et al., 2003; Vecerek et al., 2003a,b) in which micro-mineral deficiencies certainly play a role in view of their impact on the locomotor apparatus and overall health status.

The purpose of the study was to evaluate the status of selected microelements in various bovine categories (dairy cows, calves, heifers and bulls)

Table 1. List of surveyed farms and their basic characteristics (D – dairy cows, C – calves, H – heifers, B – bulls)

Farm	Region, town	Bovine categories	Farmer stated that diet included mineral supplementation		
			D	H	B
1	Jihomoravsky, Ivancice	D, C, H, B	+	–	–
2	Vysocina, Bystrice nad Pernštejnem	D, C, H, B	–	–	–
3	Jihomoravsky, Ricany	D, C, H, B	–	–	–
4	Vysocina, Nove Mesto na Morave	D, C, H	+	+	+
5	Vysocina, Velka Bites	D, C, H	+	+	+
6	Jihomoravsky, Zdanice	D, C, H, B	+	+	+
7	Jihomoravsky, Tesany	D, C, H	+	+	+
8	Vysocina, Vecov	D, C, H	+	+	+
9	Pardubicky, Litomysl	D, C, H	+	+	+
10	Pardubicky, Policka	D, C, H	+	+	+
11	Moravskoslezsky, Novy Jicin	D, C, H	+	–	–
12	Jihomoravsky, Zabcice	D, C, H, B	+	+	+
13	Vysocina, Havlickuv Brod	D, C, H	+	–	–
14	Vysocina, Ujcov	D, C, H	+	–	–
15	Jihomoravsky, Slavkov u Brna	D, C, H, B	+	+	+
16	Jihomoravsky, Slapanice	D, C, H, B	+	+	+
17	Olomoucky, Hranice	D, C, H, b	+	–	–
18	Zlinsky, Valasske Mezirici	D, C, H, b	+	–	–
19	Moravskoslezsky, Novy Jicin	D, C, H, B	+	+	+
20	Moravskoslezsky, Bruntal	D, C, H	+	+	+

and thus to obtain objective information on the quality of their micromineral nutrition.

## MATERIAL AND METHODS

Blood samples were collected on 20 farms (Table 1) in various regions of the Czech Republic to diagnose the selenium (Se), copper (Cu) and zinc (Zn) status of various bovine categories. In each case, blood samples for tests were collected via the tail or jugular vein into two Hemos sets (one containing heparin, the other without any anticoagulants) from 5 dairy cows and their calves aged 2–6 days, 5 heifers aged 8–16 months and, if there were any bullocks reared on the farm, also from 5 of the bulls (10 farms). Farmers were asked to provide basic information on used diets, and, specifically, whether these were diets with mineral supplementation.

The Se status was assessed on the basis of glutathione peroxidase (GSH-Px) activity in whole heparinized blood according to the method described by Paglia and Valentine (1967) with the use of the RANSEL-RANOX set and COBAS MIRA automatic biochemical analyzer. To assess the Cu and Zn status of the organism, blood serum concentrations of the two elements were determined by flame AAS (AA Series spectrometer SOLAAR M Thermo Electrocorporation). All the tests were performed in the biochemical laboratory of our institute. The incidence of microelement deficiencies was evaluated not only from results obtained from individual animals but also from calculated mean concentrations in animals from individual farms. Selenium deficiency was defined when the GSH-Px activity in whole blood was below 600  $\mu\text{kat/l}$ , which corresponds to whole blood selenium concentrations approaching 100  $\mu\text{g/l}$  (Pavlatá et al., 2000). Cu and

Zn deficiency was defined as blood serum concentrations of the respective element below 12  $\mu\text{mol/l}$  (Jagos et al., 1981).

Calculations of basic statistical characteristics of the sets of values obtained and statistical comparisons of significance of differences between mean concentrations of individual elements in different bovine categories on the same farm by the *t*-test were performed in Microsoft Excel 97.

## RESULTS

Selected categories of cattle were examined on 20 farms to obtain mean Zn and Cu concentrations and mean GSH-Px activity as an indirect index of the Se status of animals. The results, together with some statistical characteristics of the sets of values ascertained in individual categories of cattle are given in Tables 2 and 3. It follows from the tables that the studied elements were found in a relatively broad range of values (Zn: 5.46–36.70  $\mu\text{mol/l}$ , Cu: 2.91 to 19.65  $\mu\text{mol/l}$ , GSH-Px: 8.61–1 309.00  $\mu\text{kat/l}$ ), which suggests that the supply of microelements to cattle in the Czech Republic differs greatly from farm to farm. Results obtained in individual bovine categories from different farms (including their statistical comparisons) are in Figures 1–20. The data suggest that there are significant differences in the supply of microelements not only between individual farms but also between individual bovine categories on the same farm. In spite of differences in absolute values of individual parameters, a number of definite trends among them may be identified and highlighted by a statistical comparison of individual data sets. In dairy cows, higher mean Cu and lower mean Zn concentrations were found than in the other bovine categories studied, and the differences were statistically highly

Table 2. Basic statistical characteristics of the set of Zn and Cu concentrations found in bovine categories (<sup>a</sup>:<sup>b</sup>  $P < 0.001$ )

	Zn ( $\mu\text{mol/l}$ )				Cu ( $\mu\text{mol/l}$ )			
	cows	calves	heifers	bulls	cows	calves	heifers	bulls
Mean	12.21 <sup>a</sup>	18.91 <sup>b</sup>	17.80 <sup>b</sup>	16.69 <sup>b</sup>	13.62 <sup>a</sup>	10.18 <sup>b</sup>	10.96 <sup>b</sup>	11.18 <sup>b</sup>
S.D.	3.19	5.78	2.76	3.08	2.62	3.22	2.52	2.40
Min	5.51	5.46	11.44	9.59	8.30	4.37	2.91	7.55
Max	20.25	36.70	24.54	21.99	19.65	17.79	18.52	18.92
$\nu$ (%)	26	31	16	18	19	32	23	20
<i>n</i>	100	100	100	50	100	100	100	50

Table 3. Basic statistical characteristics of the set of GSH-Px activity values found in bovine categories (<sup>a</sup>:<sup>b</sup>  $P < 0.001$ )

	Se-GSH-Px ( $\mu\text{kat/l}$ )			
	cows	calves	heifers	bulls
Mean	720.47 <sup>a</sup>	688.34 <sup>a</sup>	555.69 <sup>b</sup>	516.17 <sup>b</sup>
S.D.	174.47	204.12	318.36	214.70
Min	260.00	286.50	8.61	62.32
Max	1 193.00	1 222.00	1 309.00	938.50
$\nu$ (%)	24	30	57	42
$n$	100	100	100	50

significant ( $P < 0.001$ ). Compared with dairy cows and calves, heifers and bulls showed lower GSH-Px activity levels, and the difference was statistically highly significant ( $P < 0.001$ ). It follows from the above data that the lowest Zn concentrations were found in dairy cows, while low Se and Cu concentrations were most frequently found mainly in heifers and bulls. In calves, low serum Cu concentrations were recorded.

Zn deficiency was found in 9 dairy cow herds and 1 herd of bulls. On all farms, cows had lower Zn concentrations than their calves, and the differences were statistically significant on 12 farms. In fact, dairy cows also had lower mean serum Zn concentrations than bulls and heifers (the differences were statistically significant in 16 and 7 heifer and bull herds, respectively). In four herds of bulls, statistically significantly lower Zn concentrations than in heifers were found. On other five farms, however, Zn concentrations in bulls were higher than in heifers, but those differences were not statistically significant.

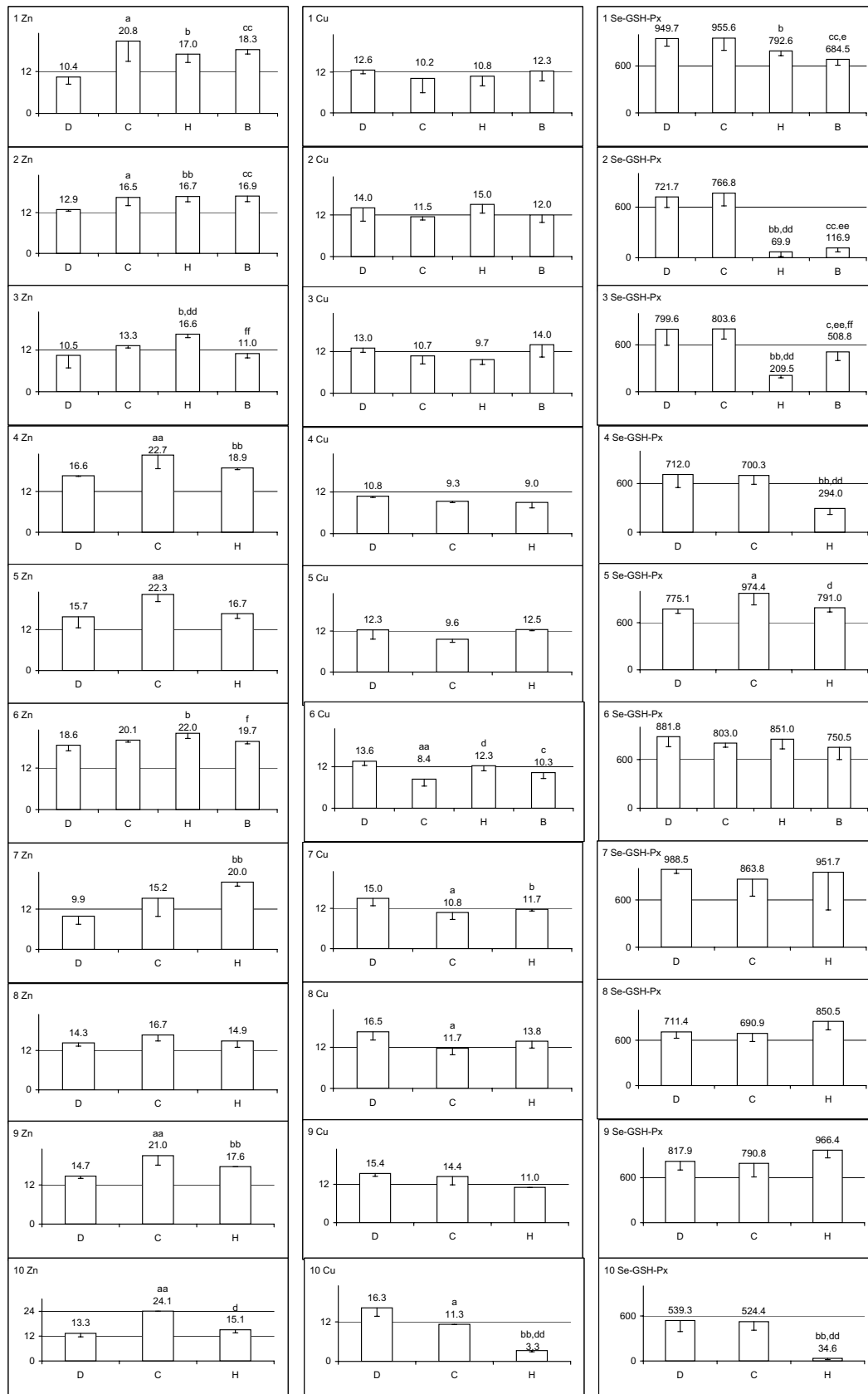
Cu concentrations below  $12 \mu\text{mol/l}$  were found in 4 dairy cow herds, 16 calf herds, 15 heifer herds and 6 bull herds. In most cases, the lowest Cu concentrations were found in calves. In 18 cases, mean serum Cu concentrations in calves were lower than those

of respective dams (the differences were statistically significant in 11 herds). In 13 herds, Cu blood concentrations were lower in calves than in heifers. In 17 heifer herds and 8 bull herds, Cu concentrations were lower than those in dairy cows. A comparison between bulls and heifers showed lower mean serum Cu concentrations in the latter in most (6) cases. The Cu status in individual bovine categories can generally be described as relatively balanced, but with a high incidence of Cu deficiencies.

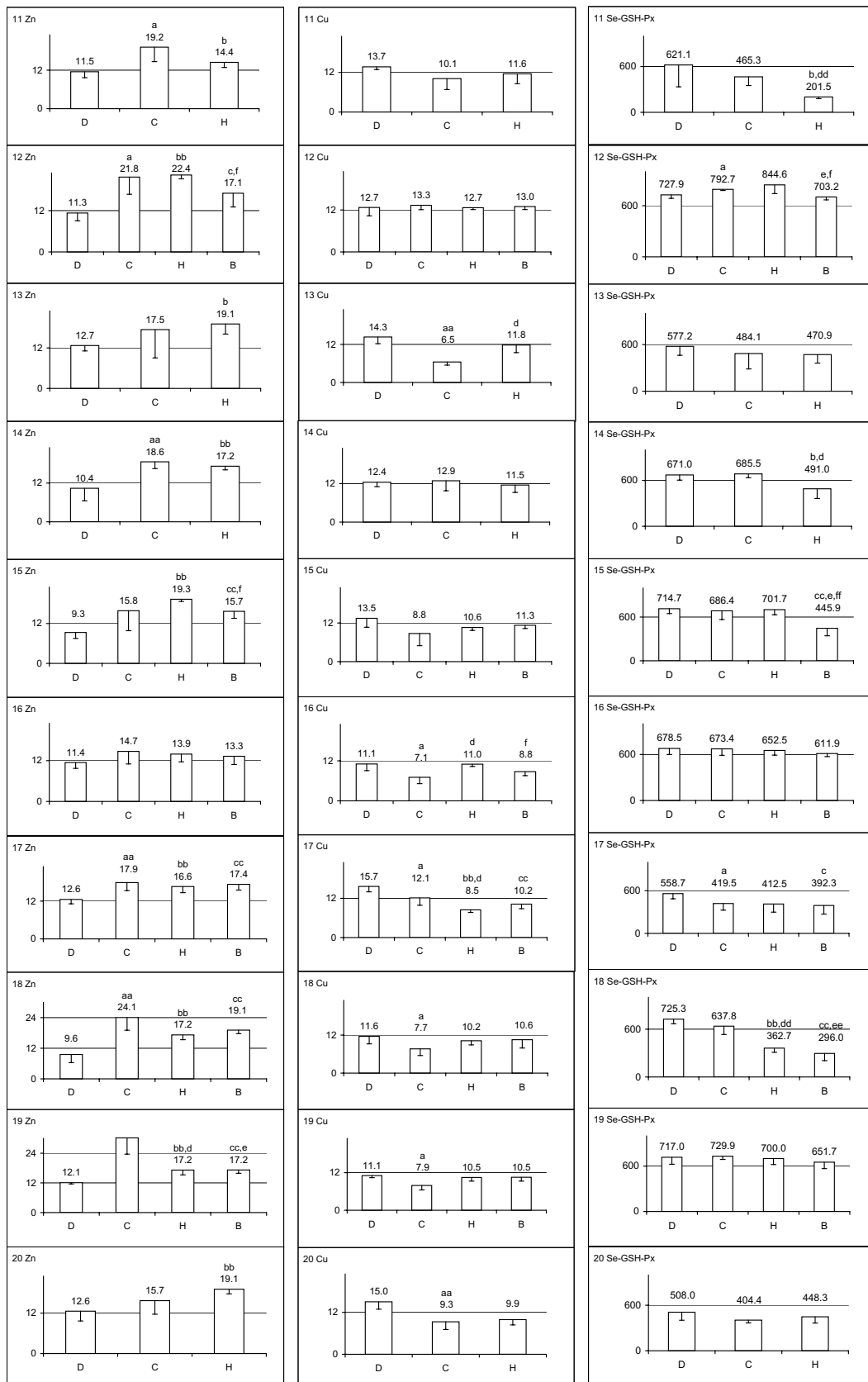
The assessment of GSH-Px activity in whole blood indicated insufficient Se concentrations in dairy cows in 4 herds, calves in 5 herds, heifers in 10 herds and bulls in 5 herds. Of the microelements studied, Se status showed the largest differences between different bovine categories. In some cases, the mean GSH-Px activity in heifers and also in bulls was around 10 to 20% of the levels found in cows and calves. Selenium status in heifers and bulls tended to be generally lower than Se status in dairy cows and calves. On 16 farms, the lower GSH-Px activity in blood was found in heifers than in dairy cows (differences were statistically significant in 8 herds), and in all 10 herds the lower GSH-Px activity was found in bulls than in dairy cows (statistically significant in 6 herds). Se deficiency was demonstrated in heifers and bulls on many farms

Table 4. Incidence of zinc, copper and selenium deficiencies (in %) when concentrations of individual animals and mean values of animals in herds were evaluated

	Concentrations of animals				Mean values of animals			
	cows	calves	heifers	bulls	cows	calves	heifers	bulls
Zn	41	9	1	13	45	0	0	10
Cu	28	70	65	70	20	80	75	60
Se	23	31	51	58	20	25	50	50



Figures 1 – 20: Mean Zn and Cu concentrations in blood serum ( $\mu\text{mol/l}$ ) and mean GSH-Px activity in whole blood ( $\mu\text{kat/l}$ ) for individual bovine categories (D – dairy cows, C – calves, H – heifers, B – bulls), standard deviations and statistical significance levels of differences between the values of bovine categories on respective farms



<sup>a</sup>  $P < 0.05$  and <sup>aa</sup>  $P < 0.01$  for D : C; <sup>b</sup>  $P < 0.05$  and <sup>bb</sup>  $P < 0.01$  for D : H  
<sup>c</sup>  $P < 0.05$  and <sup>cc</sup>  $P < 0.01$  for D : B; <sup>d</sup>  $P < 0.05$  and <sup>dd</sup>  $P < 0.01$  for C : H  
<sup>e</sup>  $P < 0.05$  and <sup>ee</sup>  $P < 0.01$  for C : B; <sup>f</sup>  $P < 0.05$  and <sup>ff</sup>  $P < 0.01$  for H : B

Table 5. Incidence of mineral deficiencies in bovine categories on surveyed farms (deficiency of the particular element is indicated by +)

Farm	Cows			Calves			Heifers			Bulls		
	Zn	Cu	Se	Zn	Cu	Se	Zn	Cu	Se	Zn	Cu	Se
1	+				+			+				
2					+				+			+
3	+				+			+	+	+		+
4		+			+			+	+	[Redacted]		
5					+							
6					+						+	
7	+				+			+		[Redacted]		
8					+							
9								+				
10			+		+	+		+	+	[Redacted]		
11	+				+	+		+	+			
12	+									[Redacted]		
13			+		+	+		+	+			
14	+							+	+	[Redacted]		
15	+				+			+				
16	+	+			+			+			+	
17			+			+		+	+		+	+
18	+	+			+			+	+		+	+
19		+			+			+			+	
20			+		+	+		+	+	[Redacted]		
Σ	9	4	4	0	16	5	0	15	10			

although a sufficient GSH-Px activity was found in the blood of dairy cows and calves. On none of the farms was a significantly higher GSH-Px activity demonstrated in the blood of heifers and bulls compared with dairy cows and calves.

A general overview of deficiencies of monitored microelements is given in Tables 4 and 5. It follows from Table 5 that a deficiency of at least one microelement in at least one bovine category was diagnosed on all surveyed farms. It also follows from the data that deficiencies of more than one element on one and the same farm are relatively frequent not only in different bovine categories but also within one category. In 15 of the 20 farms surveyed, the deficiency of at least 2 of the 3 elements studied was demonstrated, and deficiency of all the 3 elements was found on 5 farms.

## DISCUSSION

Although it is generally true that the microelement status of newly born calves is largely determined by the microelement status of the dams during pregnancy because microelements can be transferred via the placenta to the foetus (Hostetler et al., 2003), our results have shown that the situation may differ from microelement to microelement. The results of the present study showed that serum Zn concentrations of the majority of calves were considerably higher than those of their dams. Even on farms with relatively major dairy cow deficiencies (e.g. farms 7, 15 and 18 with mean Zn serum concentrations below 10 µmol/l) Zn serum concentrations in calves were sufficiently high. The results thus document a good transplacental transfer of Zn from the dam

to the foetus or that calves concentrate Zn from the dams, and that assessments of Zn status in calves are very unlikely to produce values below 12  $\mu\text{mol/l}$ . Such values were obtained in only 8% of calves tested in the present study. It follows that Zn concentrations in calves are not a very good indicator of the microelement status of their dams. These results are in agreement with our previous findings of markedly higher blood serum Zn concentrations in calves at birth even before they were given colostrum, and also at day 5 *post partum* compared with maternal concentrations of that element (Pavlata et al., 2004b). This was probably a major reason why dairy cows tested in the present study had the lowest Zn concentrations of all the bovine categories studied. The Zn concentrations found in calves and also in heifers and bulls seem to suggest that basic diets fed to the tested animals contained enough Zn to cover the needs of their age groups. But Zn levels were insufficient for the late pregnancy and early lactation periods. This is probably so because large quantities of Zn are used up by the developing foetus and because high concentrations of Zn are present also in colostrum and milk (Muehlenbein et al., 2001; Kracmar et al., 2003; Pavlata et al., 2004b). It seems that in the last stage of pregnancy and around calving, dams lose more Zn than they ingest, their Zn reserves are depleted and Zn plasma concentrations decrease.

The evaluation of Cu concentrations, however, revealed a very different situation. The highest Cu concentrations were found in dairy cows, while Cu deficiency was observed in a large proportion of the other bovines. Thus the rations fed to heifers and bulls do not seem to be a sufficient source of copper in most cases. The evaluation of Cu metabolism in the cow-calf relationship shows that the situation is basically opposite compared with Zn. The absolute majority of calves had lower Cu concentrations than their dams. Serum Cu concentrations found in calves cannot thus be used as direct indicators of the Cu status of their dams, and vice versa. These results are in agreement with the findings of Enjalbert et al. (2002), who also reported low plasma Cu concentrations in calves in week 1 *post partum* compared with those found in their dams, and with our previous findings (Pavlata et al., 2004b). Lower Cu concentrations are related not only to generally lower dietary Cu intake but also they may be due to reduced Cu utilization by the animals. Lower utilization of Cu has, for instance, been blamed to high intakes of molybdenum and

sulphur (Smart et al., 1992; Ward et al., 1993; Ward and Spears, 1997; Randhawa and Randhawa, 2002). Cu concentration may negatively be influenced by high Fe intake, and the microelement status of animals may also be influenced genetically or by the breed (Ward et al., 1995, Mullis et al., 2003). In spite of Cu supplementation, marginally deficient concentrations are relatively frequent (Dargatz et al., 1999).

Our evaluation of the Se status of cows and their calves has confirmed that the Se status in calves is very similar to that of cows, and if an adequate (or, alternatively, deficient) Se status is found in one of the two groups, it may be assumed that it also applies to the other group (Pavlata et al., 2003, 2004b). Although statistically significant differences between GSH-Px activities in calves and their dams were found on 4 farms, the differences could not have caused a fundamentally wrong interpretation of the Se status, i.e. whether the herd was Se deficient or not. In one herd only, slightly lower values in calves were found at the GSH-Px activity above 600  $\mu\text{kat/l}$ . This finding is in agreement with our previous results when we reported a very close correlation between Se concentrations and GSH-Px activity in the blood of newborn calves and their dams ( $r = 0.91$  and  $0.93$ , respectively) (Pavlata et al., 2003). Relatively good results were obtained in cows and their calves, when Se deficiencies were found in 20 and 25% of farms, respectively. This marks a major improvement over the results of the previous study when selenium deficiency was found in 40% of dairy cow herds (Pavlata et al., 2002). Results in heifers, however, were very unfavourable. The 50% Se deficiency may be markedly better than the values reported in the previous study when Se deficiency was found in all the heifers tested, but GSH-Px values on those 50% of farms are often very low. This may not only have a major effect on the health of heifers but also it may be negatively manifested in calves born to such severely Se deficient primiparous dams. Because Se deficiency may cause serious health problems including nutritional muscle dystrophy, reduced resistance to cold in the young, an increase in the counts of somatic cells in milk and higher incidence of mastitis, placenta retention, and reproduction cycle disorders (Smith, 1996; Smith et al., 1997; Galyean et al., 1999; Underwood and Suttle, 1999; Pavlata et al., 2001c), selenium deficiency in heifers must be interpreted as a very negative factor. And because many health disorders can be linked to the negative influence of



deficiency of selenium (and also of other microelements) on the immune system, deficiencies may be expected to lead to higher prevalence of infectious diseases, particularly among calves. Furthermore, sufficient microelement supplementation also influences the colostrum quality (Swecker et al., 1995; Pavlata et al., 2004a), and negative consequences of microelement deficiencies in heifers may thus add up and increase the risk of diseases in their calves compared with calves from cows at higher lactations when microelement deficiencies (and mainly Se deficiencies) are encountered less frequently. This is one of the reasons why any analysis of higher calf morbidity should include a comparison between the test results of calves from primiparous and from multiparous cows.

The microelement status of cattle, and of dairy cows in particular, is undoubtedly influenced also by the usually reduced dietary nutrient profiles during the dry period. Microelement concentrations in cows during the dry period are usually lower than in the lactation period when rations contain more concentrates with more mineral supplements (Hain et al., 2003). Another factor that may affect the mineral status of cattle is seasonality (Prosbova et al., 1982). For instance, Mee et al. (1994) found very low concentrations of microelements in more than three quarters of the Irish cattle herds in autumn compared with 54% in spring. At present, cattle in the Czech Republic are predominantly fed uniform diets based on preserved feeds throughout the year, and seasonal influences are probably not responsible for any major fluctuations in their microelement status.

It proved relatively difficult to assess the relation between diets fed to cattle and their microelement status. Surprisingly, most farmers were unable to give us a clear answer to our question what microelements, if any, were supplemented to basic diets fed to different bovine categories. Diets are frequently designed by animal nutrition specialists and farmers are not usually interested in the details about mineral supplementation, assuming that their animals receive all mineral elements that are necessary. Although most farmers stated that diets they fed included mineral supplements, the incidence of deficiencies was high. A general conclusion may be drawn that the importance of Se and Cu status in heifers and bulls is often underestimated and that feed rations are not a sufficient source of minerals. If basic diets are not supplemented with the minerals, the incidence of

deficiencies among cattle categories surveyed in this study is then relatively high.

The results of the study clearly demonstrated that preventive diagnosis of the mineral status must be given more attention in individual bovine categories and that, based on the results of those analyses, diets must be supplemented in a targeted manner. Whenever supplementation of feed rations is used, metabolic tests should be conducted to check the effectiveness of the measures.

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Received: 04–10–07

Accepted: 05–09–09

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