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*Short communication*

# Zinc, copper and selenium deficiencies in broodmares in south-eastern Poland

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## Abstract

Microelement deficiencies are a current problem in horse breeding, causing infertility and fetal development disorders. The aim of the study was to control the concentration of zinc, copper and selenium in the blood serum of pregnant herd mares. The study included 154 mares in the second half of pregnancy, kept in five regions of south-eastern Poland: Łęczyńsko-Włodawska Plain, Chełmskie Hills, Łukowska Plain, Beskid Niski and Działy Grabowieckie. The concentration of zinc, copper and selenium in the obtained blood serum was determined using atomic absorption spectrophotometry. The serum zinc concentration in all studied mares did not exceed 10.7  $\mu\text{mol/l}$ , while the adopted reference range is 14.9 - 29.2  $\mu\text{mol/l}$ . The lowest concentration of copper and selenium was found in mares from Beskid Niski. In all the studied regions, herd mares had zinc deficiency, and in Beskid Niski additionally copper and selenium deficiency. Despite the identified deficiencies, all the studied mares gave birth on time and registered newborn foals. Since no clinical symptoms of deficiencies of the tested trace elements were observed, it can be assumed that if occurred, they were subclinical.

**Keywords:** mares, microelements, serum, trace elements



## Introduction

The supply of microelements to animals depends on the content of individual elements in the soils used to grow crops for animal feed, and on a supplementation (Mirowski 2014, 2023, Mirowski and Didkowska 2015). Serum microelement concentrations below the optimal ranges are constantly being detected in horses (Pilarczyk et al. 2014, Kósa et al. 2021, Deride et al. 2023). Insufficient content of microelements may cause characteristic clinical symptoms, or occur as a subclinical deficiency, without noticeable symptoms. Zinc (Zn) deficiency is known to disturb stallion fertility, whereas copper (Cu) deficiency is responsible for the lack of mare successful fertilization and an early embryo loss (Danek 2002, Kędzierski and Wałkuska 2007). Moreover, foals are born with very low blood Cu level, and Cu deficiency induces osteochondrosis (Cymbaluk and Christensen 1986, Bridges and Harris 1988). Selenium (Se) deficiency causes various myopathies (Gomez et al. 2015, Deride et al. 2023). On the other hand, over-supply of Zn leads to Cu deficiency, whereas an excess of Cu could be a cause of haemolysis (Bridges and Moffitt 1990, Kędzierski and Wałkuska 2007, Belli et al. 2021). Thus, the aim of the study was to screen the concentration of Zn, Cu and Se in the blood serum of pregnant mares reared in Poland.

## Materials and Methods

The study used randomly selected blood serum samples collected from 154 mares by veterinarians directly supervising the horse breeding farms. All procedures of blood sampling from studied mares were performed as a part of health examination. Therefore, according to the European directive EU/2010/63 and Polish regulations regarding experiments on animals, there was no need to obtain a special approval of Ethical Committee for the described procedures, qualified as non-experimental clinical veterinary practices. The owners of the animals tested were aware of the purpose of blood sampling, and gave verbal consent to the publication of the study's results.

Collecting samples took place before the grazing season, from February 4 to March 15, within one foaling season. Only the samples collected from mares in the second half of pregnancy with known mating day were analysed. The mares used for sampling were in good and very good body condition. They appeared healthy throughout their pregnancies and did not show any clinical signs of health disorders at the time of sampling. They were maintained in breeding farms located in five regions of south-eastern Poland (Table 1, Fig. 1),

and were entered in studbooks or were registered by the Polish Horse Breeders Association (PHBZ-PZHK). The mares were kept in stables with free access to paddocks and fed with oats, hay, and commercial feed for breeding mares. Data on the dates of birth of individual mares was obtained from studbooks and from the PHBZ database.

The Zn and Cu concentrations in serum samples were determined using flame atomic absorption spectrometer, as was described in details by Maško et al. 2024. The Se concentration was determined by the GFAAS atomic absorption spectrometry method according to the modified Neve and Molle method (Banasik et al. 2011). The methods were controlled by analysing the series of samples from a certified reference material

A normal distribution of the analyzed data was confirmed using the Shapiro-Wilk test. Thus, data were analyzed using MANOVA and Tukey's comparison test (Graph Pad Software, USA).

## Results and Discussion

All studied mares gave birth to live foals on their expected due date ( $\pm 2$  weeks). All the born foals were registered in the studbooks or the PZHK register, according to their breed, within 3 months after birth.

Mean serum Zn concentration ranged from 9.03 to 10.7  $\mu\text{mol/l}$  and did not differ significantly in mares from studied regions (Table 2). These values are below the reference range (Krumrych 2003, Winnicka 2021). However, low serum Zn levels not exceeding 10  $\mu\text{mol/l}$  (0.7 mg/l) were often reported in horses without any visible signs of health disturbances (Krumrych et al. 1996, Deride et al. 2023). Moreover, the feeding season may have some influence on the Zn supply of horses. For example, Górski et al. (2017) stated higher levels of serum Zn before grazing season than after it in riding horses. On the other hand, serum Zn concentrations gradually increased during the pasture season in Polish Konik mares (Maško et al. 2024).

Serum Cu concentration in mares reared in Beskid Niski amounted to 14.5  $\mu\text{mol/l}$ , and was significantly lower than in other regions, except Chełmskie Hills (Table 2). Serum Cu concentrations in studied mares were generally within the reference range (Krumrych 2003, Winnicka 2021). An evident deficiency of this element was only stated in mares reared in Beskid Niski. However, low serum Cu levels have been frequently reported in different regions and different breeds of horses (Krumrych et al. 1996, Deride et al. 2023, Maško et al. 2024). Generally, the discussion about reference values and normal blood Cu levels has been going on



Fig. 1. Localisation of places of origin of studied mares.

Table 1. Demographic data of the mares studied.

Abreviation	Name of region	Breed or type	n
LP	the Łuków Plain	Anglo-Arabian	14
		Purebred Arabian	25
GH	the Grabowiec Heights	Purebred Arabian	33
LWP	the Łęczna-Włodawa Plain	Coldblooded	25
CH	the Chełm Hills	Coldblooded	28
LB	the Low Beskids	Hucul	29
Total number:			154

Table 2. Serum zinc (Zn), copper (Cu) and selenium (Se) concentrations in pregnant mares reared in studied regions in south-eastern Poland (Means  $\pm$  SD).

Region studied	Zn ( $\mu\text{mol/l}$ )	Cu ( $\mu\text{mol/l}$ )	Se ( $\mu\text{mol/l}$ )
the Łuków Plain	$10.7 \pm 3.57$	$21.1 \pm 2.73^a$	$0.96 \pm 0.17$
the Grabowiec Heights	$9.88 \pm 1.18$	$24.1 \pm 3.76^a$	$2.10 \pm 0.23^b$
the Łęczna-Włodawa Plain	$9.93 \pm 1.25$	$25.8 \pm 3.10^a$	$1.11 \pm 0.51^a$
the Chełm Hills	$9.03 \pm 1.17$	$17.7 \pm 2.59^{ab}$	$1.30 \pm 0.42^a$
the Low Beskids	$9.78 \pm 1.75$	$14.5 \pm 2.15^b$	$0.42 \pm 0.10^c$
Physiological range*	$<15.0 ; 29.0>$	$<19.0 ; 21.0>$	$<0.90 ; 2.10>$

<sup>a,b,c</sup> – different litters mean statistically significant differences at  $p < 0.05$

\* according to Krumrych 2003 and Winnicka 2021

for almost a century (Sarata 1938, Auer et al. 1988, Cieśla and Janiszewska 2000). Mares should be fertilized in a short time after delivery, while Cu deficiencies causes reproductive malfunctions. For this reason, monitoring the Cu supply of bred mares is of particular importance.

In mares from Beskid Niski, serum Se concentration was lower than in other regions (Table 2) whereas mares from Działy Grabowieckie had significantly higher values of this element than those from other regions. Values of Se below the reference ranges were stated only in Hucul mares reared in Beskid Niski

(Krumrych 2003). A low level of Se is a common problem in horses around the world (Finno et al. 2015, Delesalle et al. 2017, Kósa 2021). On particular note is the case of uterine atonia due to severe Se deficiency described in a parturient mare (Busse and Uberti 2020). Thus, the proper Se supplementation for horses, especially for breeding mares is widely recommended (Mirowski 2014). Generally, Hucul mares bred in Beskid Niski should be supplemented with Cu and Se.

Nevertheless, trace elements supplementation is not always necessary. For instance, an experimental supplementation with a premix formulated to meet the requirements of pregnant mares at a dose four times higher than recommended, did not influence mare milk, mare and foal serum Cu and Zn concentrations, as well as foal growth and development (Kavazis et al. 2002). Moreover, in newborn foals, the initially low Cu content increases significantly within a few days, independently of the health status of these animals (Stahl et al. 2024). Adult horses with serum Cu concentration below 10 µmol/l exhibited pica behaviour (geophagia). It means that horses with mineral deficiency tended to reduce the problem by seeking non-feed sources of deficient micronutrients (Aytekin et al. 2011). On the other hand, lowered values in serum microelement levels could be related to the occurrence of some inflammatory diseases, not only to the supply of trace elements in the diet (Youssef et al. 2012).

Moreover, the assessment of metal content in animal tissues gives insights into environmental conditions. Aragona et al. (2024 a) reported elevated values of Zn and Cu in the blood of horses reared in rural areas. From this point of view, our results may indicate the absence of industrial pollution, especially Zn and Cu, in the studied regions.

The other factor which should be mentioned here is the fact that the concentration of trace elements in serum did not represent the contents of these elements in other tissues (Cymbaluk and Christensen 1986, Massayi et al. 2014, Paßlack et al. 2014, Aragona et al. 2024b). Moreover, the content of Se is significantly higher in the muscle, liver and kidney tissues obtained from young horses (6-18 months) as compared to horses aged from 10 to 13 years (Szkucik et al. 2014). Thus, the results of trace mineral determination in the serum are not representative of their content in the body.

In conclusion, Zn deficiency occurred in mares in all regions studied, according to current reference values. Additionally, Cu and Se deficiencies were noted in Hucul mares from the Beskid Niski. Despite the identified deficiencies, the mares studied did not show any health disorders, they delivered on time and gave live foals. Nevertheless, the health of the horses and the level of supply of the discussed microelements should

be monitored, as maintaining a deficit at a subclinical level may lead to clinical symptoms in the future. We propose that a discussion be initiated within the scientific community on lowering the lower reference ranges for the microelements in question, especially for Zn and Cu.

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