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Effectiveness of copper supplementation in mares during reproduction season according to the feed zinc : copper ratio

Efektywność uzupełniania niedoboru miedzi u klaczy w sezonie rozrodczym w zależności od proporcji cynku do miedzi w dawce pokarmowej

Summary. Intestinal absorption of copper is inhibited by zinc. The aim of this study was to define the maximal value of Zn:Cu ratio in feed ration sufficient to increase the supply of Cu in mares with diagnosed Cu deficiency. The research was conducted in the stud, in which 46 percent of broodmares remained barren because in the previous reproductive season they had shown various reproduction malfunctions: the lack of estrus and ovulation, silent estrus, the lack of pregnancy after insemination as well as fetal resorptions on the 21st–35th day of pregnancy. The levels of Cu, Mn, Fe and Zn were determined in blood plasma from 10 barren mares and in fodder samples by atomic spectrophotometry absorption (ASA). During the study two common mineral supplements were given in doses recommended by the manufacturers, which decreased the Zn:Cu ratio in fodder from 9.0 to 8.3 or 6.0. When Zn:Cu fodder ratio was 8.3, no effects of eight weeks of supplementation were shown. After decreasing this ratio to 6.0, plasma Cu level in mares increased from 5.5 to 12.1 μmol/l and the mating period has gone without disturbances since then.

Key words: copper deficiency, fertility, mares, mineral supplements, zinc

INTRODUCTION

Supply of copper in females is still a currant problem in humans and animals because the content of this trace element in food and soils is still dropped in comparison to previous decades [Kabata-Pendias and Pendias 1999, Kocjan *et al.* 2002, Kubik *et al*. 2004, Ghayour-Mobarhan *et al*. 2005, Murawski *et al*. 2006]. The enrichment of the feed in-

take by Cu is especially important in pregnant mares. In this species, Cu storage in fetus liver during the final half of gestation takes place, because Cu content in milk is low and does not satisfy the demand of the foal for the discussed element. Therefore, the demand for Cu of mares during late pregnancy is especially big great. Cu deficiency can cause various disorders in animals, e.g. anemia, increased susceptibility to infections, disorders of growth and long bones development, as well as malfunctions of the reproductive system. In colts at intensive growth, the shortage of this element can cause osteochondrosis and joints and limbs deformation, whereas in mares it causes increased fetal mortality and more frequent abortions [Pearce *et al.* 1998a, Cieśla and Janiszewska 2000, van Weeren *et al.* 2003, Sanders 2005, Gee *et al.* 2007]. The supply of microelements varies greatly in horse populations depending on maintenance conditions, trace elements contents in soils and fodder and on the potential use of mineral supplements in feeding [Ott and Asquith 1995, Bis-Wencel *et al.* 2002]. Furthermore, it was found that microelement supplements do not always increase the level of these elements in the tissues of the tested animals [Pearce *et al.* 1998b]. One of the reasons for limited Cu bioavailability can be the interactions of micronutrient like zinc, iron and other divalent cations [Wapnir and Balkman 1991, Barany *et al.* 2005, Arredondo *et al.* 2006]. Moreover, the deficit of Fe, Mn and Zn also has a major impact on reproductive inefficiency.

The study aims at defining the effectiveness of copper supplementation of stud mares with diagnosed reproductive malfunctions according to the value of feed Zn:Cu ratio.

MATERIAL AND METHODS

The tests were carried out on a stud farm where the fertility percentage in mares had dropped significantly in comparison to the results in the previous years. A herd consisted of tens of warm-blood mares aged from 4 to 17, and 46 percent of them remained barren. The bacteriology and virusology tests in reproductive tracts swabs have given negative results. Horses were fed with fodder produced at the farm. During the grazing season the diet was supplemented with 3 kg of oats, in autumn and winter except oats 5 kg of hay and 5 kg of carrots were provided regularly, and mash twice a week. During the mating period regular USG examinations of the reproductive systems of mares were carried out to determine the optimum insemination date, as well as to prove the presence of fetus on the 14^{th}, 21^{st} and 35^{th} days of pregnancy.

Our investigations took place during the reproduction season, which for the tested horses took place in winter and spring. Fodder samples of oats and hay were collected to determinate content of copper, manganese, zinc and iron. Before the start of trace elements application, as well as after 8 weeks of diet supplementing, blood samples were taken from 10 randomly chosen non-pregnant mares from the external jugular vein to test tubes containing heparin. In the gathered blood plasma and in fodder samples the concentrations of Cu, Fe, Mn and Zn were determined by the method of atomic spectrophotometry absorption (ASA), apart from Fe content in blood plasma, which was determined by the colorimetric method with the diagnostic set of the Cormay (Lublin, Poland) and expressed as mg/kg d.m. or μmol/l.

At first, during eight weeks the feed ratio was enriched with a 0.5% addition with a mineral supplement composed on the basis of sunflower oil cake (Marlibo, Boleslaw,

Poland). Afterwards, a mineral–vitamin premix Horsemix 'K Universal' (Dolfos, Piotrków Trybunalski, Poland) was used as feeding supplement in a dose of 90 g daily.

The obtained results were statistically analyzed with the test t-Student (Microsoft Excel NT).

RESULTS

In Table 1, the content of Cu, Fe, Mn and Zn in the samples of oats and hay are shown. Table 2 presents the content of Cu, Fe, Mn and Zn, counted from the obtained results, in daily fodder dose consisting of three kg of oats, five kg of hay and supple-

Table 1. Cu, Fe, Mn and Zn content in oats and hay samples (mg/kg d.m.)
Tabela 1. Zawartość Cu, Fe, Mn i Zn w próbkach owsa i siana

Element Pierwiastek	Oats Owies		Hay Siano	
	RMC	samples	RMC	samples
Cu	1.0–5.2	2.33	2.2–21	2.50
Fe	54–140	16.0	60–140	45.0
Mn	17–121	19.0	20–665	21.0
Zn	12–75	25.0	15–61	20.0

RMC – ranges of mean elements content in plants in Poland [Kabata-Pendias i Pendias 1999]; średnia zawartość pierwiastków w roślinach w Polsce

Table 2. The content of Cu, Fe, Mn and Zn in daily fodder dose consisting of 3 kg oats and 5 kg hay fed to the mares during reproduction seasons before and after 8 weeks of trace elements supplementation (mg/kg d.m.)
Tabela 2. Zawartość Cu, Fe, Mn i Zn w dziennej dawce pokarmowej składającej się z 3 kg owsa i 5 kg siana stosowanej w żywieniu klaczy w sezonie rozrodczym przed i po 8 tyg. uzupełniania diety dodatkami mineralnymi

Element Pierwiastek	Demand* Zapotrzebowanie*	Oats and hay only Wyłącznie owies i siano	Daily fodder dose with mineral supplement Dawka pokarmowa uzupełniona dodatkiem mineralnym	Daily fodder dose with mineral-vitamin premix Dawka pokarmowa uzupełniona dodatkiem mineralno-witaminowym
Cu	10	2.43	14.4	8.14
Fe	50	34.0	62.5	61.2
Mn	40	20.2	40.0	47.5
Zn	40	21.9	120	49.1
Zn:Cu ratio	4	9	8.3	6.0

*Demand of mares during pregnancy and lactation for the discussed elements [Nutrient Requirements of Horses 1997]; zapotrzebowanie na omawiane mikroelementy klaczy w czasie ciąży i laktacji

ments (in doses recommended by the manufacturers) fed to the mares during the period of the study. The addition of the first mineral supplement enhanced the content of all determined elements and decreased Zn:Cu ratio to 8.3. Enrichment of fodder dose with the mineral-vitamin premix decreased fodder copper and zinc content and Zn:Cu ratio to 8.14 mg/kg, 49.1 mg/kg and 6.0, respectively, as compared to the results obtained during the first supplementation.

The concentrations of Cu, Fe, Mn, and Zn in the blood plasma of mares before mineral supplementation and after eight weeks of their application are presented in Table 3. The mean Fe and Zn concentrations in blood plasma of mares did not differ significantly during the period of testing. After the use of fodder in which Zn:Cu ratio was at the level of 8.3, a considerable rise of Mn concentration was recorded: from 0.73 μmol/l before supplementation to 0.91 μmol/l ($p \leq 0.05$). In the next part of the study after the use of the mineral-vitamin premix, the mean copper concentration in blood plasma rose statistically significantly from 5.5 μmol/l to 12.1 μmol/l ($p \leq 0.05$).

Table 3. Cu, Fe, Mn and Zn concentrations in blood plasma of mares before and after diet supplementation (μmol/l)
Tabela 3. Zawartość Cu, Fe, Mn i Zn w osoczu krwi klaczy przed i po podaniu dodatków mineralnych

Element Pierwiastek	RVR	Before supplementation Przed uzupełnianiem	After 8 weeks of use Zn:Cu fodder ratio at the level 8.3 Po 8 tyg. podawania karmy, w której stosunek Zn:Cu wynosił 8,3	After 8 weeks of use Zn:Cu fodder ratio at the level 6.0 Po 8 tyg. podawania karmy, w której stosunek Zn:Cu wynosił 6,0
Cu	19–21	5.5 ±1.0	5.5 ±1.0	12.1 ±4.1*
Fe	13–25	23.2 ±4.0	22.4 ±6.4	22.9 ±6.1
Mn	0.3–0.9	0.73 ±0.18	0.91 ±0.18*	0.87 ±0.21
Zn	15–29	52 ±26	65 ±33	57 ±18

RVR – referential values range [Krumrych 2003]; zakres wartości referencyjnych
*The means differs significantly from the values obtained in the first test at the confidence level $p \leq 0.05$; średnie istotnie wyższe od uzyskanych w pierwszym badaniu przy $p \leq 0,05$

During the full study period, the course of deliveries, health and growth of the offspring were satisfactory. However, after foaling the majority of the mares showed various reproductive malfunctions: the lack of estrus and ovulation, silent estrus, the lack of pregnancy after insemination as well as fetal resorptions on the 21^{st}–35^{th} days of pregnancy, similarly to the previous reproduction season. The first fodder supplementation with Zn:Cu ratio at the level of 8.3 did not bring about the expected results. About a half of the mares in the stud were still left without foal, the rate of fetal resorption also remained high, at 26%. After the enrichment of the feed intake with the mineral-vitamin premix causing decrease Zn:Cu ratio to 6.0, the fetal resorptions in the stud were found only in 4% of mares and the percentage of barren broodmares was at the level of 10%.

DISCUSSION

The recorded concentrations of Cu, Fe, Mn and Zn in the samples of oats and hay fed to the tested mares can be described as low in relation to the mean concentration values of these elements in plants in Poland [Kabata-Pendias and Pendias 1999]. A daily feed ration prepared on the basis of these components did not satisfy the horses' demand for the discussed elements [Nutrient Requirements of Horses 1997]. The results of the analysis of blood plasma in mares indicated that despite the deficiencies of trace elements found in fodders, their level in blood plasma was satisfactory, except Cu, the content of which remained below the referential values [Krumrych 2003].

At present the recommended Cu level in a daily feed ration for horses is 10 mg/kg d.m. [Nutrient Requirements of Horses 1997]. However, in tests with fodder containing the lower content of this element, for example 4.8 mg/kg Cu for young pure-breeds, and even as low Cu value as 3.5 mg/kg for grown-up pony, no significant health damage or condition drop was found [Cymbaluk *et al.* 1981, Ott and Asquith 1995, Pearce *et al.* 1998a, Bis-Wencel *et al.* 2002].

The definition of the Cu deficiency concept and the minimum content of this element in the blood of clinically healthy horses are still under discussion. The difficulties in defining these concepts result from the lack of a uniform referential values range [Mee and McLaughlin 1995]. The Cu level differences in particular reports are caused by factors such as place of exploitation and breed of horses, prophylactic diet supplementation with minerals, the horses' age and physiological condition [Suttle *et al.* 1996, Cieśla and Janiszewska 2000, Wichert *et al.* 2002]. However, in none of these reports was the Cu content in blood plasma lower than 8 μmol/l. Krumrych [2003] claims that the physiological range of Cu concentration in horse blood plasma is 19–21 μmol/l. The average concentration of Cu in plasma of 5.5 μmol/l recorded in the initial phase of tests, can thus be regarded as a deficient one.

A separate consideration is due to the Zn level in blood plasma of the investigated mares, whose values were much higher than the referential ones. Similarly, high concentrations of this element were also observed in horses from other home studs [Górecka *et al.* 2002, Gralak *et al.* 2004]. It is possible that the antagonism which appears in the process of the absorption of these elements from the alimentary tract occurred in the discussed case. Copper deficiency in fodder at a significantly higher Zn supply could have resulted in excessive Zn absorption [Wapnir and Balkman 1991, Arredondo *et al.* 2006].

The fodder enrichment with a mineral additive with high Zn content did not bring about the expected results. After eight weeks of supplementation, about a half of broodmares in the stud were left without foal, and the rate of fetal resorption still remained high. These observations together with blood plasma tests results proved the continuing deficiency of this element in the observed group of mares, despite the applied supplementation.

After eight weeks of feeding with an addition of the mineral-vitamin premix with low Zn level, the mean Cu concentration in blood plasma of mares increased from 5.5 to 12.1 μmol/l, despite the decrease of Cu content in a daily feed ratio below the recommended level. At the same time the mating period stopped showing disturbances and the reproductive efficiency improved. Fetal resorptions were found only in two mares, in the

plasma of which the level of this element was the lowest in the investigated horses. These individual cases confirm the validity of the performed observations pointing out at a significant relationship between Cu deficiency in a mare' organism and the disorders of the sexual cycle and early pregnancy. The occurrence of similar abnormalities was described in sheep and rats, as well as in people [Soltan and Jenkins 1983, Wachnik *et al.* 1993, du Plessis *et al.* 1999]. These reports point out at a relationship between Cu deficiency and the occurrence of the reproductive system malfunction due to disorders in the hormonal regulation of the sexual cycle.

The explanation of the reasons for the reproductive function disorders in mares due to Cu deficiency requires a further, more detailed research. The described relationship is especially unfavorable in horses, as these animals are characterized by late maturity, large generation gaps as well as low proliferacy and fertility.

CONCLUSIONS

1. Availability of fodder Cu in mares during reproductive season was limited by Zn:Cu ratio when it was at the level of 8.3. Decreasing this ratio to 6.0 was enough to the with drawal of all signs of Cu deficiency observed previously in studied broodmares.

2. The original deficiencies of trace elements still remain one of the reasons for the lack of success in horse breeding.

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Streszczenie. Wchłanianie miedzi w układzie pokarmowym jest hamowane przez cynk. Celem badań było określenie maksymalnej wartości stosunku Zn:Cu w paszy, pozwalające na uzupełnienie niedoboru miedzi u klaczy. Badania prowadzono w stadninie, w której odsetek klaczy jałowiących wynosił 46%. Już w poprzednim sezonie rozrodczym u większości klaczy w stadzie obserwowano zaburzenia funkcjonowania układu rozrodczego: brak rui i owulacji, ciche ruje, brak ciąży po inseminacji oraz resorpcje zarodków w 21–35 dniu ciąży. Zawartość Cu, Mn, Fe i Zn

w osoczu krwi pobranej od 10 jałowych klaczy i w próbkach owsa i siana badano metodą spektrofotometrii absorpcji atomowej (ASA). Zastosowano dwa rodzaje dodatków mineralnych w dawkach zalecanych przez producentów, co spowodowało obniżenie stosunku Zn:Cu w dawce pokarmowej z 9,0 do 8,3 i 6,0. Utrzymywanie stosunku Zn:Cu w paszy na poziomie 8,3 przez 8 tygodni nie spowodowało dających się zauważyć zmian w przebiegu rozrodu i w poziomie Cu oznaczanym w osoczu klaczy. Po kolejnych 8 tyg. żywienia klaczy paszą, w której wartość Zn:Cu obniżono do 6,0 odnotowano istotny wzrost stężenia Cu w osoczu krwi z 5,5 do 12,1 μmol/l oraz ustąpienie opisanych wcześniej zaburzeń w przebiegu rozrodu.

Słowa kluczowe: niedobór miedzi, płodność, klacze, dodatki mineralne, cynk