

EFFECT OF THE INTENSITY OF RECREATIONAL HORSE USE IN THE SUMMER HOLIDAY SEASON ON SERUM SE CONCENTRATION AND CHOSEN HAEMATOLOGICAL PARAMETERS

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Abstract. The aim of this study was to determine the effect of exploitation of recreation horses in the summer season on the selenium concentration in serum and the values of chosen haematological parameters. Blood to analyse was sampled from horses used in recreation in two horse riding centres located in the Western Pomeranian Province. Stable 1 (8 horses), situated in a seaside resort, with rides being led mostly in a riding arena, less often in the field. Large rotation of riders with different level of riding advancement has been observed, with prevalence of novice riders and horses working on the lunge. Stable 2 (18 horses), situated in a rural area, far away from large cities; in the holiday season, horses work maximum 4 hours a day, with prevalence of rides of weak and moderate intensity, being led in the field, with riders with average and high level of riding advancement and small rotation of riders. Blood samples were drawn twice: in the first days of July (start of the holiday season) and in the last days of August (end of the holiday season). Average selenium concentration in the blood serum of horses in Stable 1 decreased significantly during the holiday season. Mean values of chosen analysed haematological parameters of the horses in both riding centres did not undergo any significant changes during their use in the holiday season. Significant increase in the leukocyte and platelet counts after the holiday season was observed in the blood of horses in Stable 1. The horses of Stable 2 were characterised by significantly higher mean haematocrit value (39.14%) when compared to those in Stable 1. No significant correlations were found between the Se concentrations and chosen haematological parameters. The observations being carried out confirm the occurrence of Se deficiencies in horses in the Western Pomerania. As it appears from the performed study, intensive recreational horse use (5–6 hours of work per day) affects the significant decrease in serum selenium concentration,

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which suggests that application of appropriate supplementation in the nutrition of intensively working horses is necessary. Insufficient number of studies and ambiguous results concerning the effect of horse use on the WBC and the platelet counts prompt to undertake more broader observations in this respect.

Key words: blood, hematological parameters, horses, selenium

INTRODUCTION

It is a commonly known fact that selenium is a trace mineral, that is essential to good health. Appropriate concentrations of this chemical element determine the proper course of many biochemical processes and the appropriate function of many systems. Selenium deficiency and diseases induced by it have been repeatedly found and described in horses all over the world [Meyer et al. 1995, Lee et al. 1995, Avellini et al. 1996, Ludviková et al. 2005 a, b]. The region of Western Pomerania has been considered for a long time as a deficit area in respect of the presence of selenium in the environment [Zabłocki 1990], while threshold and deficit values have been repeatedly found in earlier studies on the Se concentrations in the blood of horses being kept in this region [Ramisz et al. 2000, Balicka-Ramisz et al. 2002]. These results are confirmed by the recent studies by Pilarczyk et al. [2011], determining the Se concentrations in the blood serum of mares and stallions from different areas of Poland; deficit Se values have been found in them in 90% of the examined horses. In the face of Se deficiencies being frequently found in horses, it is important to monitor the concentrations of this bioelement in organisms of different horse groups. To date, studies on the effect of recreational horse use in serum Se concentration have not been conducted and therefore the aim of this study was to determine the effect of exploitation of recreation horses in the summer season on the selenium concentration in blood serum and the values of chosen haematological parameters.

MATERIAL AND METHODS

Blood to analyse was sampled from horses used in recreation in two horse riding centres located in the Western Pomeranian Province. Stable 1 (8 horses) is situated in a seaside resort; due to specific location of this centre, horses are used on average for 5–6 hours a day in the holiday season (July–August), with rides being led mostly in a riding arena, less often in the field. Large rotation of riders with different level of riding advancement has been observed, with prevalence of novice riders and horses working on the lunge. Stable 2 (18 horses) is situated in a rural area, far away from large cities; in the holiday season, horses work maximum 4

hours a day, with prevalence of rides of weak and moderate intensity, being led in the field, with riders with average and high level of riding advancement and small rotation of riders. Warm-blood horses, not in foal mares and geldings were selected for the study. The horses were clinically healthy. Horses in both stables were fed a standard, feed originating from the Western Pomeranian Province. The hay was given *ad libitum*, the horses had free access to water. Blood samples were collected from horses in the morning hours, at rest, from the external jugular vein. Blood samples were drawn twice: in the first days of July (start of the holiday season) and in the last days of August (end of the holiday season). Blood was collected into two tubes: without anticoagulant – to determine Se concentration in serum and with K₃EDTA – to determine hematological parameters. In total, 52 blood samples were analysed.

The content of selenium was determined by the spectrofluorimetric method using 2,3-diaminonaphthalene. The Se determination was conducted in accordance with the method described in the earlier paper by Pilarczyk et al. (2010). Accuracy of the analytical method was determined based on the BCR–185R (bovine liver) reference material (European Commission Joint Research Centre Institute for Reference Materials and Measurements-LGC Standards GmbH, Germany). Se concentration was also determined in samples of hay and water from both stable. Chosen haematological parameters were determined using a SYSMEX F800 haematological analyser in accordance with the recommendations of devices's manufacturer. The following was determined in the analysed material: red blood count (RBC), white blood count (WBC) and platelets count (PLT), as well as haemoglobin concentration (HGB) and haematocrit value (HCT). The obtained data were statistically analyzed by means of Statistica 9.0 software package.

RESULTS

As shown in Table 1, average selenium concentration in the blood serum of horses being used recreationally in Stable 1 decreased significantly during the holiday season, amounting on average to $26.56 \mu\text{g} \cdot \text{l}^{-1}$. The Se concentration in the blood serum of horses being used in Stable 2 did not undergo any significant changes during the holiday season, while its mean value proved to be significantly higher than that determined in the blood serum of horses of Stable 1 and amounted to $67.81 \mu\text{g} \cdot \text{l}^{-1}$.

In Table 2 the values of chosen haematological parameters of horses in the riding centres under observations are presented. In the blood of the examined horses, erythrocyte (RBC), leukocyte (WBC) and platelet (PLT) counts, haemoglobin concentration (HGB) and haematocrit (HCT) were determined. As it appears from Table 2, mean values of most analysed haematological parameters of the horses

in both riding centres did not undergo any significant changes during their use in the holiday season. Significant increase in the leukocyte and platelet counts after the holiday season was observed in the blood of horses in Stable 1. The horses of Stable 2 were characterised by significantly higher mean haematocrit value (39.14%) when compared to those in Stable 1. No significant correlations were found between the Se concentration and chosen haematological parameters.

Table 1. Average selenium concentration ($\mu\text{g} \cdot \text{l}^{-1}$) in the blood serum of horses used in recreation in the stables under study

Tabela 1. Średnie stężenie selenu ($\mu\text{g} \cdot \text{l}^{-1}$) w surowicy koni użytkowanych rekreacyjnie w stajniach objętych badaniami

		Mean Średnia	S
Stable 1 Stajnia 1 n = 8	Start of holiday season Początek sezonu wakacyjnego	32.13 x	6.90
	End of holiday season Koniec sezonu wakacyjnego	21.00 x	5.40
	Total Ogółem	26.56 a	8.29
Stable 2 Stajnia 2 n = 18	Start of holiday season Początek sezonu wakacyjnego	64.50	9.95
	End of holiday season Koniec sezonu wakacyjnego	71.11	13.63
	Total Ogółem	67.81 a	12.23

x – mean values between the start and the end of season differ significantly at $P \leq 0.05$.

a – total mean values between stables differ significantly at $P \leq 0.05$.

x – średnie pomiędzy początkiem i końcem sezonu różnią się istotnie przy $P \leq 0,05$.

a – średnie ogółem pomiędzy ośrodkami różnią się istotnie przy $P \leq 0,05$.

DISCUSSION

Western Pomerania has been regarded for a long time to be a region where Se deficiencies are being observed, both in soil and plants and farm and wild animals [Zabłocki 1990, Ramisz et al. 2000, Balicka-Ramisz et al. 2002, Pilarczyk et al. 2009]. There are large differences in the literature in the values of serum Se concentrations, both in the blood serum and the whole blood of horses, with the values determined in the whole blood being higher than the concentrations in the blood serum. According to Puls [1988], the biochemical criteria being applied to diagnose the occurrence of selenium deficiency in the blood serum of horses are as follows: below $0.053 \mu\text{g} \cdot \text{ml}^{-1}$ – deficiency; from 0.054 to $0.12 \mu\text{g} \cdot \text{ml}^{-1}$ – threshold level; above $0.13 \mu\text{g} \cdot \text{ml}^{-1}$ – appropriate level (optimal) for animals. Ramisz et al. [2000], based on the findings of other authors and own studies, propose to consider as an optimal value the Se concentrations above $100 \mu\text{g} \cdot \text{l}^{-1}$, as

Table 2. Mean values for chosen haematological parameters in the horses used in recreation in the stables under study

Tabela 2. Średnie wartości podstawowych parametrów hematologicznych koni użytkowanych rekreacyjnie w stajniach objętych badaniami

		RBC, $\times 10^6 \cdot \mu\text{l}^{-1}$		WBC, $\times 10^3 \cdot \mu\text{l}^{-1}$		HGB, $\text{g} \cdot \text{dl}^{-1}$		HCT, %		PLT, $\times 10^3 \cdot \mu\text{l}^{-1}$	
		Mean Średnio	S	Mean Średnio	S	Mean Średnio	S	Mean Średnio	S	Mean Średnio	S
Stable 1 Stajnia 1 n = 8	Start of holiday season Początek sezonu wakacyjnego	7.94	0.69	5.73 x	1.87	12.88	0.75	37.00	1.80	98.9 x	37.6
	End of holiday season Koniec sezonu wakacyjnego	8.05	0.68	7.90 x	1.90	12.09	0.76	36.60	3.40	563.0 x	217.0
	Total Ogółem	8.00	0.66	6.81	2.14	12.48	0.84	36.80 a	2.60	331.0	283.0
Stable 2 Stajnia 2 n = 18	Start of holiday season Początek sezonu wakacyjnego	8.04	0.50	7.18	1.82	13.11	0.98	38.80	2.80	162.9	49.0
	End of holiday season Koniec sezonu wakacyjnego	8.39	1.10	7.89	1.34	12.76	0.82	39.5	5.20	212.0	86.7
	Total Ogółem	8.22	0.86	7.54	1.61	12.93	0.91	39.14 a	4.15	187.0	73.7

x – mean values between the start and the end of season differ significantly at $P \leq 0.05$.a – total mean values between stables differ significantly at $P \leq 0.05$.x – średnie pomiędzy początkiem i końcem sezonu różnią się istotnie przy $P \leq 0.05$.a – średnie ogółem pomiędzy ośrodkami różnią się istotnie przy $P \leq 0.05$.

threshold values those ranging $50\text{--}100 \mu\text{g} \cdot \text{l}^{-1}$, and as deficiency those below $50 \mu\text{g} \cdot \text{l}^{-1}$. The horses examined by Ramisz et al. [2000] and Balicka-Ramisz et al. [2002], being kept in private farms in Western Pomerania, were characterised by serum Se concentrations ranging $24.7\text{--}27.0 \mu\text{g} \cdot \text{l}^{-1}$. In these farms, foal deaths had been observed, probably due to Se deficiency. Similar values were determined in the present study in the blood serum of horses in Stable 1 (Table 1). These are the values being comparable to the mean value determined in the blood serum of horses from different areas of Poland at a level of $29 \mu\text{g} \cdot \text{l}^{-1}$ [Pilarczyk et al. 2011]. In view of the opinions being mentioned earlier, these values should be considered as deficiency ones. On the other hand, the total Se concentration being determined in the blood serum of horses in Stable 2 proved to be significantly higher than that determined in the horses of Stable 1. This was probably induced by higher Se concentration in the water being provided to the horses of Stable 2

in this time period: $0.025 \mu\text{mol} \cdot \text{l}^{-1}$ (water Se concentration in Stable 1: $0.013 \mu\text{mol} \cdot \text{l}^{-1}$). Also the hay being fed to horses in Stable 2 was characterised by twice as high Se content ($26 \mu\text{g} \cdot \text{kg}^{-1}$) when compared to that in Stable 1 ($0.11 \mu\text{g} \cdot \text{kg}^{-1}$). Higher Se concentration in hay ($53 \mu\text{g} \cdot \text{kg}^{-1}$) was determined by Mihailović et al. [1996], with the Se concentration in the blood serum of horses being fed with it amounting to $73.3 \mu\text{g} \cdot \text{l}^{-1}$, thus being comparable to the mean value determined in the blood serum of horses in Stable 2 (Table 1). In view of the opinions of Ramisz et al. [2000], Se concentrations being determined in the blood serum of horses in Stable 2 (Table 2) should be considered as threshold ones.

The studies on the Se concentration in the blood serum of horses being carried out so far have focused mainly on the effect of supplementation with additives containing Se and vitamin E on the Se concentration and the GSH-Px activity in race horses [Shellow et al. 1985, Mihailović et al. 1996, Avellini et al. 1996, 1999]. Górecka et al. [2002], when determining the Se concentration according to horse breed, age and sex, did not observe any effect of horse gender [Pilarczyk et al. 2011 also didn't notice similar relation] or age or stable on the concentration of this chemical element but found the effect of horse breed. These authors determined the lowest Se concentration in cold-blooded horses ($14.22 \mu\text{g} \cdot \text{l}^{-1}$), while the highest one in Anglo-Arabian horses ($33.18 \mu\text{g} \cdot \text{l}^{-1}$). Balicka-Ramisz et al. (2002), when examining the Se concentration in horses from state stud farms and stallion studs, found that in 74% of mares from stud farms the Se content was below $50 \mu\text{g} \cdot \text{l}^{-1}$, whereas in stallions being kept in stallion studs it ranged 70– $100 \mu\text{g} \cdot \text{l}^{-1}$. It should be emphasised that in view of the suggestion of Ramisz et al. [2000] none of the above mentioned Se concentrations can be considered as optimal ones since they do not exceed $100 \mu\text{g} \cdot \text{l}^{-1}$.

There are no data in literature referring to the effect of similar recreational use of horses in the summer season on the Se concentration in their blood serum. Few studies referred to changes in Zn, Mg, Fe, Cu and Mn concentrations [Bis-Wencel et al. 2002, Cieřła 2009, Ogoński and Cieřła 2009], with their findings not being clear-cut. As it appears from Table 1, the Se concentration during work in the holiday season in the horses being used in Stable 1 significantly decreased, whereas that in Stable 2 did not change. The observations being carried out in both stables show that the horses in the seaside resort are more heavily loaded with work during the summer season, which, perhaps, probably affected the decrease in serum Se concentration. At the same time, a significant increase in the leukocyte and the platelet counts was found in these horses (Table 2) but no significant changes were observed in the RBC, HGB and HCT values. Studies on the effect of horse recreational use on the values of haematological parameters were carried out occasionally and did not give clear-cut results either. This is largely due to different loading of the horses under respective studies with recreational

work. In earlier own studies [Cieřła 2009, Ogoński and Cieřła 2009], a significant decrease in the RBC, HGB and HCT values at rest was found at the end of the holiday season when observing the horses being intensively used recreationally in a seaside resort in the holiday season, which is evidence of horse condition deterioration. In the same studies, an increase in the values of these parameters at rest was observed in the riding centre where horses had been used like in Stable 2, which is, according to many authors, a favourable phenomenon and is evidence of rationally carried out training [Cieřła 2009]. Bis-Wencel et al. [2009] found no effect of recreational and hippotherapy use half-bred horses in the summer season on the WBC, RBC and HGB values. It should be mentioned that the WBC, RBC, HGB and HCT values were in the reference ranges [Winnicka 1997] both in the aforesaid studies and in that being presented in this paper (Table 2).

As can be seen from Table 2, the WBC and the platelet counts significantly increased in the horses in Stable 2 during the holiday season. Bis-Wencel et al. [2004, 2009] did not show any significant effect if recreational work on the WBC count in the blood of the Biłgoraj and half-bred saddle horses. In earlier own studies, the leukocyte count in the half-blood horses working several hours a day in recreation did not undergo any significant changes, with simultaneous decrease in the platelet count [Ogoński and Cieřła 2009]. This is a different result from the observations being made in this study in the horses in Stable 1 (Table 2). Platelets are an extremely important component taking part in many physiological processes, among others in haemostasis and blood coagulation. They are also an important element of the immune system; their reduced amount favours the development of infections. Thrombocytopenia is being observed in intoxications, viral infections with vascular endothelium damage, bone marrow damages as well as may be of auto-immunological background. On the other hand, thrombocytosis accompanies, among others, pneumonias and pleurisy, equine polysaccharide storage myopathy, neoplastic diseases, iron deficiencies and haemorrhages [Winnicka 1997]. Despite the important role of platelets in organism, there are only few papers referring to the platelet count values in the horses being used recreationally, which may make the results presented in Table 2 difficult to interpret. According to Winnicka [1997], the reference range for the platelet count is $150\text{--}400 \times 10^3 \cdot \mu\text{l}^{-1}$. As can be seen from Table 2, mean platelet count in the horses in Stable 1 was not in the given range and did not exceed $100 \times 10^3 \cdot \mu\text{l}^{-1}$ at the start of the holiday season, while significantly increasing to over $500 \times 10^3 \cdot \mu\text{l}^{-1}$ at the end of the holiday season. Low platelet count values, like in the horses of Stable 1 at the start of the holiday season, were also observed in athletic and sedentary Stella Italiano horses: $89\text{--}141 \times 10^3 \cdot \mu\text{l}^{-1}$ [Piccione et al. 2001]. In the study of Cieřła [2009], the platelet count range in the horses used recreationally was definitely broader and amounted in respective animals to 50–1559, with none of the

examined horses showing any external symptoms of disease, like the horses under the present study. In the horses working in Stable 2 (Table 2), mean platelet count did not significantly change and was in the reference range (Winnicka 1997). The platelet counts, similar to those being determined in the horses of Stable 2, were observed by Ogoński et al. (2008) in trotters exposed to 3-month long training programme.

CONCLUSIONS

The observations being carried out confirm the occurrence of Se deficiencies in horses in the Western Pomerania. As it appears from the performed study, intensive recreational horse use (5–6 hours of work per day) affects the significant decrease in serum selenium concentration, which suggests that application of appropriate supplementation in the nutrition of intensively working horses is necessary. Insufficient number of studies and ambiguous results concerning the effect of horse use on the WBC and the platelet counts prompt to undertake more broader observations in this respect.

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WPLYW INTENSYWNOŚCI UŻYTKOWANIA REKREACYJNEGO KONI W SEZONIE WAKACYJNYM NA STĘŻENIE SELENU W SUROWICY I WYBRANE WSKAŹNIKI HEMATOLOGICZNE

Streszczenie. Celem badań było określenie wpływu użytkowania rekreacyjnego koni w sezonie wakacyjnym na stężenie Se w surowicy i wartości wybranych wskaźników hematologicznych. Krew do badań pobrano w dwóch ośrodkach jeździeckich zlokalizowanych na terenie województwa zachodniopomorskiego. Stajnia 1 (8 koni) usytuowana była nad morzem, gdzie konie pracują 5–6 godzin dziennie, głównie na ujeżdżalni, rzadziej w terenie. Obserwuje się dużą rotację jeźdźców o różnym poziomie zaawansowania, z przewagą jeźdźców początkujących. Stajnia 2 (18 koni) usytuowana była na terenach wiejskich, w czasie wakacji letnich konie pracują maksimum cztery godziny dziennie, z przewagą jazdy w terenie o małej i umiarkowanej intensywności, mała rotacja jeźdźców o wysokich i średnich umiejętnościach. Konie żywione były standardowo, paszami pochodzącymi z regionu województwa zachodniopomorskiego. Próby krwi pobrano dwukrotnie: w pierwszych dniach lipca (początek sezonu wakacyjnego) i w ostatnich dniach sierpnia (koniec sezonu wakacyjnego). Średnie stężenie Se w surowicy określone u koni ze stajni 1 obniżyło się istotnie w czasie sezonu wakacyjnego. Średnie wartości większości podstawowych wskaźników hematologicznych koni w obu stajniach nie zmieniły się istotnie podczas sezonu wakacyjnego. Istotny wzrost ilości leukocytów i płytek krwi po sezonie wakacyjnym zaobserwowano w krwi koni ze stajni 1. Konie w stajni 2 charakteryzowały się istotnie wyższym stężeniem hematokrytu w porównaniu z końmi ze stajni 1. Nie stwierdzono istotnych korelacji pomiędzy stężeniem Se a wartościami wskaźników hematologicznych. Przeprowadzone obserwacje potwierdzają występowanie niedoborów Se u koni na Pomorzu Zachodnim. Jak wynika z przeprowadzonych badań, intensywne użytkowanie rekreacyjne koni (5–6 godzin pracy dziennie) wpływa na istotne obniżenie stężenie selenu w surowicy, co sugeruje konieczność zastosowania odpowiedniej suplementacji w żywieniu koni intensywnie pracujących. Niedostateczna ilość badań i niejednoznaczne wyniki odnośnie wpływu użytkowania na wartości WBC i płytek krwi skłaniają do podjęcia szerszych obserwacji w tym zakresie.

Słowa kluczowe: konie, krew, selen, wskaźniki hematologiczne

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