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SERUM CONCENTRATION OF FOLIC ACID IN POLISH KONIK HORSES KEPT IN A STABLE SYSTEM AND A FREE-ROAMING HERD SYSTEM

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ABSTRACT

Folic acid has multi-faceted effects on the body. Its biological activity ensures genome stability and the normal course of the cell cycle. A deficiency of this acid can lead to serious consequences for health and life. Folic acid is the most oxidized form of folates taken with food. Folic acid is not produced in horses and must be ingested with food. Its bioavailability is also affected by environmental factors. The aim of the study was to analyse the content of folic acid in the blood serum of Polish Konik horses kept in a free-roaming herd system and a stable system. The folic acid concentrations in all horses were within the normal range of reference values for the species. Higher folic acid concentrations were noted in the free-roaming horses. Variation in the content of folic acid in the serum of stabled horses was higher than in the free-roaming horses. The management system was not found to significantly affect the serum content of folic acid in the horses.

Key words: Polish Konik horses, folic acid, free-roaming herd system, stable system

INTRODUCTION

The Polish Konik is a primitive horse breed. The breed is extremely valuable, genetically and environmentally, not only because of the primitive traits inherited from its direct ancestors, the tarpans, but mainly because in nature, alongside Przewalski's horse, for representatives of Equus caballus these horses are an invaluable genetic reserve of primitive biological traits, especially those associated with longevity and health [Pasicka 2013]. Polish Konik horses are a valuable genetic reservoir due to their unique inherited constitutional traits and unique ability to adapt to environmental changes, unknown in other horse breeds. These abilities allow them to survive even in extremely difficult living conditions [Pasicka 2010].

Polish Konik horses are raised in three different systems. Reserve system (horses are kept year-round in a natural forest or forest-and-meadow environment, in large areas with valuable natural assets, surrounded by other wildlife, in harems). Stable-free system (horses are kept year-round in large pastures, where under certain unfavourable weather conditions they have access to a shelter or trees for shelter). Stable system (horses are kept in pastures during the pasture season and in the stable during the winter, using paddocks every day). [PZHK 2019]. The distinguishing features of this breed include numerous practical benefits associated with their adaptation to local environmental conditions, e.g. strong herd behaviour [Łuczyńska et al. 2008], modest requirements, rational utilization of poor-quality feed, very good reproductive parameters, a strong constitution, and strong pulling power relative to their body weight [Jaworski 2003]. Years of research have shown that Polish Konik horses are able to accumulate fat reserves during periods of abundant feed, which enables them to survive periods of food shortages in the winter and early spring. Representatives of this breed can live a long time, and mares are highly fertile. An example is the mares from the Popielno reserve: Lalka (21 foals) lived 33 years, Niwa (20 foals) lived 29 years, Nuta (21 foals) lived 27

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years, Ożyna (24 foals) lived 25 years, and the nearly 33-year-old Tarka (25 foals) was the record-holder in terms of fertility in the post-war history of the Polish Konik. Currently, other mares, especially those kept in free-roaming herds, also live a long time in good condition and have very high fertility rates [Jaworski 2003, Jaworski et al. 2015].

Maintenance of optimal health in horses depends on a proper diet that provides adequate amounts of balanced nutrients. These nutrients must be appropriately balanced because either an excess or a deficiency can pose a health threat to horses. Folates are considered to be one of the groups of nutrients that are supplied to horses in inadequate amounts. Folic acid, also called pteroylmonoglutamic acid, is the most oxidized form of folate ingested with food. Horses do not produce it themselves, so it must be supplied with the diet [Czeczot 2008, Babicz and Pastwa 2014].

Natural folic acid is present in the form of folates, which differ from one another in the degree of oxidation of the ring and glutamic acid residues [Czeczot 2008]. They undergo rapid degradation under the influence of factors such as high temperature, low pH, and light. Folic acid is synthesized in negligible amounts in the in the digestive tract by enteric bacteria, so it must be supplied with food. Intestinal enzymes cause it to be reduced to dihydrofolates and then to tetrahydrofolates [Kalemba-Drożdż 2010].

Folic acid has multi-faceted effects on the body [Czeczot 2008]. Its biological activity has indirect and direct effects on cell division and the cell cycle. A deficiency of folic acid can lead to serious health consequences, including cardiovascular and neurodegenerative disease, megaloblastic anaemia, and neural tube defects [McNulty and Pentieva 2004, Czyżewska-Majchrzak and Paradowska 2010]. It is not only important in human nutrition and health, but also essential in animal nutrition and genome functioning. Folic acid belongs to a group of acids that protect genome stability. A folic acid deficiency results in genome instability, which leads to tumours and infertility [Kim 2006, Koziołkiewicz 2009]. Folic acid plays a key role in DNA synthesis and methylation, which are of biological importance in carcinogenesis [Urlich and Potter 2006, Ferguson and Philpott 2008, Rock et al. 2000].

Folic acid deficiencies lead to a reduction in levels of thymine, which is essential for DNA polymerization and repair. This can lead to misincorporation of uracil into DNA in place of thymine. The presence of uracil in DNA results from spontaneous deamination of deoxycytosine. This disturbance has high mutagenic potential, because uracil is located opposite adenine, which during replication can lead to exchange of GC base pairs for AT base pairs in the original DNA molecule. Hundreds of uracil molecules appear in the bodies of vertebrates every day, but when there is a deficiency of folic acid, this number grows to four million [Kalemba-Drożdż 2010, Gętek et al. 2013].

The aim of the study was to analyse the content of folic acid in the blood serum of Polish Konik horses kept in a stable system and in a free-roaming herd system.

MATERIAL AND METHODS

The study was conducted on Polish Konik horses living in Roztocze National Park. Treatment of animals during the experiment was in compliance with national and EU regulations. The analysis was carried out in compliance with Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.

Twenty horses were used in the study, of which 10 were kept in a free-roaming herd system and 10 in a stable system. The analysis was conducted in November 2019. Table 1 presents data on the horses. Blank spaces indicate that data was unavailable. Blood for analysis was drawn from the jugular vein into sterile tubes without an anticoagulant. The sample was centrifuged to separate the serum from the blood cells. The concentration of folic acid in the serum was determined by chemiluminescence in an Immulite 2000 XPi analyser at the Lab-Wet laboratory in Warsaw. Statistical analysis of the results was performed and presented in Tables. The effect of the management system on the serum content of folic acid was assessed by one-way analysis of variance using the following mathematical model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where:

Y_{ij} – trait level,
μ – mean for population,
a_i – effect of i-th level of the factor (management system),
e_{ij} – random error.

The significance of differences between groups was determined by Tukey's test at $P \leq 0.05$.

RESULTS AND DISCUSSION

According to the objective of the experiment, the folic acid level was tested in the serum of Polish Konik horses kept in a free-roaming herd and in a stable system. Table 2 presents detailed results on the folic acid concentrations in the groups of free-roaming and stabled horses. The range of folic acid content in the serum of horses is relatively wide, from 17 to 59 mmol $\cdot$ l^{-1}. The mean folic

Table 1. Horses used in the experiment

No. of individual	Management system	Sex	Date of birth
1	Free-roaming herd	Female	04.2014
2	Free-roaming herd	Female	03.2013
3	Free-roaming herd	Female	04.2014
4	Free-roaming herd	Female	06.2007
5	Free-roaming herd	Female	03.2015
6	Free-roaming herd	Female	04.2012
7	Free-roaming herd	Female	unknown
8	Free-roaming herd	Female	03.2019
9	Free-roaming herd	Female	03.2019
10	Free-roaming herd	Female	03.2019
11	Stable	Female	03.2010
12	Stable	Female	04.2005
13	Stable	Female	03.2013
14	Stable	Female	04.2015
15	Stable	Female	06.2003
16	Stable	Female	03.2018
17	Stable	Female	03.2018
18	Stable	Female	03.2018
19	Stable	Female	unknown
20	Stable	Female	unknown

acid content in the horses was 30.32 mmol · l^{-1}. It was higher in free-roaming horses (30.89 mmol · l^{-1}) than in stabled horses (29.74 mmol · l^{-1}). The level of folic acid ranged from 23.6 mmol · l^{-1} in stabled horse no. 4 to 37.8 mmol · l^{-1} in free-roaming horse no. 4. The serum levels of folic acid of all horses were normal, within the range of reference values for the species. Greater variation in this parameter was found in stabled horses (Sd = 4.7), while the standard deviation in free-roaming horses was 3.35. Table 3 presents the results of the analysis performed to test the effect of the management system on the serum content of folic acid. Analysis of the results showed that the management system had no significant effect ($P \leq 0.05$) on the serum content of folic acid in the horses.

Horses are closely connected with the environment and possess traits characteristic of free-living animals [Dmoch et al. 2008]. It has long been known from many studies that concentrations of nutrients differ in horses of the same breed but living in different conditions [Dmoch et al. 2008]. The average concentrations of folic acid in the serum of horses indicate that free-roaming horses have more of this vitamin than stabled horses. Free-roaming horses make use of pastures, and thus the level of folic acid in the blood is associated with its content in the soil and grassland. Free-roaming horses eat what they find under their feet, and meadows contain many plants with nutritional value and health-promoting properties for horses. Thus natural absorption of folic acid is more beneficial than supplementation with various dietary supplements. This is supported by research by Roberts [1983], who found that physical activity and access to a paddock have a positive effect on the serum content of folic acid. Roberts [1983] determined the concentrations of folic acid and vitamin B12 in horses by radioimmunoassay. The highest serum levels of vitamin B12 and folic acid were found in horses with more physical activity. Physically active horses with access to a paddock, including pregnant and nursing mares, had much higher folate activity in the serum than less active animals [Roberts 1983].

A study on the effect of folic acid on animals was conducted by Hsin-Ling et al. [2019]. They tested the reaction of rats with hepatic cirrhosis to folic acid. Cirrhosis is characterized by increased intrahepatic resistance, vasodilation, and the formation of collateral blood vessels. Left untreated, it can cause complications such as oesophageal bleeding. Higher levels of homocysteine were noted in the rats with cirrhosis. Folic acid reversed the effect of homocysteine, alleviating the adverse effects of

Table 2. Folic acid concentration (mmol · l^{-1}) in the serum of Polish Konik horses

Free-roaming horses		Stabled horses	
1	27.4	1	27.2
2	30.6	2	24.2
3	30.4	3	29.0
4	37.8	4	23.6
5	27.4	5	31.7
6	28.1	6	37.4
7	34.7	7	30.1
8	32.2	8	32.6
9	31.5	9	35.8
10	28.8	10	25.8
Mean for groups			
30.89		29.74	
Mean for all horses			
30.32			
Standard deviation for groups			
3.35		4.70	
Standard deviation for all horses			
4.01			
Coefficient of variation for groups			
10%		15%	

Table 3. Analysis of the effect of the management system on the content of folic acid (mmol · l^{-1}) in the serum of Polish Konik horses

Management system	Mean	Standard deviation
Free-roaming herd	30.89[a]	3.35
Stable	29.74[a]	4.70
Total	30.32	4.01

Means with the same superscript letter (a) are not significantly different at $p \leq 0.05$.

the high homocysteine level in rats with cirrhosis [Hsin-Ling et al. 2019].

Research by Zehra and Khan [2020] assessed the need for folic acid in fish of the species Corydoras punctatus by measuring their growth parameters and the concentration of folic acid added to their feed. They used 7 doses of folic acid (0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg · kg^{-1} of feed). Folic acid concentrations from 0.75 to 1.0 mg · kg^{-1} were determined to be essential for the optimal growth of Corydoras punctatus [Zehra and Khan 2020].

Diet is one of the most important factors affecting health. Feedstuffs for animals may contain various substances with both beneficial and adverse effects on the health and functioning of the body. These include folic acid [Czyżewska-Majchrzak and Paradowska 2010]. Increasing attention is paid to genetic predispositions, including individual needs for folic acid resulting from single nucleotide polymorphism in certain genes [Czyżewska-Majchrzak and Paradowska 2010]. Determination of the optimum concentration of folic acid to preserve genome stability is an important task for nutrigenetics. It is becoming clear that information in DNA can undergo changes for which diet is largely responsible. Bioactive substances transfer information from external environments and have a quantitative and qualitative influence on gene expression. Most studies are conducted in vivo on model tumour cells. However, the relation-

ships between metabolic and signalling pathways and cellular and tissue specificity should be taken into account [Pieszka and Pietras 2010]. It remains unclear whether the potential harmful effects of a high level of folic acid outweigh the known and potential benefits. Moreover, this balance many vary between individuals in a population [Koziołkiewicz 2009].

SUMMARY

Appropriate intake of individual vitamins is important for health in general, normal parturition and lactation, the postpartum period in horses, the development and maturation of foals, mammary gland health, and maintaining good hoof condition. In economic terms, rational supply of vitamins makes it possible to minimize losses caused by metabolic disorders and treatment costs. Vitamin supplementation is necessary in the case of deficiencies, but also for prevention, especially in animals with higher levels of physical exertion (sport or work), which have much higher nutrient requirements. Such dietary supplementation involves administration of suitable vitamin preparations, which may be additionally enriched with macro- and microelements. Cases of effects of excessive use of these preparations are rare. Prevention of health problems resulting from vitamin deficiencies is ascribed to the increasing knowledge of breeders. To sum up, folic acid is used both in prevention and to address the effects of deficiencies, particularly at times of greater demand for this nutrient, such as late pregnancy, the peripartum period, and lactation. It is also used in animals that are susceptible to disease, frequently or continuously if there are visible signs of illness, such as poor body condition. In each case, deficiencies of folic acid and B vitamins lead to consequences in the functioning of animals and thus to economic losses. Rational diet supplementation with vitamins ensures animals' health and welfare and at the same time increases the benefits for the breeder. While all vitamins are important for vital processes, the most important role is often ascribed to supplementation with vitamins that must be supplied externally and cannot be produced by the animal's body.

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STĘŻENIE KWASU FOLIOWEGO W SUROWICY KRWI KONIKÓW POLSKICH UTRZYMYWANYCH W SYSTEMIE TABUNOWYM I STAJENNYM

STRESZCZENIE

Kwas foliowy ma wielokierunkowe oddziaływanie na organizm. Jego aktywność biologiczna wpływa na stabilność genomu i prawidłowy przebieg cyklu komórkowego. Niedobór tego kwasu może prowadzić do poważnych konsekwencji zdrowia i życia. Kwas foliowy to najbardziej utleniona forma folianów, przyjmowanych z pożywieniem. Organizm koni nie wytwarza ich samodzielne, przez co muszą być dostarczane wraz z pokarmem. Czynniki środowiskowe mają również wpływ na jego przyswajalność. Celem pracy była analiza zawartości kwasu foliowego w surowicy krwi koników polskich utrzymywanych w systemie tabunowym i stajennym. Wartości stężenia kwasu foliowego u wszystkich koni były na poziomie prawidłowym mieszczącym się w zakresie wartości referencyjnych dla tego gatunku. Stwierdzono wyższe stężenie kwasu foliowego w surowicy koni tabunowych. Zmienność zawartości kwasu foliowego w surowicy krwi koni stajennych była wyższa, niż u koni tabunowych. Stwierdzono brak istotności wpływu systemu utrzymania na zawartość kwasu foliowego w surowicy krwi koni.

Słowa kluczowe: konik polski, kwas foliowy, system tabunowy, system stajenny

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