Ukrainian Journal of
Veterinary and Agricultural Sciences
http://ujvas.com.ua

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

Volume 1
Number 2

original article | UDC 619:636.1:616-07:577.118:613.3 | **doi: 10.32718/ujvas1-2.03**

Hypocobaltosis and hypocuprosis in pregnant mares in the western biogeochemical zone of Ukraine (distribution, diagnosis)

A.R. Shcherbatyy, L.G. Slivinska, B.O. Lukashchuk

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Pekarska Str., 50, Lviv, 79010, Ukraine

Article info
Received 10.09.2018
Received in revised form 15.10.2018
Accepted 16.10.2018

Correspondence author
Andriy Shcherbatyy
Tel.: +38-097-758-07-19
E-mail: ua-andrea@ukr.net

Contents
1. Introduction 11
2. Materials and methods 12
3. Results and discussion 12
4. Conclusions 14
References 14

Abstract

The article presents theoretical and experimental substantiation of relationship between the content of trace elements in the soil, water, feed and blood of mare with hypocobaltosis and hypocuprosis in biogeochemical province of Transcarpathia. The total content of Zn in soils is 50.96 mg/kg, Cu – 16.32, Co – 7.14, and Mn – 493.97 mg/kg. It was established decrease in the concentration of mobile forms of Cu and Co (0.01mg /kg), Cr (1.93), Pb (1.66), Ni (0.67), Zn (1.42). Also, in water from artesian wells of research farm it was established decreased content of Co (by 3.3 times), Cu (111), Zn (1.19), Mn (1.31), Cd (5.0 times) and on the contrary increased content of Pl by 5.6, Ni – 3.3 and Fe – 43.5 times. Providing of mares by Co is 54.7%, Cu – 88.5, Zn – 83.3, Fe – 88.4, Mn by 29%. The negative effect of Cobalt and Copper deficiency on erythrocytopoesis is shown. In particular, the number of erythrocytes, hemoglobin level, hematocrit value, and hemoglobin content in the erythrocyte indicate a development of alimentary anemia in pregnant mares. The established low level of Co and Cu that indicates the development of polymicroelementosis in mares. The Cobalt content in blood of non-pregnant mares was on the lower physiological limit and on average it was 0.48 ± 0.02 μmol/l. In the mares, at 7 month of pregnancy, the Cobalt content was lower ($P < 0.01$) than that of non-pegnant. In the 9–11 months of pregnancy, its level was on average 0.19 ± 0.02 μmol/l, which is lower by 2.5 and 1.9 times than in non-pregnant mares ($P < 0.001$) and at 4 months of pregnancy ($P < 0.05$). The Copper content in blood of mares at 9–11 months of pregnancy was lowest and the average was 2.09 ± 0.07 μmol/l (1.98–2.27), which is lower on 46.8 and 22.6% ($P < 0.001$) compared to non-pregnant and mares at 4 month of pregnancy. Hypocopperemia was established in 100% of studied mares. In non-pregnant mares, the Copper content was on average 3.93 ± 0.14 μmol/l. Taking into account the role of these microelements in metabolic transformations, changes in the composition of blood and the development of metabolism disorders in the organism of mares during the period of pregnancy can be explained.

Key words: mares, pregnancy, trace elements, ration, soil, water, blood, erythrocytopoiesis, cobalt, copper.

Citation:
Shcherbatyy, A.R., Slivinska, L.G., & Lukashchuk, B.O. (2018). Hypocobaltosis and hypocuprosis in pregnant mares in the western biogeochemical zone of Ukraine (distribution, diagnosis). *Ukrainian Journal of Veterinary and Agricultural Sciences*, 1(2), 11–14.

1. Introduction

Constant microelemental insufficiency in animals often occurs in biogeochemical provinces for insufficient income of certain trace elements in organism (absolute microelemental insufficiency). Inconstant (short-term) microelemental insufficiency develops in animals not only in biogeochemical provinces, but may occur as a result to increased need of the body in them for pregnancy, high lactation, and physical stress (Shcherbatyi and Slivinska, 2016).

In farms, microelementosis is not clinically manifested in all animals. It depends on many factors and, above all, on the individual adaptability of animals to the environment (Shcherbatyi and Slivinska, 2016). It should also be taken into account that high-performance animals are more susceptible to lack or excessive amounts of trace elements, and the clinical symptoms of microelementosis are better expressed in them than in unproductive and adult animals (Shcherbatyy et al., 2017).

The diagnosis of microelementosis is based on the general principles: taking into account peculiarities of the biogeochemical zone, the content of trace elements in soils, water, feed and blood of animals. Among the factors that influence on full feeding of farm animals, an important place belongs to macro- and microelements that are involved in energy, protein, carbohydrate and lipid metabolism, are part of the tissues and organs, are components of blood, a number of hormones and enzymes (Shcherbatyi and Slivinska, 2016; Shcherbatyy et al., 2017; Gutyj et al., 2017).

Mares during the period of pregnancy are very sensitive to malnutrition, conditions of retention and their use. Particularly it concerns the feeding of mares, as in the time of pregnancy the needs of animals in biologically active substances are increasing, and in their organism there are changes in all aspects of metabolism (Shcherbatyi et al., 2010; Slivinska, et al., 2012; Golovakha et al., 2017).

Disorder of mineral metabolism is more often manifested in the form of polypathology, since the manifestation of deficiency of an element is rare. The soils and water springs of the western zone, which includes the Transcarpathian region, are depleted on moving forms of Cobalt, Zinc, Copper and Mangan (Shcherbatyi, 2012). The deficiency of these elements is the most important etiological factor in microelementosis of horses. For pregnant mares, this question becomes of particular importance, since in the last trimester intensively develops 60–65% of fetal tissues. At the same time, the need for mares in nutritious and biologically active substances increases by 20%, and in pregnant mares, during the winter-spring period, by 40–50% (Beythien et al., 2017).

Therefore, the development of early diagnostic methods of microelementosis in mares, at present, is very relevant and needs to be addressed.

2. Materials and methods

The research was conducted at the Scientific-Production Association "Plemekonecenter" (Transcarpathian region). The objects of research were pregnant and non-pregnant mares of Hutsul breed, aged 4–18 years old, with a weight of 400–450 kg. Material of the study was blood. All the mare were in the same conditions of retention and feeding. A daily ration of mares included (kg): hay of cultivated hayfields – 2.5. high altitude hay – 2, meadow hay – 2.5, wheat bran – 0.5, corn bran – 1, oat grain – 1, sunflower oil – 0.5, dry pulp, granulated – 1. Each mares drank about 30.3 ± 0.21 liters of water per day, which is extracted from an artesian well.

Clinical examination of mares and blood tests were performed according to generally accepted techniques (Vlizlo et al., 2012).

The content of Copper and Cobalt in the blood of mares was determined by atomic absorption spectrophotometry method on apparatus AAS-30.

Water and soils research on the content of trace elements was determined by the method of emission spectral analysis on coupled spectrographs using the three standards with decoding at the recording microfotometer; determination of ferrum – for B. Price and X-ray fluorescence analysis. Feeds were determined for the content of trace elements and nutrition according to the methods outlined in the manual (Vudmaska and Prylutskyi, 1975).

In conducting experimental studies, all bioethical norms were observed in relation to animals that meet the requirements of the Law of Ukraine "On the protection of animals from cruel treatment" and European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Official Journal of the European Union).

3. Results and discussion

Taking into account the peculiarities of the Western biogeochemical zone, characterized by a lack of essential microelements such as Iodine, Zink, Cobalt, Copper, Mangan, Selenium, we conducted a series of researches aimed at detecting microelementosis (Co, Cu) in the mares of Hutsul breed in the biogeochemical province of Transcarpathia. Our studies were conducted according to the theory of V.I. Vernadskyy: soil – plant – animal.

The analysis of the soils of Scientific-Production Association "Plemekonecenter" shows that the total content of Zinc is 50.96 mg/kg, Copper – 16.32, Cobalt – 7.14, and Mangan – 493.97 mg/kg. At the same time was established decrease in the concentration of mobile forms of Cu and Co (0.01 mg/kg), Cr (1.93), Pb (1.66), Ni (0.67), Zn (1.42) (Slivinska et al., 2012).

An important source of trace elements for animals is water, but water sources in the Transcarpathian region are impoverished due to the content of trace elements in them. We established that in water from artesian wells of research farm it was established decreased content of Cobalt (by 3.3 times), Copper (111), Zinc (1.19), Mangan (1.31), Cadmium (5.0 times) and on the contrary increased content of Plumbum by 5.6, Nickel – 3.3 and Ferrum – 43.5 times (Shcherbatyi, 2012).

Consequently, the low content of mobile forms of trace elements in the soil and water of the biogeochemical province gives grounds to argue about the probability of a lack of microelements in feeds.

The analysis of the ration showed that the Providing of mares by Cobalt is 54.7%, Copper – 88.5, Zinc – 83.3, Ferrum – 88.4, Mangan by only 29%, which is the basis for the development of microelementosis in mares.

The revealed deficiency of essential microelements in feed requires addition of relevant biologically active substances to animal's ration, which will be aimed at eliminating their imbalance and metabolic disorders in animals (Gutyj et al., 2017).

The methodological basis for the prevention of these diseases is clinical examination. At the time of clinical examination, 82 horses of the Hutsul breed were kept in the "Plemekonecenter", of which 40 were mares, 9 stallions, and the rest – young animals under 2 years of age (Shcherbatyi et al., 2010).

Clinical examination has been established that 57.5% of mares have satisfactory fattening, medium body structure, and dense constitution. They also had dim hair cover, dry and low elasticity skin. The highest number of such animals was found among the mares at 9–11 months of pregnancy (50%). The body temperature in 80% of the studied mares was within the normal range (37.5–38.5 °C), hypothermia was established in 20% of mares. In 32.5% of the studied mares, visible mucous membranes (conjunctiva, nose, mouth) are anemic, moderately moist and without swelling. The highest number of mares with pallor of mucous membranes was at 9–11 months of pregnancy (40%). In the area of mane, neck, trunk, on limbs and around the eyes was detected alopecia. The pulse rate in 87.5% of the experimental mares fluctuated within the normal range (24–42 beats per minute). In 17.5% of the mares was established tachypnoea.

There was decrease in appetite and distortion of taste, which is a characteristic indicator of mineral deficiency (osteodystrophy, hypocobaltosis and hypocuprosis). In 20% of the mares was observed lameness during movement and incorrect position of the limbs. In 40% of the mares was detected damage of hoof's integrity.

The most pronounced disturbances of the bone resisting apparatus were observed in the mares at 9–11 months of pregnancy, which may be due to the more intensive use of minerals for development of the fetus or development of microelementosis in pregnant mares.

A group of 40 mares was formed to determine the state of erythrocytopoiesis and biochemical status of the pregnant mares were kept in the "Plemekonecenter". The dispensary group included 10 non-pregnant mares, 5 mares at 4 month of pregnancy, 5 mares – at 7 and 20 mares – at 9–11 months of pregnancy.

The content of hemoglobin in blood of mares at 9–11 months of pregnancy was in the range 80.0–94.0 g/l (86.4 ± 1.22) and was lower by 28.0% ($P < 0.001$) than in non-pregnant (120.0 ± 4.7 g/l), and at 7 month ($P < 0.01$) and tended to decrease compared to mares at 4 month of pregnancy (Table 1).

Table 1
Hematological parameters in mares (Lim, M ± m)

Parameter	Non-pregnant, n = 10	Pregnant		
		at 4 month, n = 5	at 7 month, n = 5	at 9–11 months, n = 20
Hemoglobin, g/l	105.0–147.0 120.0 ± 4.70	85.0–95.0 91.2 ± 2.15 P < 0.001	82.0–101.1 91.3 ± 1.20 P < 0.001	80.0–94.0 86.4 ± 1.12 P < 0.001 P_1 < 0.01
Erythrocytes, T/l	5.9–8.3 7.3 ± 0.25	5.3–6.6 5.9 ± 0.23 P < 0.001	4.8–5.9 5.3 ± 0.18 P < 0.001	3.7–6.7 5.1 ± 0.28 P < 0.001
Mean corpuscular hemoglobin (MCH), pg	15.8–18.2 16.7 ± 0.29	15.5–19.0 17.2 ± 0.85	15.6–17.5 16.7 ± 0.30	14.2–18.2 16.1 ± 0.24
Hematocrit, l/l	0.28–0.39 0.33 ± 0.03	0.28–0.39 0.32 ± 0.07	0.19–0.27 0.24 ± 0.01 P < 0.05	0.16–0.29 0.24 ± 0.02 P < 0.05
Mean corpuscular volume, (MCV), fl	41.2–67.9 48.5 ± 1.59	41.4–52.4 46.9 ± 2.19	41.2–47.8 45.6 ± 1.18	41.2–46.2 43.2 ± 0.32 P < 0.01
ESR, mm/hr	51.0–61.0 56.4 ± 1.02	50.0–60.0 53.8 ± 1.85	36.0–62.0 52.4 ± 1.51	43.0–58.0 48.2 ± 2.71 P < 0.01
Leukocytes, G/l	6.4–8.4 7.1 ± 0.42	6.5–7.7 6.9 ± 0.35	6.5–8.3 7.2 ± 0.20	6.1–8.4 6.8 ± 0.47

Note: $P < 0.05$; $P < 0.01$; $P < 0.001$ – compared to non-pregnant mares
$P_1 < 0.01$ – compared to mares at 7 month of pregnancy

According to the results of research hemopoiesis parameters, it was established that number of erythrocytes in blood of non-pregnant mares on average it was 7.3 ± 0.25 T/l. In the mares at 4 month of pregnancy, their numbers were lower by 19.2% ($P < 0.001$) than average in non-pregnant. The number of erythrocytes in mares at 7 month continued to decrease, by 27.4% and 10.2%, respectively, compared to non-pregnant and mares at 4 month of pregnancy. In 80% of mares at 9–11 months of pregnancy was established oligocythemia, and the number of erythrocytes averaged 5.1 ± 0.28 T/l. By the content of hemoglobin, it can be interpreted that anemia established in 65% of mares at 9–11 months of pregnancy. As the results of our studies have shown, in 100% of mares at 7 and 9–11 months, the amount of hematocrit has been reduced. The results of our research showed that in 100% of mares at 7 and 9–11 months lowered hematocrit. On average, it is by 27.3 and 27.3% lower in mares at 7 (0.24 ± 0.01 l/l) and 9–11 (0.24 ± 0.02 l/l) months of pregnancy than in non-pregnant mares ($P < 0.05$).

In mares at 9–11 months of pregnancy, the mean corpuscular volume was in range from 41.2 to 46.2 (norm is 50–58 fl), which is by 10.9% ($P < 0.01$) lower than in non-pregnant mares, and there was a tendency towards its decrease (by 7.9 and 5.3%) compared to parameters of mares at 4 and 7 months of pregnancy.

Thus, the number of erythrocytes, hemoglobin content, hematocrit, mean corpuscular hemoglobin and mean corpuscular volume indicate the development of alimentary anemia in pregnant mares, which is evidently due to the lack of Cobalt, Copper, Mangan and Ferrum (Wood, 2004; Sessions-Bresnahan et al., 2018).

Taking into account the specific peculiarities of western region of Ukraine, we determined Co and Cu content in the blood of mares, since they are the most important in erythrocytopoiesis (Hoffman et al., 2003; Maksymovych et al., 2017).

The content of Cobalt in the blood of non-pregnant mares was on the lower physiological limit, which averaged 0.48 ± 0.02 μmol/l (Table 2).

In 20% of mares is established a decrease in its content. In the blood of pregnant mares, there there was a tendency to decrease the content of Cobalt as increase the duration of pregnancy. In mares, at 7 month of pregnancy the Cobalt content was was lower ($P < 0.01$) than of non-pregnant. At 9–11 months of pregnancy, its level was on average 0.19 ± 0.02 μmol/l (0.12–0.22), which is less in 2.5 and 1.9 times than in non-pregnant ($P < 0.001$) and mares at 4 month of pregnancy ($P < 0.05$).

It should be noted that the content of Copper in blood of mares at 9–11 months of pregnancy was lowest and the average was 2.09 ± 0.07 μmol/l (1.98–2.27), which is by 46.8 and 22.6% ($P < 0.001$) less compared to non-pregnant and mares at 4 month of pregnancy. In mares at 7 months, the content of Copper was also low – hypocopperemia was established in 100% of studied mares. In non-pregnant mares, the Copper content was on average 3.93 ± 0.14 μmol/l and was on the lower limit of physiological norm (3.5–7.1 μmol/l).

Table 2
The content of Cobalt and Copper in the blood of mares (Lim, M ± m)

Parameter	Non-pregnant, n = 10	Pregnant		
		at 4 month, n = 5	at 7 month, n = 5	at 9–11 months, n = 20
Co, µmol/l	0.41–0.58 0.48 ± 0.02	0.30–0.42 0.36 ± 0.08	0.18–0.32 0.23 ± 0.07 P < 0.01	0.12–0.22 0.19 ± 0.02 P < 0.001 P_1 < 0.05
Cu, µmol/l	3.15–4.51 3.93 ± 0.14	1.45–3.50 2.7 ± 0.12 P < 0.001	1.98–2.50 2.26 ± 0.11 P < 0.001 P_1 < 0.05	1.98–2.27 2.09 ± 0.07 P < 0.001 P_1 < 0.01

Note: P < 0.01; P < 0.001 – mares at 9–11 months of pregnancy compared to non-pregnant
P_1 < 0.05; P_1 < 0.01 – compared to mares at 4 month of pregnancy

The established low content of Co and Cu indicates the development of polymicroelementosis in mares. Taking into account the role of these microelements in metabolic transformations, can be explained changes in blood composition and the development of metabolic disorders in the mare's organism during the period of pregnancy (Muñoz et al., 2012; Padalino et al., 2017).

4. Conclusions

1. The main reasons for the development of microelementosis in mares are low content in soil of Cobalt, Zinc, Manganese and Zinc, in water sources of Copper, Cobalt, Zinc, Mangan for the excess of Ferrum, Plumbum and Chromium, and low supply of essential microelements in feed of ration.

2. Clinical criteria for hypocobaltosis and hypocuprosis are decreased appetite, taste distortion, pallor of visible mucous membranes, dim hair cover with alopecia, low elastic skin, disorders of cardiovascular and digestive functions.

3. Laboratory diagnostic criteria for hypocobaltosis and hypocuprosis in mares are: low blood content of Cobalt (0.12–0.42 µmol/l) and Copper (1.98–3.0 µmol/l), oligocythemia (3.7–5.9 T/l), oligochromemia (80.0–88.0 g/l) and hypochromia (14.2–16.7 pg). The content of Cobalt and Copper in the blood of mares decreases as increase the duration of pregnancy and is on average: at 4 month – 0.36 ± 0.08 and 2.7 ± 0.12; at 7 month – 0.23 ± 0.07 and 2.26 ± 0.11 (P < 0.01), at 9–11 months – 0.19 ± 0.02 and 2.09 ± 0.07 (P < 0.01).

References

Beythien, E., Wulf, M., Ille, N., Aurich, J., & Aurich, C. (2017). Effects of sex, pregnancy and season on insulin secretion and carbohydrate metabolism in horses. *Anim Reprod Sci.*, 184, 86–93. doi: 10.1016/j.anireprosci.2017.07.002.

Golovakha, V., PiddubnyakO., Sliusarenko, S., Slivinska, L., Maksymovych, I., Shcherbatyy, A., & Gutyj, B. (2017). Acid resistance and population structure of erythrocytes in trotter horses during and after exercise. *Regulatory Mechanisms in Biosystems*, *8*(4), 623–627. doi: 10.15421/021795.

Gutyj, B., Nazaruk, N., Levkivska, A., Shcherbatyj, A., Sobolev, A., Vavrysevych, J., Hachak, Y., Bilyk, O., Vishchur, V., & Guta, Z. (2017). The influence of nitrate and cadmium load on protein and nitric metabolism in young cattle. *Ukrainian Journal of Ecology*, 7(2), 9–13. doi: 10.15421/201714.

Hoffman, R.M., Kronfeld, D.S., Cooper, W.L., & Harris, P.A. (2003). Glucose clearance in grazing mares is affected by diet, pregnancy, and lactation. *J Anim Sci.*, 81(7), 1764–1771. doi: 10.2527/2003.8171764x.

Maksymovych, I.A., Slivinska, L.H., & Shcherbatyi, A.R. (2017). Pokaznyky hemopoezu v robochykh konei. The Institute of animal science, 117, 152–162 (in Ukrainian).

Muñoz, A., Riber, C., Trigo, P., & Castejón, F. (2012). Age- and genderrelated variations in hematology, clinical biochemistry, and hormones in Spanish fillies and colts. *Research in Veterinary Science*, 93(2), 943–949. doi: 10.1016/j.rvsc.2011.11.009.

Padalino, B., Raidal, S., Carter, N., et al. (2017). Immunological, clinical, haematological and oxidative responses to long distance transportation in horses. *Research in Veterinary Science*, 115, 78–87. doi: 10.1016/j.rvsc.2017.01.024.

Sessions-Bresnahan, D.R., Heuberger, AL., & Carnevale, E.M. (2018). Obesity in mares promotes uterine inflammation and alters embryo lipid fingerprints and homeostasis. *Biol Reprod.*, 99(4), 761–772. doi: 10.1093/biolre/ioy107.

Shcherbatyi, A.R. (2012). Analiz mikroelementnoho skladu gruntiv i vody v systemi dyspanseryzatsii kobyl hutsulskoi porody. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series "Veterinary Sciences"*, 14, 2(52), 368–372. http://nbuv.gov.ua/UJRN/nvlnu_2012_14_2%281%29__74 (in Ukrainian).

Shcherbatyi, A.R., & Slivinska, L.H. (2016). Klinichnyi status kobyl hutsulskoi porody za dyspanseryzatsii. Scientific *Messenger of LNU of Veterinary Medicine and Biotechnologies. Series "Veterinary Sciences"*, 18, 1(65), 199–204. https://nvlvet.com.ua/index.php/journal/article/view/73 (in Ukrainian).

Shcherbatyi, A.R., Slivinska, L.H., & Drachuk, A.O. (2010). Rezultaty dyspanseryzatsii kobyl hutsulskoi porody. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series "Veterinary Sciences"*, 12, 3(45), 313–318 (in Ukrainian).

Shcherbatyy, A., Slivinska, L., Gutyj, B., Golovakha, V., Piddubnyak, O., & Fedorovuch, V. (2017). The influence of a mineral-vitamin premix on the metabolism of pregnant horses with microelemetosis. *Regulatory Mechanisms in Biosystems*, 8(2), 293–398. doi:10.15421/021746.

Slivinska, L.H., Shcherbatyi, L.H., & Demydiuk, S.K. (2012). Analiz mineralnoho skladu kormiv i ratsionu hodivli kobyl. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series "Veterinary Sciences"*, 14, 3(53), 258–263. http://nbuv.gov.ua/UJRN/nvlnu_2012_14_3%281%29__49 (in Ukrainian).

Vlizlo, V.V., Fedorchuk, R.S., Ratych, I.B. et al. (2012). Laboratorni metody doslidzhen' u biologii', tvarynnyctvi ta veterynarnij medycyni: dovidnyk. L'viv, SPOLOM (in Ukrainian).

Vudmaska, V.Iu., & Prylutskyi, P.P. (1975). Vyznachennia pozhyvnosti i yakosti kormiv u hospodarstvi. K.: Urozhai (in Ukrainian).

Wood, D.R. (2004). Veterinary Laboratory Medicine, Interpretation and Diagnosis, 3rd edition. *Veterinary Clinical Pathology*, 33(3), 182–182. doi: 10.1111/j.1939-165X.2004.tb00372.x.