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ORIGINAL ARTICLE

The occurrence of osteodystrophy in cows with chronic micronutrients deficiency

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The article deals with the results of the microelements content in the soil-feed-animal system. The analysis of farm soil 1 showed that the total cobalt content was 6.8; copper – 34.7; zinc – 25.4; manganese – 70.6; iron – 198.2 mg/kg. The content of mobile forms of copper and cobalt in soils was 8.5 and 3.6 mg/kg. In farms 2 and 3, the total cobalt content was 6.4 and 5.9; copper – 33.9 and 23.4; zinc – 27.9 and 24.8; manganese – 83.3 and 215.8; iron – 179.4 and 145.9 mg/kg, respectively. The content of mobile forms of microelements in soils has been reduced, with the exception of copper, lead and nickel. Thus, the results of searches of the microelement composition of the soil in the experimental farms, found their impoverishment on cobalt, zinc and manganese. Cadmium is present in all tests of soil samples. Analysis of the fodder of all three farms showed that the copper content was less in the beets, in cereals and silage it is established as its deficiency, and excess, in straw and hay – excess. Cobalt was less in fodder beets, hay, straw and cereals, except in wheat and silage. More manganese was found in beet, hay, straw, less in cereals. Less zinc was in the hay and cereals, more – in silo and straw. The iron content was less in hay, silage, barley and rye, but more – in beet, straw and oats. The analysis of rations showed that the supply of macroelements (calcium and phosphorus) was insufficient (62.0–78.2 and 49.4–57.4%). Along with all rations there was an excess of magnesium (126.9–183.2%) and potassium (186.9–188.0%). The provision of the ration with cobalt and zinc was 25.7–71.8 and 33.6–75.1%, with copper – 21.2–116.1%. In 1 kg of feed dry matter was a low content of cobalt – 0.15–0.29 mg (optimal 0.5–0.7 mg), zinc – 13.6–21.5 (40 mg), manganese – 21.3–51.8 (40 mg), excessive copper – 8–8.7 mg (7 mg), iron – 146.5 – 268.9 mg/kg, with the optimal 60 mg. In feed rations there is an insufficient amount of vitamin D (8.9–14.1% of demand). Subclinical course of osteodystrophy in cows is manifested by loss of shine and ruffling of the hairline, moulting, decreased skin elasticity, alotriophagia, hyporexia, anemia of the conjunctiva; in blood – a decrease in cobalt up to 0.24 ± 0.01 μmol/l, copper – up to 13.4 ± 0.11, manganese – 2.1 ± 0.03, zinc – 14.5 ± 0.13 μmol/l (15–23 μmol/l). In addition to the changes described above, thinning and partial lysis of the last pair of ribs were observed, resorption of the last 2–3 caudal vertebrae, partial deformation of the spine, convexity of the ribs, overgrowth and deformation of the hoof horns. In cows with subclinical course of osteodystrophy, the total calcium content was reduced in 18.9% (2.1–2.9 mmol/l, 2.4 ± 0.02), phosphorus in 5.7% of animals (1,2–2.2 mmol/l, 1.7 ± 0.02). Sick cows with hypocalcemia were diagnosed in 95.0% (1.95–2.40 mmol/L, 2.2 ± 0.05), which was combined with hypophosphatemia in 35.0% of cows (1.1–1.9 mmol/l 1.6 ± 0.07). So, found that the main causes of osteodystrophy in barn feeding cows were a low content of vitamin D, calcium, phosphorus, and also cobalt and zinc in rations.
Key words: soil; feed; ration; cobalt; zinc; iron; manganese; biogeochemical zone

Introduction

The creation of modern dairy farms with a year-round barn feeding of cows leads to the development of internal non-communicable diseases, which are often associated with a metabolic disorder (Beattie and Avenel, 1992; Caldow et al., 1995). A special place among metabolic diseases is occupied by osteodystrophy, which is considered to be the most common disease of the barn feeding period of cows (Liesegang et al., 1998, Liesegang et al., 2003).

Diagnosis of osteodystrophy and the study of pathological conditions associated with it has an academic and practical significance. The search begins with its diagnosis. Osteodystrophic processes are mainly differentiated according to subjective data (for example, symptoms, not always specific ones), in some way inhibits the early diagnosis of the disease (Klitsenko et al., 2001; Beattie and Avenel, 1992). All this is holding back the development and practical use of scientific achievements about the characteristics of osteodystrophic processes and secondary lesions caused by the organs and systems of the animal body, which are often characterized as completely separate and independent of osteodystrophy.

In many searches, it was found that the deficiency or imbalance in soil, water, feed and micronutrient rations that actively participate in osteogenesis (Co, Se, J, Cu, Mn, Zn, etc.) contributes to the development of metabolic bone pathology in cows – osteodystrophy (Beattie and Avenel, 1992, Ytrehus et al., 1999). The Lviv region is one of the localities that belongs to the western biogeochemical zone of Ukraine with deficiency and imbalance of macroelements and especially microelements in soil and plants. In this zone, animals throughout the territory do not have enough iodine, selenium and cobalt, in most areas of manganese, zinc, and in some places iron, chromium, molybdenum and copper (Telehuz, 2011; Kabata-Pendias, 2011). The above factors cause a permanent shortage of the required amount of all bioelements. In fact, in many farms the demand for individual macro- and microelements is satisfied by no more than 50% (Doubek et al., 1989; Beattie and Avenel, 1992; Klitsenko et al., 2001). Under such conditions it is difficult to count on high productivity, reproducible ability, safety, adequate functioning of the immune and other body systems

Many questions of the etiology, pathogenesis, diagnosis, prevention and treatment of cyst osteodystrophy require clarification, supplementation and new development of its prevention and treatment (Liesegang et al., 1998). The answer to these questions requires complex studies in the system: feed-animal-metabolism, as well as studies aimed at correcting diets with macro- and micronutrient deficiencies (Underwood and Suttle, 2001, Gutyj et al., 2017).

Concerning, it is important to search the etiological role of microelements in osteodystrophy of cows, which will allow solving the issue of its effective prophylaxis.

Materials and methods

The work was performed at the Department of Internal Animal Diseases and Clinical Diagnostics of Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, three farms of the Lviv region – No1, 2, and 3 and in the conditions of scientific research laboratories of other scientific institutions. The experimental part of the work was carried out in two directions:

1-st – investigate of soils (45 samples) and feeds (23 samples) for the content of trace elements (Co, Cu, Zn, Mn, Fe, Pb, Ni, and Cd) and analysis of rations;

2-nd – investigation of the content of macro- (88 samples) and trace elements (68 samples) in the blood of cows.

Cows of Black and Spotted breed aged 3–10 years, weighing 450–550 kg were the object of the investigation. Investigation of the clinical state of cows were carried out.

The calcium content in the blood serum was determined with the help of reagent arsenazo III, magnesium – by reaction with calmagite test a set of the company "Simko Ltd" (Lviv), inorganic phosphorus – with ammonium molybdate by C.H. Fiske, Y. Subbarow. Determination of the content of trace elements (manganese, zinc, copper and cobalt) in the blood was performed by atomic absorption spectrophotometry on an AAS 30 instrument.

The probability of the indices ($P < 0.05$, $P < 0.01$, $P < 0.001$) was assessed by the t test of the Student. The results of the clinical searches of animals, laboratory analysis of blood, feeds were processed using the variational statistics method using special software (the "Statistika" program).

Results

In cows that are kept in farm conditions, osteodystrophy occurs mainly due to a lack of biologically active substances and in particular microelements in soils and forages. In compiling rations, farm specialists are guided by obsolete tabular data, and not actual indicators of the content of micronutrients in feed. Therefore, often with a "full" diet or even an excess of nutrients in animals, osteodystrophy may occur (Underwood and Suttle, 2001; Moreira et al., 2009). Bioavailability of microelements of soil is a key factor, predetermines their content in plants. A number of conditions affect the mobility and bioavailability of elements in soils, namely: pH, humus content, climatic factors, etc. (Kabata-Pendias, 2011). Soils play a major role in the circulation of microelements and is the primary source of their accumulation in plants.

To search the etiological factors of osteodystrophy of cows performing soil research on the territory of three farms of the Lviv region. It is known (Kovalenok, 2007) that when there is a lack or excess of trace elements, specific diseases are developing – microelementoses. It is also known that the deficiency of biotic microelements participating in osteogenesis undoubtedly complicates the course of osteodystrophy (Ytrehus et al., 1999; Bandgar et al., 2012).

Soil research in three farms showed that the gross content of cobalt in them was 6.8, 6.4, and 5.9 mg/kg, respectively, with the optimal – 7–10 mg/kg. In the farm 3, its content in the soil was reduced by 1.2 times. At the same time, a decrease in the concentration of mobile forms of cobalt was found to be 1.4, 1.5,and 2.0 times, respectively (at a rate of 5.0 mg/kg). In the soil of experimental farms, both the total content of zinc and the concentration of mobile forms are decreased, 25.4, 27.9, 24.8 (at a rate of 52–2370 mg/kg), respectively. The total content of manganese in the soils of experimental farms was 7.9 and 5.1 times lower than the MPC (at a rate of 1500 mg/kg). The concentration of mobile forms of manganese in the soil was low and amounted to 20.3, 30.4, and 130.8 mg/kg, respectively (at a rate of 40.0–300.0 mg/kg). According to the content of copper, its total content in farms was within the limits of the MPC (5–76 mg/kg), and the number of mobile forms – increased by 2.8, 2.6, 2.9 times, respectively (at a rate of 3.0 mg/kg). The content of iron in the soil of the farms was different. Thus, the gross content was within the norm (MPC 1000 mg/kg), and its mobile form was 2 times lower in the soil of the farm No 3. Insignificantly lower

than the lower limit of the MAC (50–74 mg/kg) 48.8 mg/kg was its content in the soils of farms No. 1 and No. 2, which corresponds to the optimal amount.
We have analyzed the content of vitally important macro- and microelements in the livestock of Lviv farms and compared them with the data in the table (Provatorov et al., 2009). The obtained results showed that the actual content of microelements in feed does not correspond to the data of the table, both their deficiency and surplus were detected.
Copper was not enough in the fodder beet of all farms – 1.3, 1.6, and 0.46 mg/kg. Its excess was in the hay (10.5–38.9 mg/kg), imbalance in cereals – in oats (excess 6.5–11.8 and deficit 2.63), wheat (5.8–11.2 and 1.8), barley (11.8 and 2.8), pea (8.4) and rye (4.9), in silage (2.9 and 0.96), and in straw there is an excess (3.9–4.14).
According to literature (Provatorov et al., 2009), these copper feeds should be: 1.5; 2.3–4.9; 1.2–2.0; 2.8–3.1. The content of cobalt was insufficient in fodder beet, hay, straw and cereals, except for wheat (0.09 mg / kg at the required 0.08) and silage (0.06, norm 0.05). Similar results were obtained in determining the manganese content (excess in beet, hay, straw, deficiency in cereals), zinc (excess – silage, straw, deficit – hay, cereals) and iron (excess beet, straw, oats, deficit – hay, silage, barley, peas, rye).

Table 1. Comparison of the content of microelements of farms fodder with tabulated data, mg/kg

Fodder	Cu			Co			Mn			Zn			Fe		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Cereal hay	>	–	>	>	–	<	>	–	<	<	–	<	<	–	>
Hay of alfalfa + cereal	–	>	–	–	–	–	–	>	–	–	<	–	–	<	–
Barley straw	>	>	–	<	<	–	>	>	–	>	>	–	>	<	–
Wheat straw	–	>	<	–	<	<	–	>	>	–	>	<	–	<	>
Beet fodder	<	=	<	<	<	=	<	>	>	<	>	>	>	>	>
Corn silo	<	–	<	<	–	<	<	–	<	<	–	>	<	–	>
Silo of annual grasses	–	>	–	–	>	–	–	>	–	–	>	–	–	<	–
Oats	>	>	<	=	<	<	<	<	<	>	<	<	>	<	>
Wheat	>	>	<	>	>	<	<	=	<	<	<	<	<	<	>
Barley	>	<	–	<	<	–	<	>	–	<	<	–	=	<	–
Peas	>	>	–	=	–	–	>	–	–	=	–	–	<	–	–
Rye	–	>	–	–	<	–	–	>	–		<	–		<	–

Notes: 1- 3 correspond to the farm numbers, > - more; <- less; = - an equal amount with the data given in the directory (Provatorov et al., 2009).

By analyzing the rations of the cows under investigation in winter, a deficiency of feed units, crude and digestible protein was established. The amount of exchange energy and raw fat in the diets was insufficient, but in 1 kg of dry matter of the two farms they were in excess, in the other there was a deficit. In cows' rations, the excess of raw fiber (16.6–30.8% in excess of demand), and its concentration in 1 kg of dry matter is less than the norm (223.7–284.3 g if necessary 230–220 g). It was also detected a significant excess of starch in the ration (+37.3–96.6%) and in 1 kg of dry matter (94.6–154.3 mg with 71 mg required). Sugar is not enough in the rations and accordingly a low concentration of 1 kg of dry matter. This caused a decrease in the sugar-protein ratio, which was 0.33–0.97 : 1 at a rate of 0.8–0.9 : 1. The ratio of the sum of sugar and starch to the digestible protein was 2.21–3.51 : 1 at a rate of 2.0–2.2 : 1.
The supply of rations with macroelements – calcium and phosphorus was insufficient. Calcium ration was provided at 62.0–78.2% (Figure 1), and phosphorus only by 49.4–57.4% (in rations 24.7–29.9 at needs and 50–55 g). The ratio between calcium and phosphorus in ration is 2.24–2.45: 1, that is high, according to the literature (Provatorov et al., 2009), the optimal is 1.7 : 1.0. It should be noted that the concentration of calcium and phosphorus in the dry matter of the rationb is insufficient – 4.6–5.6 g/ g and 1.9–2.5 g/kg at the optimal – 6.5 and 3.8 g respectively. Along with this, in all diets there was an excess of magnesium and potassium – 126.9–183.2 and 186.9–188.3% of demand, respectively. Therefore, the potassium-magnesium ratio was high – 3.40–4.85.
The ration contains an excess of iron and its high content in dry matter. As it was noted in the literature (Underwood and Suttle, 2001) cattle is tolerant to high iron content, only in water it is much more affordable, and therefore more toxic. In addition, its excess can reduce the absorption of other trace elements, in particular copper.
An analysis of the results showed that the rations of dry-barked cows of the investigated farms contain insufficient amounts of zinc and cobalt, as well as a low concentration of 1 kg of dry matter (Figure 1). According to the content of manganese, the supply of diets varies from 52.4 to 143.7% (Figure 1), but in spite of this in dry matter its quantity was insufficient (21.3–41.2 mg with the required 45), sometimes excess (52.3 mg). The provision of carotene for rations of dry cows in farms was insufficient (34.1–70.4% of demand, Fig. 1). There is also a low content of vitamin D in the ration, since it was within 8.9–14.1% of the requirement. The amount of vitamin E exceeded the requirement for all rations of the investigated farms by 147.2–295.7% (Fig. 1).
The analysis of rations showed that the supply of macroelements (calcium and phosphorus) was insufficient (62.0–78.2 and 49.4–57.4%). Along with this, in all rations there was an excess of magnesium (126.9–183.2%) and potassium (186.9–188.0%). The provision of rations with cobalt and zinc was 25.7–71.8 and 33.6–75.1%, with kuprum – 21.2–116.1%. In 1 kg of dry matter of feed it was a low content of cobalt –0.15–0.29 mg (optimal 0.5–0.7 mg), zinc – 13.6–21.5 (40 mg), sometimes manganese – 21.3–51.8 (40 mg), excessive copper – 8–8.7 mg (7 mg), iron – 146.5–268.9 mg/kg, with the optimal 60 mg.

It is insufficient amount of vitamin D (8,9–14.1% of the demand) in feed rations.

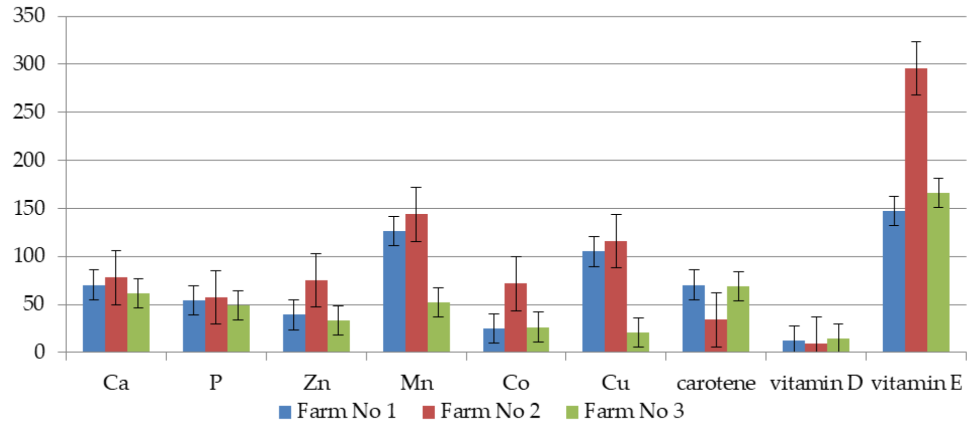

Fig. 1. Provision of farms cows with mineral substances and vitamins, %

On the basis of the detected symptoms and the results of laboratory tests, two groups of cows were created: clinically healthy, with subclinical course, patients with clinical symptoms of osteodystrophy.

In 38.7% of cows signs of latent disturbance of mineral metabolism (subclinical course) are established, in 8.9% – symptoms of osteodystrophy. Body temperature in cows was within normal limits, in 8.9% of clinically ill cows was showed tachycardia, 32.2% – tachypnea. In cows with subclinical course of osteodystrophy, the mucous membranes were pale pink or pink. In 53.6% wool lost its shine, became dull, did not stick well in the hair follicles, in 40.8% was observed molting detentions. Depigmentation of hair around the eyes ("glasses") was recorded in 26.6% of sick cows. Black hair sometimes acquired a brown color (achromotrichia). The elasticity of the skin was reduced. In the subclinical course of osteodystrophy of cows, the vulnerability of the incisors was found in 20.3%, the taste distortion in 50.0% of cows, the resorption of the last 2–3 caudal vertebrae, the thinning and partial lysis of the last pair of ribs by ¼ of their length were recorded. In some places, the ribs were narrowed or, conversely, widened, and their edges were uneven, hilly.

The content of total calcium in the serum of clinically healthy cows and with the subclinical course almost did not differ and was reduced in 18.8 and 95% of cows, respectively (Table 2). In 75% of cows, hypocalcemia and hypophosphatemia (less than 1.5 mmol/l) were established. According to the literature (Liesegang et al., 2003), due to a decrease in the total calcium content in the blood, the secretion of parathyroid hormone increases due to the feedback principle, which can also be the reason for the decrease in phosphorus in the blood.

Table 2. The content of macronutrients in the blood of cows

Index	Biometric index	Clinically healthy, n = 15	Subclinical course of osteodystrophy, n = 53
General calcium, mmol/l	Lim	2.3–3.0	2.1–2.9
	M ± m	2.6 ± 0.15	2.4 ± 0.02
	P <		0,5
Inorganic phosphorus, mmol/l	Lim	1.6–2.1	1.2–2.2
	M ± m	1.9 ± 0.09	1.7 ± 0.02
	P <		0,05

Note: P < – compared with clinically healthy animals.

There are several reasons for hypophosphataemia, first of all - phosphorus deficiency in the ration, violation of phosphorus and calcium absorption in the intestine, vitamin D takes an active part (Petrenko, 2007). It stimulates the active transport of phosphorus through the modulation of proteins, and also affects the permeability of the lipid components of the apical membrane of enterocytes (Horst et al., 1994; Sakhnyuk et al., 2007).

The reason for hypocalcemia in cows in subclinical course of osteodystrophy is insufficient quantity of calcium in rations (62.0–78.2% of demand), phosphorus deficiency (49.4–57.4% of demand) and vitamin D, the supply of which was only 8.9–14,1% of the demand.

Analysis of the contents of microelements in the blood of cows from experimental groups showed that the concentration of copper and zinc were within acceptable limits. At the same time, in the blood of cows in subclinical course of osteodystrophy, the copper content was on average 13.4 ± 0.11 µmol/l, which was significantly ($P < 0.001$) less by 29.8% compared to clinically healthy animals (19.1 ± 0.74 µmol/L, Figure 2). Copper is the most studied microelement of bone tissue, as it participates in the formation of bone collagen (Beattie and Avenel, 1992).

The concentration of zinc in the blood of cows in the subclinical couse of osteodystrophy ranged from 13.9 to 15.1 μmol/l and averaged 14.5 ± 0.15 μmol/l. In the investigated cows, the zinc content was 18.9% lower (P < 0.001) from clinically healthy animals (Fig. 2). The physiological role of zinc in metabolism is due to the fact that it is an element that is necessary for the crystallization of bone tissue, contributes to the deposition of minerals in the bone (Beattie and Avenel, 1992).

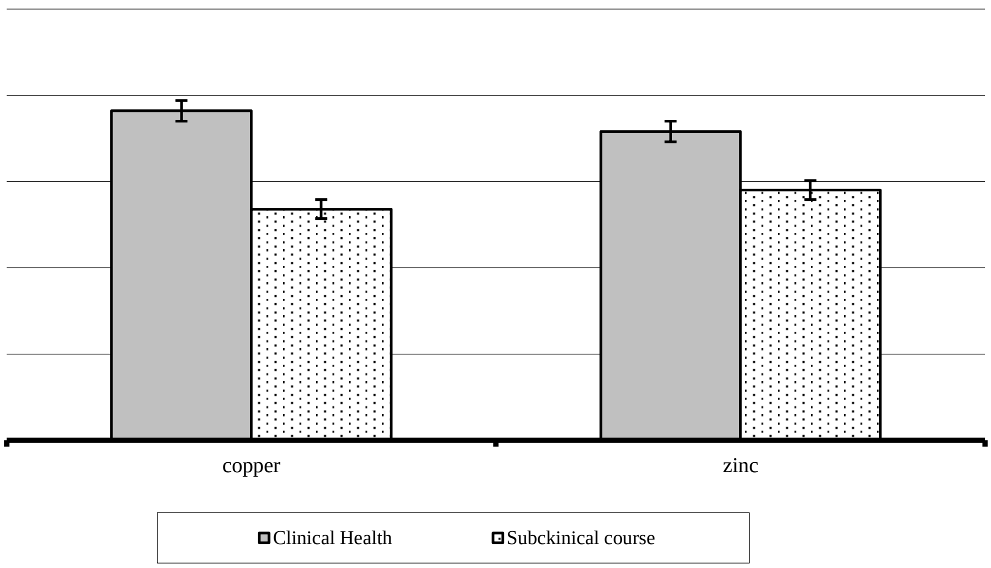

Fig. 2. Content of copper and zinc in the blood of cows, μmol/l

In the blood of clinically healthy cows, the concentration of manganese averaged 2.5 ± 0.03 μmol/l and was at the lower boundary of physiological oscillations (2.5–4.0 μmol/l, Fig. 3). The content of manganese in the blood of cows with a subclinical course of osteodystrophy averaged 2.1 ± 0.03 μmol/l and was significantly (P < 0.001) lower than clinically healthy by 16.0%.
In the activity of bone, manganese acts as an activator of a number of enzymes involved in ossification, including bone alkaline phosphatase. He is a stimulator of calcification processes, takes part in the mineralization of collagen fibrils (Beattie and Avenel, 1992).

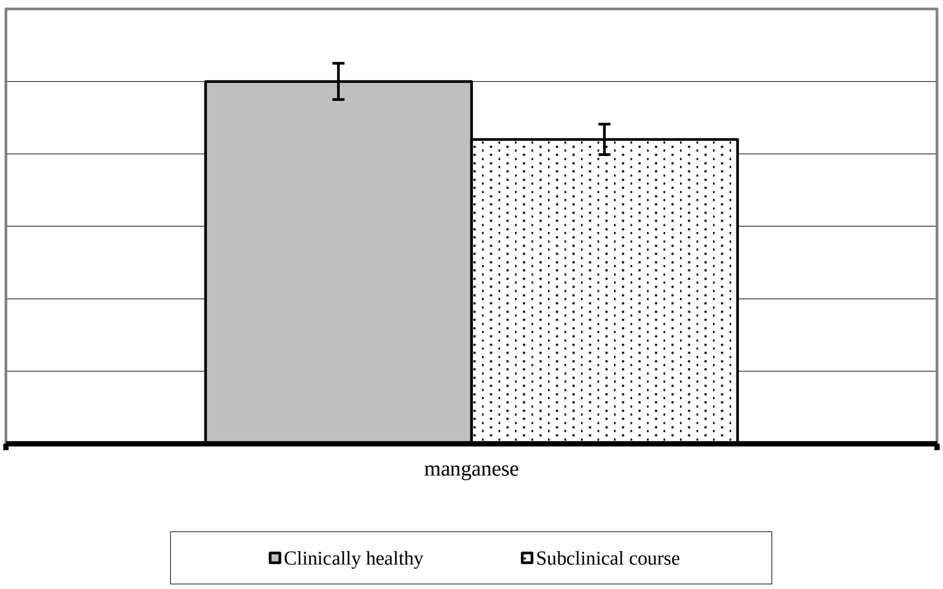

Fig. 3. The content of manganese in the blood of cows, μmol/l

The content of cobalt in the blood was very low in both the clinically healthy group and in cows with a subclinical course of osteodystrophy. Mean values of cobalt in the blood of clinically healthy cows were 0.47 ± 0.01 μmol/l at a rate of 0.5–0.8 μmol /l. In cows with subclinical course of osteodystrophy, the average cobalt content was 0.17 ± 0.01 μmol/l, which was 2.8 times less than in clinically healthy animals (Fig. 4).

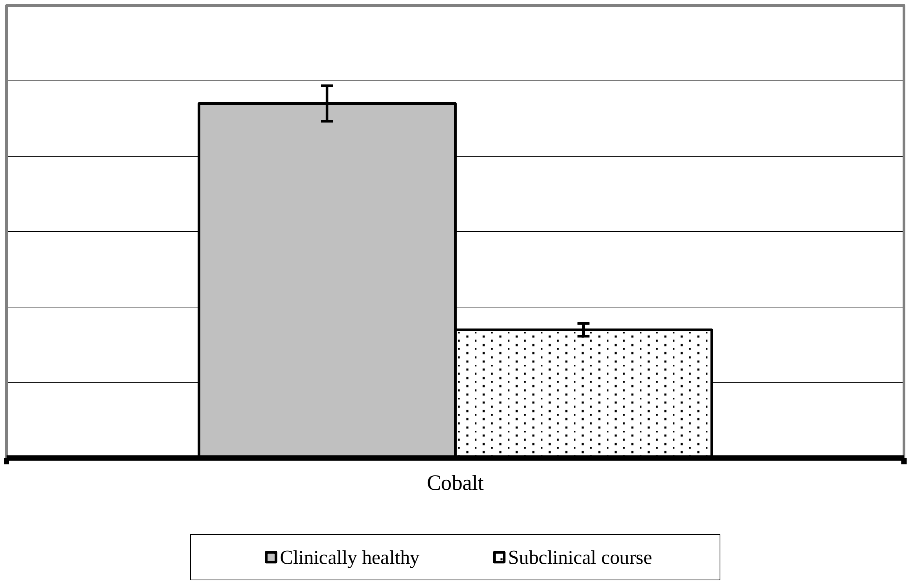

Fig. 4. Content of cobalt in the blood of cows, µmol/l

Cobalt belongs to osteogenic microelements, increasing the activity of bone phosphatase, which plays an important role in the mineralization of bone tissue. Its deficiency reduces the absorption of calcium and phosphorus in the gastrointestinal tract, which may be one of the contributing factors in the genesis of osteodystrophy (Beattie and Avenel, 1992).

Discussion

Osteodystrophy is caused by polyethological factors, the main ones are the disruption of the exchange of macro- and microelements, as well as vitamins D and A (Bandarra et al., 2011). Scientists of Ukraine found that in the etiology of osteodystrophy a certain role is played by the deficiency of microelements: cobalt, zinc, copper, manganese. This pathology has a predominantly subclinical course, in which pathobiochemical disorders are developing in the bone tissue, which to the stage of specific symptoms are still inverse. The functioning of the bone is ensured by the constant supply of energy, protein components, mineral elements, vitamins and other biologically active substances in this case the nodal mechanism of bone tissue formation is its mineralization. The course of this process occurs with the expenditure of energy, calcium, phosphorus, magnesium, sodium, potassium, chlorine, sulfur, zinc, manganese, cobalt, iodine, vitamins A, D, C, parathyroid hormone, calcitonin and other substances (Jabur, 2010, Mandıroğlu et al., 2013; Martyshuk et al., 2016; Khariv et al., 2016; Hariv and Gutyj, 2016).

The lack of essential microelements in the feed and water of the western region of Ukraine, the disruption of metabolism, the decrease in the productivity of animals and poultry, the emergence of various microelementoses. At the same time, there is no production of adequate premixes in the region with the requirements of biogeochemical zones (Stadnyk and Fedorovych, 2006, Shcherbatyy et al., 2017).

Lviv region belongs to the western biogeochemical zone with a deficiency and imbalance of many microelements in soils and plants. In which the most deficient for animals are: iodine, cobalt and selenium, in most areas manganese, zinc, and in some farms, ferment, chromium, molybdenum and copper. Therefore, the problem of osteodystrophy of cows in the Lviv region is complicated by the inadequate content of mobile forms of microelements in soils (Fedorovych, 2011).

The content of mineral elements in concentrated and coarse forages varies considerably depending on the type of soil, fertilizer, manure, plant species, the stage of vegetation and the technology of their harvesting. Therefore, a regular laboratory analysis of feed for the maintenance of macro- and microelements is important to estimate feed as sources of mineral elements.

Taking into account the peculiarities of the biogeochemical zone, which includes the Lviv region, and also with the aim of establishing the etiological factors of osteodystrophy in cows, we investigated the biological chain: soil-plant-animal. As a result of the conducted studies, a deficit in soils of total content and especially mobile forms of cobalt, manganese and zinc, which is consistent with the literature sources. The content of the microelement depends on the type and pH of the soil. The solubility of the elements increases with an acidic pH value of the soil. The total copper content was within the limits of the norm, and its mobile form exceeded the upper limit of the MPC. The effect of soil pH on the assimilation of copper is much weaker in comparison with other microelements. At an "acidic" pH value, it is better consumed by plants than with an alkaline one. Copper is retained in the surface layer, affects the biological activity of the soil and can be accessible to plants under a wide variety of conditions. The most important form of copper in soil solutions is its soluble organic chelates.

The iron content in the soils of Lviv region is usually less than the MPC, and on the average is 38 g/kg, and according to our studies it is 145.9–198.2 g/kg. At ordinary pH levels, the concentration of iron in soil solutions ranges from 30 to 550 µg/l, and in acidic soils it can reach 2000 µg/l.

Investigating the content of cobalt, we found its low total content in 1 kg of dry soil (5.9–6.8 at a rate of 7–30 mg/kg) and a mobile form, which is 1.38–2.0 times lower than MPC (5.0 mg/kg). The decrease in mobile forms of cobalt leads to an increase in soil pH, since it forms chelate-like compounds with soil amino acids. According to the authors, the content of cobalt in the soil is less than 5 mg/kg leads to its deficiency in green mass.

The content of heavy metals, in particular nickel, was normal, and its mobile form exceeded the upper limit of the maximum permissible vibrations. In soils, there is cadmium, as well as lead, its total content and mobile form exceeded the MPC. The presence of these microelements in the soils indicates its contamination with heavy metals (Gutyj et al., 2016; Gutyj et al., 2017). Summarizing the results of the search of the microelement composition of soils in the biogeochemical province, it was found that along with a low content of mobile forms of trace elements, heavy metals also exist in it. The latter cause micronutrient deficiencies in feed, therefore, honey and cobalt should be additionally used, as cadmium and lead antagonists to reduce their assimilation.

In comparison with tabular data, the content of copper was less in the beet, in cereals and silage it is established both its deficiency and surplus, in straw and hay – excess. Cobalt was less in fodder beet, hay, straw and cereals, except for wheat and silage. More than in the tables, manganese was found in beet, hay, straw, less in cereals. Less zinc was in the hay and cereals, more – in silo and straw. The iron content was less than in the tables in the hay, silage, barley and rye, but more in the beets, straw and oats. So, thanks to the search of the trace element composition of the fodder of all three farms was found both less and more content compared with tabular data (Provatorov et al., 2009; Table 1).

The conducted analysis of rations showed that the supply of macroelements (calcium and phosphorus) was insufficient (62.0–78.2 and 49.4–57.4%). Along with this, in all rations there was an excess of magnesium (126.9–183.2%) and potassium (186.9–188.0%). The provision of diets with cobalt and zinc was 25.7–71.8 and 33.6–75.1%, with kuprum – 21.2–116.1%. In 1 kg of dry matter of feed it was a low content of cobalt – 0.15–0.29 mg (optimal 0.5–0.7 mg), zinc – 13.6–21.5 (40 mg), sometimes magnesium – 21.3–51.8 (40 mg), excessive copper – 8–8.7 mg (7 mg), iron – 146.5–268.9 mg/kg, with the optimal 60 mg. In feed rations it was insufficient amount of vitamin D (8.9–14.1% of the demand).

So, it is established, that the main reasons for the development of osteodystrophy in barn feeding cows were low content of vitamin D, calcium, phosphorus, cobalt, zinc, and sometimes copper in rations and a low concentration of 1 kg of dry matter. In the ration of cows there is a violation of the ratio between the content of mineral substances, which negatively affects the course of physiological processes, in fact, with excess of some elements, the deficiency of others is increased (antagonism), their absorption is decreased.

The content of total calcium in the blood serum of clinically healthy cows and subclinical course was almost unchanged. In 75% of clinically diseased cows, hypocalcemia was established. It should be noted that in 75% of clinically diseased cows hypophosphatemia (less than 1.5 mmol/l) is established. According to the literature, as a result of a decrease in the content of total calcium in the blood, on the principle of feedback, the secretion of parathyroid hormone is increased, which can also be the cause of a decrease in phosphorus in the blood. There are several reasons for hypophosphataemia, first of all - a deficiency of phosphorus in the ration, a violation of absorption of phosphorus and calcium in the intestine, active participation in which takes vitamin D. It stimulates the active transport of phosphorus through the modulation of proteins, and also affects the permeability of the lipid components of the apical membrane of enterocytes.

According to the literature, the calcium-phosphorus ratio can be used as an auxiliary indicator of the diagnosis of osteodystrophy (Liesegang et al., 1998). Violation of its ratio leads to insufficient assimilation of both macroelements, as a consequence, the parathyroid gland activates the release of parathyroid hormone, which leads to the resorption of calcium from the bones to maintain its homeostasis and, as a consequence, develops osteodystrophy. In the literature it is noted that the optimal ratio of calcium to phosphorus should be 1.32–1.68, therefore, our results do not agree with those indicated.

Taking into account the specific features of the district of the western region of Ukraine, We determined the contents of cobalt, copper, zinc and magnesium in the blood of cows, as they participate in ossification of bone tissue.

Our research has shown, that the cobalt content in the blood of cows of all three research groups was less than normal, in particular in clinically healthy cows, the upper limit did not exceed 0.51 μmol/l. The reason for such a low content is, first of all, the deficit of its mobile forms in the soil (2.5–3.6 mg/kg of MPC 5.0), feed (mostly in coarse, juicy and partly in cereals), and hence – in the diet (25.1–71.8% of the requirement) and blood cows (Table 3). According to the literature cobalt refers to osteogenic microelements, regulates mineral and other exchanges. It increases the activity of bone phosphatase, plays an important role in the mineralization of bone tissue, carboxylase, arginase, catalase, many peptidases, reduces the activity of succinate dehydrogenase and cytochrome oxidase. In osteoblasts, cobalt catalyzes enzymatic reactions.

There is an important influence on the formation of bone tissue of manganese, which acts as an activator of a number of enzymes (Beattie and Avenel, 1992). The latter participate in the processes of ossification, including bone alkaline phosphatase, which stimulates calcification. It is involved in the enzymatic processes of mineralization of collagen fibrils. Investigating the concentration of manganese in the blood of clinically healthy cows, it was established that it was within physiological fluctuations. At the same time, in the rations of farms the amount of manganese exceeded the demand by 26.7–43.7%, but in terms of 1 kg of dry matter of ration, its amount was insufficient (21.3, 41.3) or slightly exceeded the need (according to 51.0 and 45 mg). A deficit was noted in one of the farms both in the diet (52.4% of the demand) and in dry matter (21.3 mg with the required 45 mg). In addition, it should be noted that in the feeds there was both manganese deficiency and its excess (Table 1).

As indicated in the literature, bone tissue is more saturated with zinc, the physiological role of which in osteogenesis is associated with the crystallization of bone tissue (Beattie and Avenel, 1992; Caldow et al., 1995). Investigating the concentration of zinc in the blood of cows, it was found that with the subclinical course of osteodystrophy, its content was low. Taking into account the literature data, where it is noted that with an acute zinc deficiency, the rate of its release does not compensate for the costs and there are phenomena of shortage, as well as the fact that zinc promotes the deposition of minerals and is an activator of alkaline phosphatase of bone tissue, then its deficiency, in combination with other factors, is an important contributing factor in the development of osteodystrophy. In particular, zinc was not enough in soils, feed, diet and in dry matter (Table 3).

Table 3. Comparative content of microelements in the soil, feed and blood of cows

Micro-element	The soil, (mobile forms) mg/kg	Feed		Content in the diet		Content in the blood of cows	
		less than in table	more than in table	ration	Dry matter	clinically healthy	subclinical course
Co	2.50–3.6	+		↓	↓	↓	↓
Zn	3.9–8.6	+		↓	↓	N	↓
Mn	17.4–30.4		+	↑	↓	N	↓
Cu	7.9–130.8		+	↑	↓	N	N

Copper ions in the process of growth and differentiation of osteoclasts regulate osteogenic cellular elements and ossification processes, catalyze enzymatic reactions in osteoblasts. Copper plays a significant role in the function of enzymes involved in the formation of collagen, which itself is involved in bone formation (Beattie and Avenel, 1992). In the blood of cows in subclinical flow of osteodystrophy, the content of copper was significantly ($P < 0.001$) less by 29.8% compared to clinically healthy animals (19.1 ± 0.74 μmol/l). It should be noted, that its content was in physiological limits (12.6–22.0 μmol/l). As the results of investigations have shown, the content in the soils of copper and its mobile forms is above the MAC by 2.63-2.86 times with the necessary 3.0 mg/kg, as well as its number exceeded the tables in feeds – cereals and coarse, and the latter constitute the basis of the ration (Table 3). Carrying out the analysis of ration of cows feeding, we found an excess of copper (5.1–14.5% more needs), but in 1 kg of dry matter it is small (1.8-8.7 mg). One of the reasons for reducing the concentration of copper in the blood of cows may be an excess of iron in the ration, the concentration of which in 1 kg of dry matter ranged from 146.5–268.9, if necessary 50.0 mg/kg. According to the literature (Liesegang et al., 1998; Liesegang, 2003), with an excess of iron in the dry matter, absorption of copper can be impaired. Therefore, in the blood of cows with subclinical course, a tendency to decrease in the concentration of copper is noted.

Conclusions

Clinical and laboratory searches of sick cows in the subclinical course of osteodystrophy indicate a violation of the exchange of microelements in their bodies, while in their blood it was revealed that the content of copper and zinc was within physiological fluctuations, the concentration of manganese and cobalt was below the norm. Comprehensive accounting of the feeding and keeping of cows can be a reliable basis for the development of modern prevention of osteodystrophy.

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