

EFFECTIVENESS OF INORGANIC AND CHELATE COMPOUNDS OF MICROELEMENTS FOR OSTEODYSTROPHY OF COWS

Slivinska L.G., Fedorovych V.L., Shcherbatyi A.R.

INTRODUCTION

In recent decades, the problem of providing animals with macro- and microelements has worsened, which is connected with changes in the technology of cattle breeding. Year-round keeping of livestock indoors, changing the structure of rations, irrational exploitation of premixes, numerical stress, and anthropogenic load have led to the spread of "diseases of industrialization," most of which are associated with a deficiency or excess of elements ^{1,2,3,4,5}. Inorganic salts of trace elements for preventive and therapeutic purposes do not always ensure their optimal level in body tissues. The inconsistency of the recipes of standard premixes with the biogeochemical features of the region causes this problem ^{6, 7}. Moreover, the addition of mineral salts to feed is often not practical because there is a chemical incompatibility of some ions ⁸. For example, copper sulfate is applied as a copper source in premixes, and potassium iodide, as a source of iodine. Their interaction produces insoluble copper iodide and volatile elemental iodine; it occurs in aqueous solutions and contacts with dry salts.

¹ Мікроелементози сільськогосподарських тварин / М.О. Судаков, В.І. Береза, І.Г. Погурський [та ін.]. Київ: Урожай. 1991. 144 с.

² Shcherbatyy, A.R., Slivinska, L.G., Gutyj, B.V., Golovakha, V.I., Piddubnyak, O.V., Fedorovych, V.L. The influence of a mineral-vitamin premix on the metabolism of pregnant horses with microelemetosis. *Regulatory Mechanisms in Biosystems*. 2017. Vol. 8 Iss. 2. P. 293-298. Doi: 10.15421/021746.

³ Влізлю В.В., Сологуб Л.І., Янович В.Г., Антоняк Г.Л., Янович Д.О. Біохімічні основи нормування мінерального живлення великої рогатої худоби. 2. Мікроелементи. *Біологія тварин*. 2006. Т. 8. № 1–2. С. 41–62.

⁴ Slivinska, L., Demydjuk, S., Shcherbatyy A., Fedorovich, V., & Tyndyk, I. Етіологія та клініко-біохімічні показники крові за аліментарної остеодистрофії корів. *Науковий вісник Львівського національного університету ветеринарної медицини і біотехнологій імені С. З. Гжицького*. Серія: Ветеринарні науки. 2017. Том 19. № 73. С. 79-83. <https://doi.org/10.15421/nvlvet7317>

⁵ Shcherbatyy, A., Slivinska, L., & Lukashchuk, B. Hypocobaltosis and hypocuprosis in pregnant mares in the western biogeochemical zone of Ukraine (distribution, diagnosis). *Ukrainian Journal of Veterinary and Agricultural Sciences*. 2018. № 1(2). P. 11-14. <https://doi.org/10.32718/ujvas1-2.03>

⁶ Слівінська, Л.Г., Демидюк С.К., Щербатий А.Р. Синдроматика та стан метаболічних процесів у корів за мікроелементозів. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2017. Т. 19. № 78. С. 182–186.

⁷ Федорович В.Л. Профілактика остеодистрофії корів в умовах біогео- хімічної зони регіону. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2011. Том. 13. №4 (50). Ч. 1. С. 472–476.

⁸ Slivinska, L., Fedorovych, V., Gutyj, B., Lychuk, M., Shcherbatyy, A., Gudyma, T., Chernushkin, B., Fedorovych, N. The occurrence of osteodystrophy in cows with chronic micronutrients deficiency. *Ukrainian journal of Ecology*. 2018. Vol. 8. Iss. 2. P. 24-32. Doi: 10.15421/2018_305.

Therefore, including trace elements in the diet in an inorganic form has many disadvantages. In this regard, it is advisable to administrate those mineral preparations in which microelements are chelated compounds with bioligands substances similar to natural carriers of microelements ⁹.

1. Clinical status for cows' osteodystrophy when administrating inorganic and chelated compounds of microelements

Our research was conducted based on farms in the Lviv region. Control over the prophylactic effect of trace elements was carried out established on the results of a clinical study of cows ¹⁰, and biochemical analysis of blood serum and urine ¹¹, which were maintained before the start of feeding and on the 60th and 90th day of the experiment.

Taking into account the research results and guided by the principle of treatment of trace element diseases according to M.O. Sudakov, we calculated doses based on their deficiency in the diet ¹².

According to the results of a clinical and laboratory study, we selected 45 dry cows suffering from a subclinical form of osteodystrophy, from which 3 groups were formed: one control (15 heads) and two experimental ones - 15 animals each.

The cows of the first experimental group received inorganic compounds of minerals in the amount per trace element (mg): copper – 52.5, zinc – 301, cobalt – 5.6, and manganese – 56.2. The animals of the second group received the same trace elements in the form of chelated compounds with the amino acid methionine (methionates) in the same doses. In addition, cows of experimental groups 1 and 2 received tricalcium phosphate at 80 g/day per animal, 28 g of calcium, and 14.4 g of

⁹ Федорович В.Л. Мікроелементні сполуки у профілактиці остеодистрофії корів. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2011. Т. 14. № 3 (53). Ч. 1. С. 292–297.

¹⁰ Федорович В.Л., Стадник А.М. Лабораторна діагностика субклінічного та клінічного перебігу ензоотичної остеодистрофії корів. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2007. Т. 9. №3 (34). Ч. 1 С. 225–231.

¹¹ Стадник А.М., Федорович А.М. Метаболічний профіль крові за ензоотичної остеодистрофії корів. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2006. Т. 8. № 2 (29). Ч. 1. С. 185–190.

¹² Мікроелементози сільськогосподарських тварин / М.О. Судаков, В.І. Береза, І.Г. Погурський [та ін.] – Київ: Урожай. 1991. 144 с.

phosphorus. The vitamin preparation "Kombisol AD₃E" (1 ml of the drug contains vitamin A - 50,000 MO; D₃– 10000 MO; E – 100 mg) 2 ml/day per animal. Animals of the control group received only the essential diet. Compounds of salts of trace elements, tricalcium phosphate, and vitamins were mixed with concentrated feed and fed for 90 days.

The research established that the clinical condition of the cows of the control group, which received only the essential diet, did not differ on the 60th day compared to the animals' indicators at the experiment's beginning. On the 90th day of research, 60% of cows in the control group were diagnosed with pale mucous membranes. During the entire experimental period, the general condition of the animals was satisfactory. The cows mostly lay down, did not get up long, and stepped on their limbs when standing.

Changes characterizing mineral metabolism disorders became more pronounced: distortion of taste was noted in 3 cows (20%), disheveled and dullness of hair - 5 (33.3%), depigmentation of the hair coat - 4 (26.7%), delayed molting – 7 (46.7%), decrease in skin elasticity – 4 (26.7%), incorrect regulation of limbs – in 3 (20%). Alopecia was found around the eyes, neck, back, and limbs. When palpating the last pair of ribs in 7 (46.7%) cows, their thinning was noted in 5 (33.3%) - lysis of the tail vertebrae. That is, typical symptoms of stages 1 and 2 of osteodystrophy were registered in the control group of cows. In the cows of the research groups, which, in addition to the essential diet, were fed inorganic salts and chelated compounds of microelements, positive changes in clinical status indicators were established.

In the cows of the first experimental group, the body temperature fluctuated within limits 38,7– 39,3°C (39,0±0,25 °C), pulse rate and breathing averaged 62.0±1.25 beat./min. and 22.0±1.18 breaths.mov./min. respectively.

Allotriophagy was observed in only one cow, which is 6.67% of all cows in the first experimental group, while at the beginning of the experiment, there were 7 of them (46.7%). The hair coat became shiny, the skin became elastic, the mucous membranes of 6 cows (40%) became pink, and in 9 (60%) - they acquired a pale pink color. On the 90th day of the experiment, hair depigmentation was not observed in cows.

The body temperature of the cows of the second group at the end of the experiment ranged from 38.1–38.8°C (38.4±0.25 °C), pulse rate – 61–77 beats/min, (69.0± 1.25 beats/min), and the respiratory rate is 21–27 breaths.mov./min. (23.0±1.18 resp.move./min). On the 60th day, molting was completed, and the hair coat was evenly pigmented. The mucous membranes of all cows were pink and pale pink. Allotriophagy was not observed in the animals.

Clinical study results established that the symptoms progressed in the cows of the control group. Furthermore, at the end of the experiment, signs characteristic of the 1st and 2nd stages of osteodystrophy were found, in particular, lysis of the last tail vertebrae and ribs ¹³.

When feeding chelated compounds of microelements, the clinical symptoms of the cows of the second experimental group normalized on the 60th day of the experiment. In contrast, the clinical symptoms of the cows of the first group disappeared only after the end of the study (the 90th day).

2. The content of macroelements in the blood serum of cows suffering from osteodystrophy administrating inorganic and chelated compounds of microelements

In the cows of the control group, on the 60th day of the experiment, the total calcium level in blood serum decreased significantly ($p < 0.001$) from 2.37±0.022 mmol/l to 2.18±0.011 mmol/l. On the 90th day - to 2.01±0.014 mmol/l ($p < 0.01$; 0.001; Fig. 1). The decline in the total calcium concentration in the blood serum of cows of the control group is due to its low amount in the diet.

In the cows of the first experimental group, during the use of tricalcium phosphate and inorganic salts of microelements, the concentration of total calcium in blood serum did not differ from the initial values. However, on the 60th day, its content in blood serum was 13.8% higher ($p_3 < 0,001$), and on the 90th day – 18.9% ($p_3 < 0,001$) compared to the control (Fig. 1).

¹³ Внутрішні хвороби тварин / В.І.Левченко, В.В.Влізло, І.П.Кондрахін, І.М.Карпуть, Л.Г.Слівінська, Л.М.Богатко, А.Ю.Мельник, І.В.Папченко, В.В.Чумаченко, В.Ю.Чумаченко; За ред. В.І.Левченка. Біла Церква, 2015. Ч.2. 610 с.

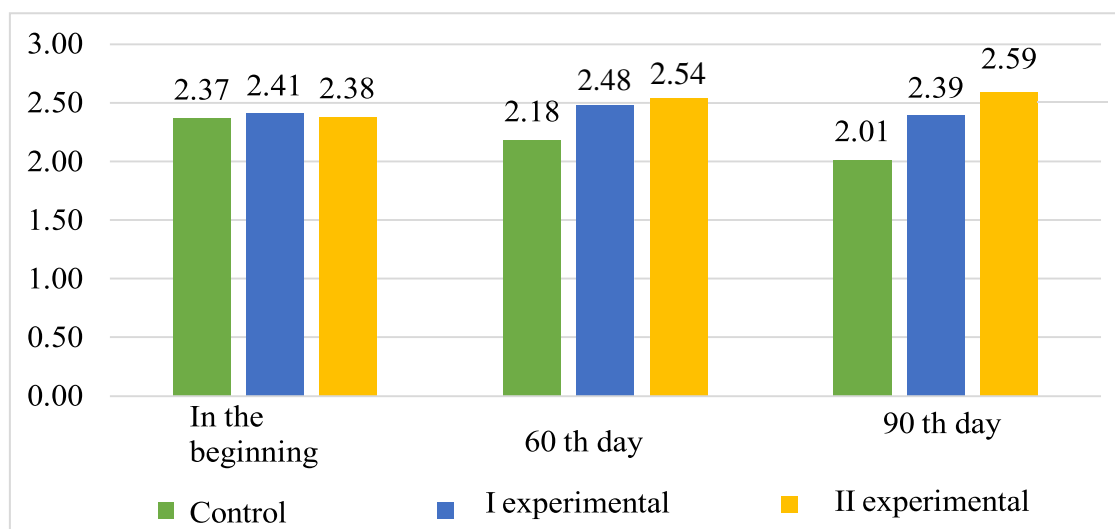


Fig. 1 – Content of total calcium in blood serum of cows, mmol/l

In the second experimental group, the total calcium concentration in blood serum increased from 2.38 ± 0.019 mmol/l at the beginning of the experiment to 2.54 ± 0.031 mmol/l ($p_2 < 0.05$) on the 60th day. At the end - up to 2.59 ± 0.030 mmol/l ($p_2 < 0.001$). The concentration of total calcium in the blood serum of cows of this group on the 60th day was probably 16.5% ($p_4 < 0.001$), and at the end - 28.9% ($p_4 < 0.001$) compared to the control group of animals.

The best therapeutic effect of using tricalcium phosphate in combination with chelated compounds of microelements was confirmed by the fact that total calcium content was 8.4% ($p_5 < 0.001$) higher than cows of the first experimental group on the 90th day of the experiment.

The inorganic phosphorus concentration in the blood serum of the control group during the entire experiment decreased from 1.80 ± 0.03 mmol/l to 1.42 ± 0.02 on the 60th day and to 1.26 ± 0.08 mmol/l on the 90th ($p < 0.001$). It was less by 21.1 and 30.0%, respectively, compared to the beginning of the study (Fig. 2).

In cows of experimental group I, the inorganic phosphorus concentration remained stable throughout the experiment (Fig. 2; Table 1). However, on the 60th and 90th day, its concentration was higher by 31.0 and 42.1%, respectively ($p_3 < 0.001$) compared to the indicators of the control group.

After the end of the experiment in group I on the 90th day, the macroelement

concentration decreased ($p_1 < 0.5$). Still, it was within the physiological range (1.79 ± 0.03 mmol/l) and remained higher by 42.0% ($p_3 < 0.001$) than the control (Fig. 2).

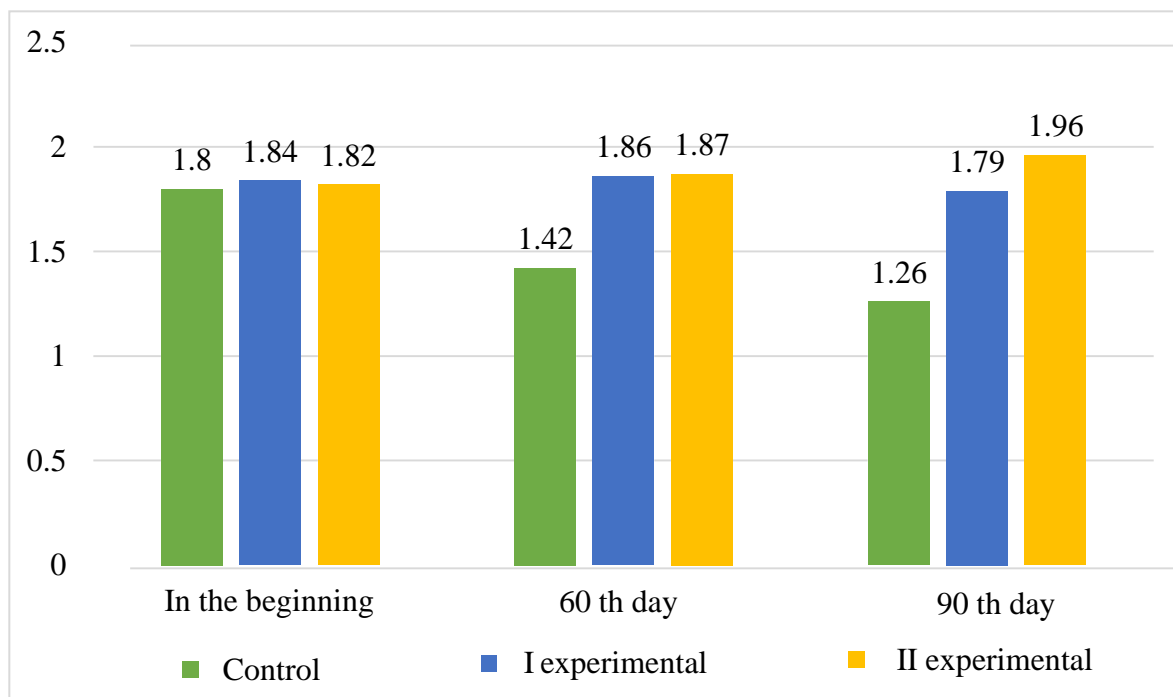


Fig. 2 – The content of inorganic phosphorus in the blood serum of cows, mmol/l

In the cows of the II experimental group, the inorganic phosphorus concentration in the blood serum on the 60th day did not change compared to the beginning. Furthermore, it did not differ from the indicator in the first group. However, only after 90 days it increased ($p < 0.001$) compared to the beginning of the experiment (+ 7.7%) and the first research group (+9.5%).

The macroelement concentration on the 60th day was higher by 31.7% ($p_4 < 0.001$) compared to the control, and after the end - by 55.6% ($p_4 < 0.001$; Fig. 2).

The calcium-phosphorus ratio at the beginning was in the range of 1.30–1.33. On the 60th day, the ratio between macroelements in the blood of cows of the control group was, on average, 1.54 ± 0.01 . After the end of the experiment – 1.59 ± 0.01 and was 19.5% higher ($p < 0.001$) compared to the beginning (Table 1).

In cows of the 1st and 2nd experimental groups, the ratio of calcium to phosphorus using tricalcium phosphate remained unchanged. Thus, in the cows of the 1st

experimental group, the ratio of macroelements did not change.

Table 1

Calcium-phosphorus ratio in blood serum of cows

A group of cows	Biometric indicator	Research period		
		In the beginning	60 th day	90 th day
Control, n=15	Lim	1,17–1,44	1,45–1,63	1,53–1,64
	M±m	1,33±0,02	1,54±0,01	1,59±0,01
	p<		0,001	0,001
I experimental, n=15	Lim	1,23–1,44	1,27–1,40	1,27–1,46
	M±m	1,31±0,02	1,33±0,01	1,34±0,01
	p ₁ <		0,1	0,5
	p ₃ <		0,001	0,001
II experimental, n=15	Lim	1,17–1,39	1,27–1,46	1,18–1,43
	M±m	1,32±0,02	1,36±0,02	1,29±0,02
	p ₂ <		0,05	0,5
	p ₄ <		0,001	0,001
	p ₅ <		0,5	0,05

In the beginning, it was 1.31±0.02, and at the end - 1.34±0.01 (p₁<0.5), while it was smaller (p₃<0.001) compared to the control.

In the II experimental group, the calcium-phosphorus ratio did not change. It was 1.32±0.01 at the beginning of the experiment and 1.29±0.02 on the 90th day. Compared to the control on the 60th and 90th day, it was smaller (p₄<0.001) by 11.7 and 18.9%, respectively. In addition, in the end, the calcium-phosphorus ratio in 5 cows (33.3%) was in the range of 1.18–1.31 and was lower (p₅<0.05) by 3.7% compared to the index in cows I of the research group.

According to literature data ¹⁴, the ratio of calcium to phosphorus is a reasonably informative diagnostic indicator of osteodystrophy in cows. Therefore, its calculation can be used as an additional criterion for diagnosing osteodystrophy.

¹⁴ Slivinska, L., Demydjuk, S., & Shcherbatyу А.. Діагностика хвороб, пов'язаних з порушенням обміну речовин у великої рогатої худоби в ННВЦ «Комарнівський» Городоцького району Львівської області. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. Серія: Ветеринарні науки. 2016. Том 18. №1. С. 220-225.

The optimal value of the ratio is 1.32–1.68, and its reduction signifies the development of hypocalcemia. Conversely, its increase indicates hypophosphatemia and the development of the aphosphorous form of osteodystrophy ¹⁵.

The magnesium concentration in the blood serum of cows of the control group tended to decrease from 0.97 ± 0.03 mmol/l at the beginning of the experiment to 0.79 ± 0.02 on the 60th and 90th days of the study ($p < 0.001$) (Fig. 3).

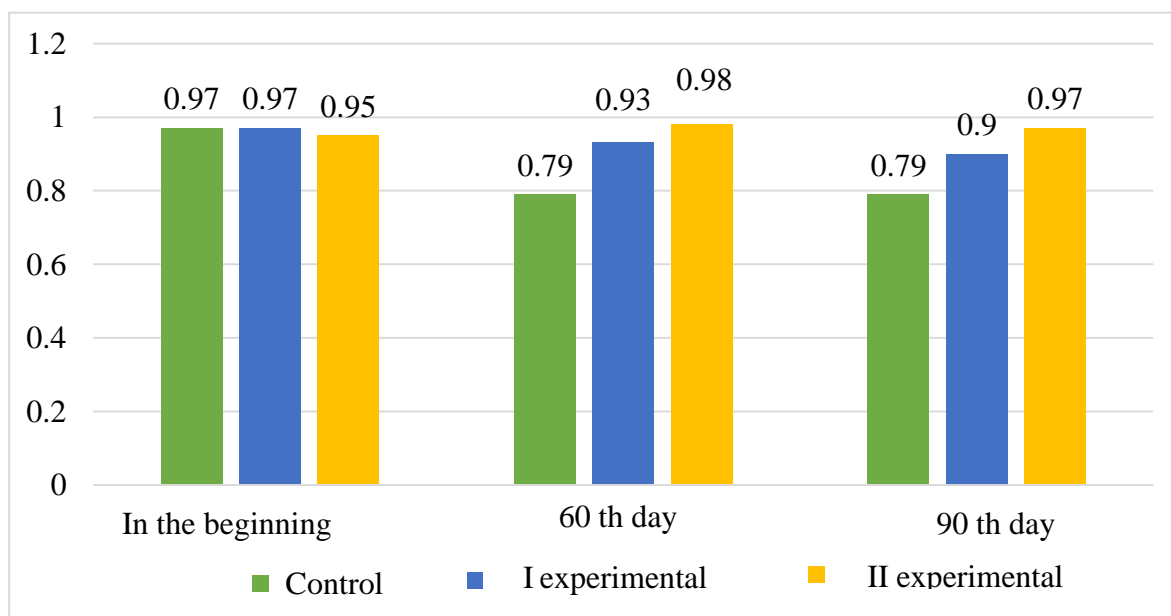


Fig. 3 – Magnesium content in blood serum of cows, mmol/l

The insufficient intake of magnesium in the diet of animals does not always decrease since magnesium is mobilized from depots, primarily muscles and bone tissue ¹⁶. But in our case, the amount of magnesium in the diet was in excess (+132.4% of the need). This leads to the displacement of calcium ions from the mineralization processes of bone tissue, resulting in osteodystrophy.

The development of osteodystrophy is deepened by a lack of calcium in the diet, which leads to the activation of mechanisms to support its homeostasis. As a result,

¹⁵ Долецький С. П. Теоретичне та клініко-експериментальне обґрунтування профілактики порушень мінерального обміну в корів у біогеохімічних зонах України: дис... д-ра вет. наук: 16.00.01. Нац. у-тет біоресурсів та природокористування України. Київ. 2015. 38 с.

¹⁶ Ветеринарна клінічна біохімія [текст]: підручник / В.І.Левченко, В.В.Влізло, І.П.Кондрахін та ін.; за ред. В.І. Левченка і В.Л. Галяса. Біла Церква. 2019. 400 с.

even more, calcium depletion of the bone occurs^{17, 18, 19}. In the cows of the control group, a decrease in magnesium concentration in blood serum was noted at the end of the experiment. The cause may be an excess of potassium in the diet, which is its antagonist. The potassium in the diet exceeded the need by 188.3%, which increased its ratio to magnesium, which was 4.45.

During the study, the magnesium concentration in blood serum in group I, tended to decrease from 0.97 ± 0.03 to 0.93 ± 0.03 mmol/l ($p_1 < 0.5$) on the 60th day, and on the 90th, the macroelement content was lower by 7.2% ($p_1 < 0.05$), compared to the beginning. However, it remained within the normal range (Fig. 3). On the 60th and 90th days of the experiment, the average concentration of magnesium in the blood serum of the cows of the first group was higher by 17.7 and 13.9% ($p_3 < 0.01$; 0.001), respectively, compared to the indicator in the cows of the control group. The magnesium concentration was at the same level in the blood serum of cows of the II experimental group, where chelated compounds of microelements were used. In particular, at the beginning of the experiment, its average concentration was 0.95 ± 0.02 mmol/l. On the 60th day, – 0.98 ± 0.03 , and at the end of the experiment, the average magnesium content was 0.97 ± 0.02 mmol / l. On the 60th and 90th days, it was higher by 24.0 and 22.8% ($p_4 < 0.001$), respectively, compared to the indicators of cows of the control group. Compared to the first group of cows, the concentration of magnesium in the blood serum of animals of the second experimental group on the 90th day was higher by 7.8% ($p_5 < 0.05$).

3. The content of microelements in the blood of cows with osteodystrophy in the case of using mineral compounds

Among the trace elements indirectly involved in osteogenesis, an important role

¹⁷ Петренко О.С. Гіпокальціємія і гіпофосфатемія високопродуктивних корів: автореф. дис. на здобуття наук. ступеня канд. вет. наук: спец. 16.00.01 “Діагностика і терапія тварин”. Білоцерківський національний аграрний університет. Біла Церква. 2011. 24 с.

¹⁸ Slivinska, L., Fedorovych, V., Gutyj, B., Lychuk, M., Shcherbatyy, A., Gudyma, T., Chernushkin, B., Fedorovych, N. The occurrence of osteodystrophy in cows with chronic micronutrients deficiency. *Ukrainian journal of Ecology*. 2018. Vol. 8. Iss. 2. P. 24-32. Doi: 10.15421/2018_305.

¹⁹ Bodiako, O., Golovakha, V., Tyshkivskyi, M., & Shcherbatyi, A. Metabolism macroelements in foals for hypercalcitoninemia. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*. Series: Veterinary Sciences. 2017. T. 19. № 77. P. 80-85. <https://doi.org/10.15421/nvlvet7719>

belongs to cobalt, copper, manganese, and zinc. The supply of cobalt and zinc in the diet of farm cows was insufficient^{20, 21}. Therefore, we analyzed the effectiveness of using inorganic salts and chelated compounds of trace elements for osteodystrophy of cows.

In the blood of cows of the control group, the cobalt content at the beginning of the study was, on average, $0.28 \pm 0.011 \mu\text{mol/l}$. However, during the experiment, its concentration decreased to 0.19 ± 0.009 and $0.18 \pm 0.004 \mu\text{mol/l}$ on the 60th and 90th days, respectively.

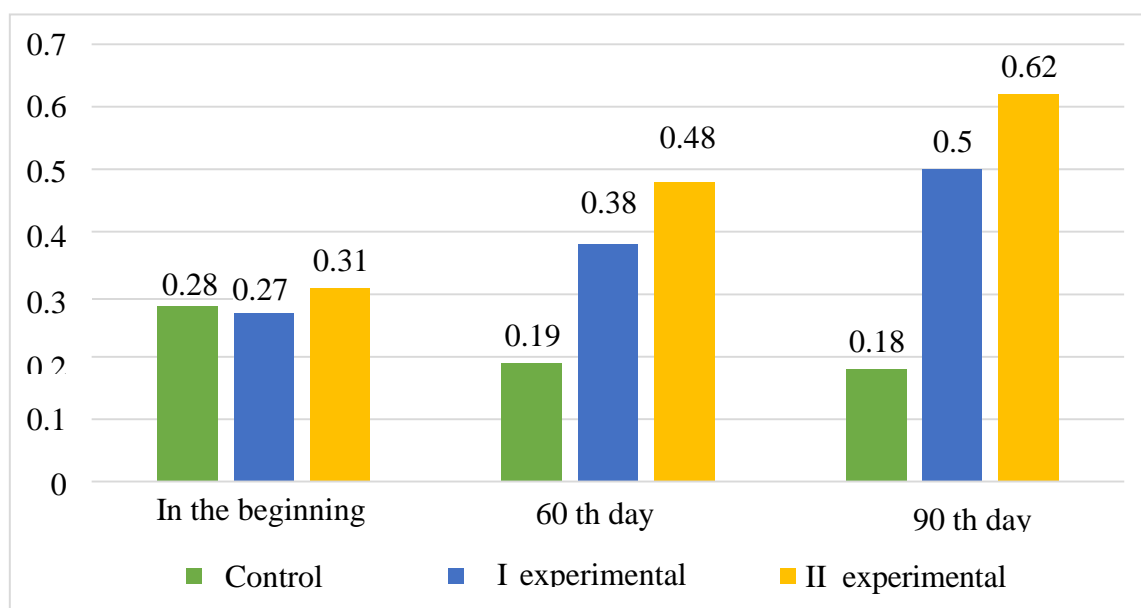


Fig. 4 – Cobalt content in the blood of cows, $\mu\text{mol/l}$

Changes in the level of cobalt in the blood of cows of the control group lead to hematopoiesis disorders^{22, 23}, which generally complicates the course of

²⁰ Slivinska, L. G., & Fedorovuch, V. L. Clinical status of cows osteodystrophy. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*. Series: Veterinary Sciences. 2015. T. 17. № 1. P. 170-175.

²¹ ShcherbatyyA., & Slivinska, L. Effect on indices premix Marmiks exchange trace elements in the pregnant mare. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*. Series: Veterinary Sciences. 2015. T. 17. №1. P. 225-230.

²² Slivinska, L.G., Shcherbatyy, A.R., Lukashchuk, B.O., Zinko, H.O., Gutyj, B.V., Lychuk, M.G., Chernushkin, B.O., Leno, M.I., Prystupa, O.I., Leskiv, K.Y., Slepokura, O.I., Sobolev, O.I., Shkromada, O.I., Kysterna, O.S., Usiienko, O.V.. Correction of indicators of erythrocytopoesis and microelement blood levels in cows under conditions of technogenic pollution. *Ukrainian journal of Ecology*. 2019. Vol. 9. Iss. 2. P.127-135.

²³ Щербатий А.Р., Слівінська Л.Г. Лікувально-профілактична ефективність мінерально-вітамінного преміксу Мармікс за гіпокобальтозу і гіпокупрозу кобил. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2013. Том 15, №3 (57). Частина 1. С. 378-385.

osteodystrophy.

The cobalt content in the blood of cows of the first experimental group raised on the 60th day to $0.38 \pm 0.011 \mu\text{mol/l}$ ($p_1 < 0.001$) and was 40.7% higher compared to the beginning and by 90– to $0.50 \pm 0.008 \mu\text{mol/l}$ or by 85.2% ($p_1 < 0.001$). Compared with the control, the level of cobalt on the 60th and 90th days of the study was also 2 and 2.8 times more elevated ($p_3 < 0.001$), respectively (Fig. 4).

In the blood of cows of the II experimental group, whose diet included chelated compounds of microelements, the cobalt content on the 60th day of the experiment increased by 54.8% ($p_2 < 0.001$) compared to the beginning and on the 90th day by 100% ($p_2 < 0.001$; Fig. 4). Along with this, its level was higher on the 60th and 90th day of the study by 2.5 and 3.4 times ($p_4 < 0.001$), compared to animals of the control group.

In experimental cows, its content was higher ($p_5 < 0.001$) on the 60th and 90th days of the experiment by 26.3 and 24% compared to the indicator in the first group of animals.

Physiologically, cobalt ions play an indirect role in the processes of osteogenesis. In particular, they mineralize bone tissue through interaction with copper, which generally ensures the synthesis of bone collagen^{24, 25}. Cobalt also increases the activity of bone phosphatase, which plays an essential role in the mineralization of bone tissue. In osteoblasts, it catalyzes enzymatic reactions^{26, 27}.

The content of copper in the blood of cows of the control group decreased during the entire study period. In particular, on the 60th day, compared with the beginning of the experiment, its level in the blood of cows reduced by 11.9% ($11.8 \pm 0.05 \mu\text{mol/l}$; $p < 0.001$) and by 15.0% at the end of the experiment ($11.4 \pm 0.02 \mu\text{mol/l}$, $p < 0.001$)

²⁴ Слівінська Л.Г. Вміст заліза, кобальту, міді, вітаміну В₁₂ та фолієвої кислоти у крові корів, хворих на хронічну гематурію. *Вісник Білоцерківського державного аграрного університету*: Зб. наук. праць. 2008. Вип. 51. С. 85–90.

²⁵ Slivinska, L.G., Shcherbatyy, A.R., Lukashchuk, B.O., Zinko, H.O., Gutyj, B.V., Lychuk, M.G., Chernushkin, B.O., Leno, M.I., Prystupa, O.I., Leskiv, K.Y., Slepokura, O.I., Sobolev, O.I., Shkromada, O.I., Kysterna, O.S., Usienko, O.V. Correction of indicators of erythropoiesis and microelement blood levels in cows under conditions of technogenic pollution. *Ukrainian journal of Ecology*. 2019. Vol. 9. Iss. 2. P.127-135.

²⁶ Shcherbatyy, A.R., Slivinska, L.G., Gutyj, B.V., Fedorovych, V.L., Lukashchuk, B.O. Influence of Marmix premix on the state of lipid peroxidation and indices of non-specific resistance of the organism of pregnant mares with microelementosis. *Regulatory Mechanisms in Biosystems*. 2019. Vol. 10. Iss. 1. P.87-91. Doi: 10.15421/021914.

²⁷ Федорович В.Л. Мікроелементні сполуки у профілактиці остеодистрофії корів. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2011. Т. 14. № 3 (53). Ч. 1. С. 292–297.

(Fig.5).

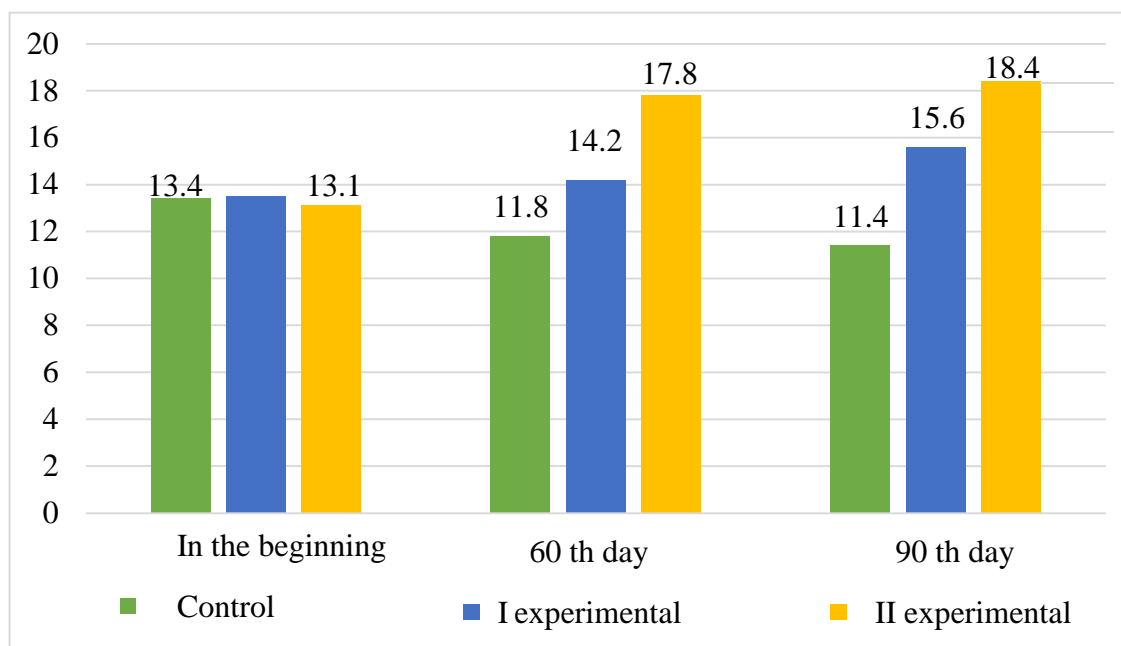


Fig. 5 – Copper content in the blood of cows, $\mu\text{mol/l}$

In the blood of cows of the 1st experimental group fed with inorganic salts of trace elements on the 60th and 90th days of the experiment, the average copper content was 14.2 ± 0.12 and 15.6 ± 0.05 $\mu\text{mol/l}$, which was higher 5.2 and 15.5% ($p_1 < 0.001$) compared to the beginning (13.5 ± 0.06 $\mu\text{mol/l}$). It was also established that its content in the blood of cows was 20.3 and 36.8% higher ($p_3 < 0.001$), respectively, compared to the control.

The copper content in the blood of cows of the II group fed with chelated compounds of trace elements on the 60th day of the study averaged 17.8 ± 0.22 $\mu\text{mol/l}$ and was higher by 35.8% ($p_2 < 0.001$) compared to the beginning.

Subsequently, its content also continued to increase ($p_2 < 0.001$). After the end of the experiment, it averaged 18.4 ± 0.16 $\mu\text{mol/l}$ and was 40.5% higher ($p_2 < 0.001$) compared to the beginning of the experiment (Fig. 5).

On the 60th and 90th days of the research, the copper content in the blood of the cows of the second group was higher by 50.8 and 61.4% ($p_4 < 0.001$) compared to the control group of animals. In the blood of cows of the II group, fed with chelated compounds of microelements, the copper content was higher by 25.3 and 18.0% ($p_5 < 0.001$), compared to the first group, whose diet included inorganic salts of trace elements.

Cuprum ions catalyze enzymatic reactions in osteoblasts, thus contributing to the synthesis of bone tissue and its remodeling^{28, 29}. Therefore, using mineral feeding in cows of the I and II experimental cows helps reduce resorption processes; on the other hand, it helps build bone tissue.

The manganese content in the blood of cows of the control group ranged from 1.95 to 2.20 $\mu\text{mol/l}$. During the experiment, it decreased from the initial values of 2.09 ± 0.02 to 1.97 ± 0.04 $\mu\text{mol/l}$ at the end of the experiment, which was 5.7%. In the blood of the cows of the first group, the manganese content on the 60th and 90th days increased by 29.8% ($p_1 < 0.001$) compared to the beginning. The indicator was also 37.0% ($p_3 < 0.001$) higher compared to the control group of cows (Fig. 6).

In the blood of cows of the II experimental group, the average manganese content on the 60th day was 2.8 ± 0.05 $\mu\text{mol/l}$ on average. However, on the 90th day - 3.0 ± 0.03 $\mu\text{mol/l}$, and was higher by 40.0 and 50.0% ($p_2 < 0.001$), compared to the beginning of the study (2.0 ± 0.02 $\mu\text{mol/l}$).

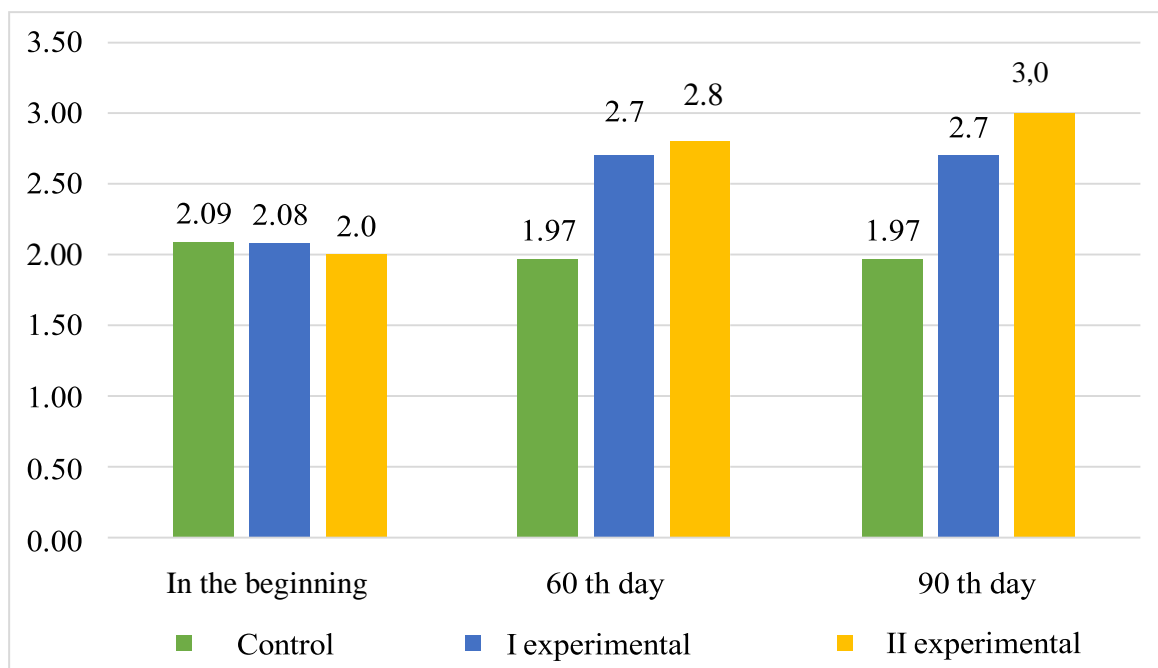


Fig. 6 – Manganese content in the blood of cows, $\mu\text{mol/l}$

On the 60th and 90th days of the experiment, the manganese content was higher

²⁸ Rondanelli M., Faliva M.A., Infantino V., Gasparri C., Iannello G., Perna S., Riva A., Petrangolini G., Tartara A., Peroni G. Copper as Dietary Supplement for Bone Metabolism: A Review. *Nutrients*. 2021. №13(7). P. 2246. doi: 10.3390/nu13072246

²⁹ Slivinska L.G, Shcherbatyy A.R., Lukashchuk B.O. Hypocobaltosis and hypocuprosis in pregnant mares in the western biogeochemical zone of Ukraine (distribution, diagnosis). *Ukrainian journal of veterinary and agricultural sciences*. 2018. Vol 1 (2). P. 11-14. doi: 10.32718/ujvas1-2.03

by 42.1 and 52.3% ($p_4 < 0.001$) compared to the control group of animals (Fig. 6).

In addition, in the blood of cows of the II group, which were fed chelated compounds of microelements, on the 90th day of the experiment, the manganese content was higher by 11.1% ($p_5 < 0.001$), compared to the index of cows of the I group, which included the diet included inorganic compounds of trace elements (Fig. 6.).

Manganese, in ossification processes, activates bone alkaline phosphatase and participates in the mineralization of collagen fibrils of bone tissue^{30, 31, 32}. In the I and II experimental groups, under the influence of the use of inorganic and chelated compounds of microelements, the mechanism of mineralization of bone tissue was restored.

The zinc content in the blood of the cows of the experimental and control groups at the beginning of the research was at the lower limit of physiological fluctuations. During the study, it continued to remain unchanged in the blood of the cows of the control group, as its deficiency was detected in the diet (39.2% to the requirement), which does not make it possible to ensure its proper level in the blood of the animals of this group.

In the blood of cows of the 1st experimental group, an increase in zinc content was established by 9.6% ($p_1 < 0.001$) (60th day) and by 14.1% (90th day; $p_1 < 0.001$) than in the beginning of the experiment and at 11.8 and 16.3% ($p_3 < 0.001$) compared to the control group of animals (Fig. 7). In the blood of cows of the II research group, the use of chelated zinc compounds on the 60th and 90th days caused an increase in its content by 21.8 and 31.1% ($p_2 < 0.001$) compared to the beginning of the study. The level of zinc was also higher by 20.2 and 29.4% ($p_4 < 0.001$), respectively, compared to animals of the control group.

³⁰ Rondanelli M., Faliva M.A., Peroni G., Infantino V., Gasparri C., Iannello G., Perna S., Riva A., Petrangolini P., Tartara A. Essentiality of Manganese for Bone Health: An Overview and Update. *Natural Product Communications*. 2021. № 16(5). doi:10.1177/1934578X211016649

³¹ Beattie J.H., Avenel A. Trace element and bone metabolism. *Nutrition Research Reviews*. 1992. №5. P.167–188.

³² Underwood E.J., Suttle N. F. The Mineral Nutrition of Livestock. CABI Publishing. 2021. 614 p.

The chelated compounds of microelements in cows of the II research group showed a better therapeutic effect since the zinc content was higher on the 60th day of the study - by 7.6% ($p_5 < 0.001$), on the 90th - by 11.2% compared to the I group.

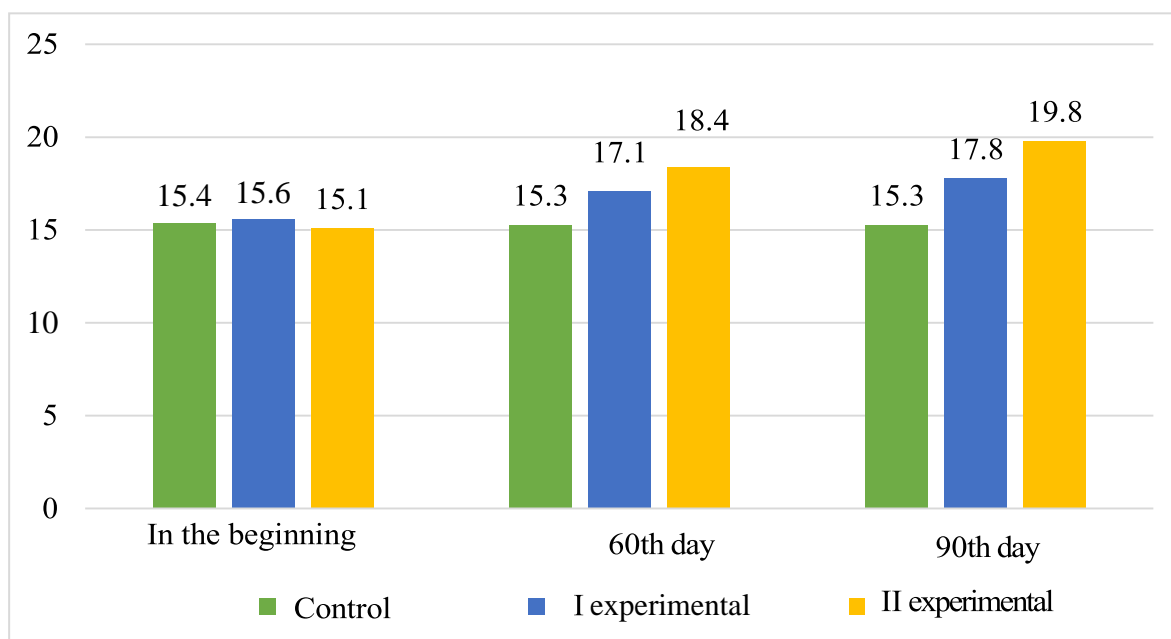


Fig. 7 – Zinc content in the blood of cows, $\mu\text{mol/l}$

In the blood of cows of the I and II experimental groups, restoring the zinc level to optimal values indicates the cessation of bone tissue demineralization processes since its biological role in osteogenesis is related to bone mineralization^{33, 34}.

Therefore, the use of inorganic and chelated compounds of trace elements for osteodystrophy of cows contributes to the normalization of the blood's cobalt, copper, manganese, and zinc content.

4. Changes in the indicators of connective tissue metabolites in the blood serum of cows with osteodystrophy during the application of trace elements

Bone tissue in its composition contains many organic components (collagen and non-collagen proteins, glycosaminoglycans, etc.), the determination of which can be used to diagnose disorders of mineral metabolism, in particular, osteodystrophy in

³³ Beattie J.H., Avenel A. Trace element and bone metabolism. *Nutrition Research Reviews*. 1992. №5. P.167–188.

³⁴ Судаков М.О., Береза В.І., Погурський І.Г. Мікроелементози сільськогосподарських тварин. Київ: Урожай. 1992.

animals³⁵.

The total glycosaminoglycans (GAG) content in the blood serum of cows of the control group was, on average, 26.6 ± 0.63 mg/100 ml. However, on the 60th and 90th days of the experiment, the content of total GAG grew to 39.6 ± 0.67 and 44.1 ± 0.47 mg/100 ml, respectively ($p < 0.001$). An increase in the total GAG content in the cows' blood serum indicates the destruction and resorption of the organic part of the bone, which is an indicator of the clinical stage of osteodystrophy.

In the cows of the first experimental group, the average content of total GAGs during the study also increased from the initial - 26.4 ± 0.56 to 31.1 ± 0.39 mg/100 ml at 60 and 32.0 ± 0.40 mg/100 ml - 90 days of the experiment. In addition, it was greater by 17.8 and 21.2% ($p_1 < 0.001$), compared to the initial indicator. However, relative to the control group, the content of total GAGs on the 60th and 90th day of the study was lower by 21.4 and 27.4% ($p_3 < 0.001$), respectively.

The increase in the content of total GAGs in the blood serum of the cows of the first research group to the indicated values is the result of the decay of the organic part of the bone, which is characteristic of the subclinical course of osteodystrophy.

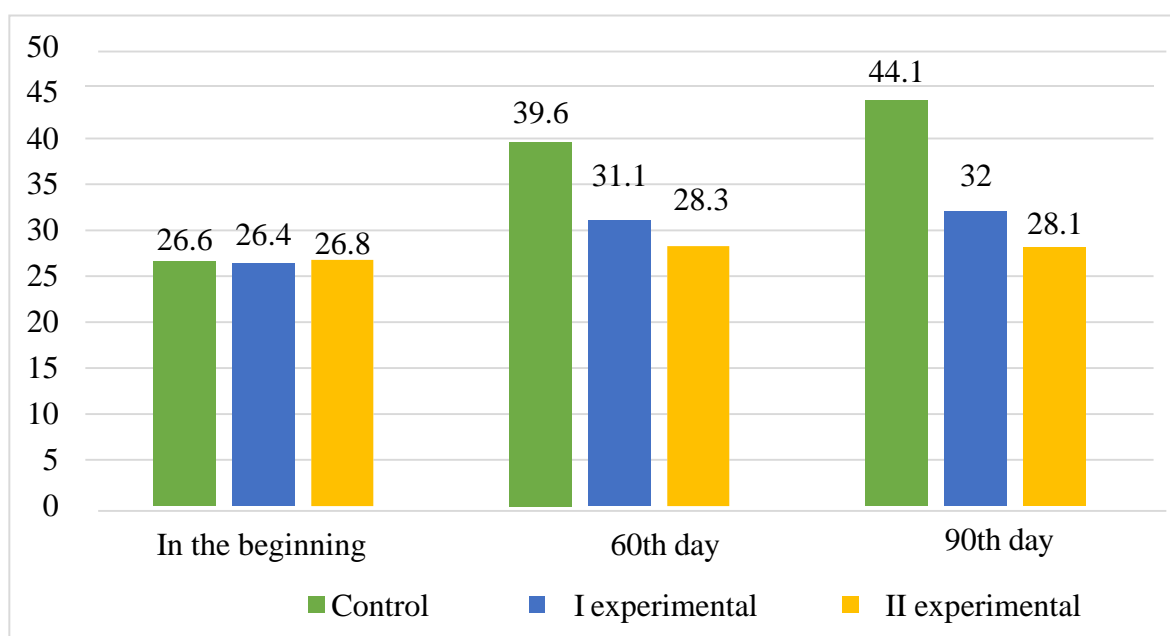


Fig. 8 – Content of total GAGs in blood serum of cows

³⁵ Маслак Ю.В., Собакар А.В. Етіопатогенез остеодистрофії у кіз зааненської породи. *Вісник Полтавської державної аграрної академії*. 2015. №1-2. С.119-123.

In the cows of the second experimental group, which were fed with chelated compounds of microelements during the study, the content of total GAG in the blood serum increased on the 60th day ($p_2 < 0.05$; Fig. 8), but on the 90th day - it remained at the level of $28, 1 \pm 0.34$ mg/100 ml, which is 4.9% more ($p_2 < 0.05$) compared to the beginning of the experiment (26.8 ± 0.55 mg/100 ml). In contrast to the control group, the content of total GAGs on the 60th and 90th days was 1.40 and 1.57 times lower ($p_4 < 0.001$) and 9.0 and 12.2% descending than the values of the first experimental group groups.

Analysis of the fractional composition of total GAGs in blood serum showed that the content of all three fractions increased in cows of the control group during the study. In particular, on the 90th day of the experiment, the content of X-6-C - by 63.2%, X-4-C - by 49.1%, and the third fraction - by 87.3% increased significantly ($p < 0.001$) compared to the beginning of the experiment.

In the cows of the first group, the indicators of each of the three fractions of GAG increased during the experiment. On the 90th day, they were higher by 20.4, 14.4, and 21.2% ($p_1 < 0.001$), compared to the beginning, and relative to the control group of cows, their concentration at the end of the study was lower by 27.2%; 21.6 and 27.4% ($p_3 < 0.001$).

In the cows of the second experimental group, chelated compounds of trace elements were used by an increase in the content of X-6-C, keratan, heparan and dermatan sulfates, and heparin. On the 90th day, their content was higher by 6.1 and 9.7% ($p_2 < 0.001$), respectively, compared to the beginning of the experiment. It was lower by 33.2%, 36.4 and 42.4% ($p_4 < 0.001$) than in the control group and by 8.2; 18.8 and 15.0% ($p_5 < 0.001$) compared to the indicators of cows of the first research group.

An increase in the content of all fractions of GAG in the blood serum of cows of the control group indicates the breakdown of the components of the intercellular matrix of bone tissue.

In addition, we determined the redistribution of GAG fractions - X-6-C, X-4-C, and keratan-, heparan-, dermatan sulfate, and heparin (Table 2). In the cows of the first experimental group, the percentage of the first and second fractions of GAG remained

stable, while the redistribution took place at the expense of the third. Thus, the use of chelated compounds of microelements showed a better therapeutic effect, compared to inorganic salts, as it contributed to the stabilization of the content of biopolymers in the cows of the second research group.

Table 2

Redistribution of HAG fractions during the study, in percent

GAG factions	Control group			The first is experimental			The second is experimental		
	In the beginning	60 th day	90 th day	In the beginning	60 th day	90 th day	In the beginning	60 th day	90 th day
Chondroitin-6-sulfate	54,1	56,5	53,3	53,8	55,3	53,4	55,2	52,6	55,9
Chondroitin-4-sulfate	22,2	20,2	19,9	22,7	21,9	21,6	21,7	23,0	19,9
Keratan, heparan, dermatan sulfates and heparin	23,7	23,3	26,8	23,5	22,8	25,0	23,1	24,4	24,2
The amount of GAG	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0

The concentration of another metabolite of connective tissue - chondroitin sulfate at the beginning of the experiment was higher in all groups compared to the indicators of clinically healthy cows. In the blood serum of cows of the control group, the concentration of chondroitin sulfates continued to increase from 0.36 ± 0.01 mg/100 ml to 0.38 ± 0.01 mg/100 ml ($p < 0.5$) on the 60th day and up to 0.46 ± 0.01 mg/100 ml after the end of the experiment ($p < 0.001$; Fig. 9).

Before feeding inorganic salts of microelements in the cows of experimental group I, the content of chondroitin sulfates was, on average, 0.35 ± 0.01 g/l and did not change during the experiment (0.34 ± 0.01 g/l). At the same time, on the 90th day of the experiment, their concentration was lower by 26.1% ($p_3 < 0.001$) compared to animals of the control group, which is explained by the therapeutic effect of inorganic salts of trace elements.

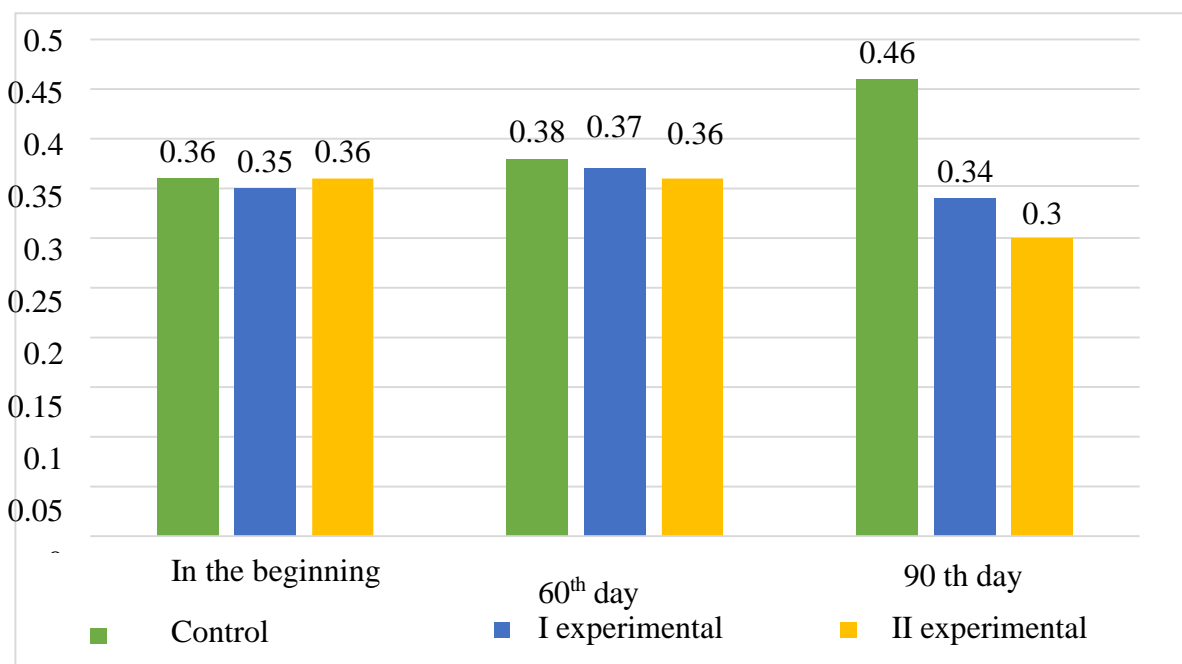


Fig. 9 – Content of chondroitin sulfates in blood serum of cows, g/l

The chondroitin sulfates concentration in the cows of the second experimental group decreased from 0.36 ± 0.01 g/l at the beginning of the experiment to 0.30 ± 0.01 g/l. At the end of the experiment ($p_2 < 0.001$), and corresponded to the indicators of clinically healthy animals. On the 90th day of the experiment, their content was probably ($p_4 < 0.001$) lower by 34.7% compared to the indicators in the control group of cows and by 11.7% ($p_5 < 0.05$) compared to the first experimental group (Fig. 9).

At the beginning of the study, the sialoglycoproteins concentration in the blood serum of the cows of the experimental groups did not differ from the indicators of clinically healthy cows. However, in the blood serum of cows of the control group, the concentration increased on the 60th day and averaged 3.28 ± 0.05 mmol/l, and on the 90th day – 3.88 ± 0.06 mmol/l, which was higher on the 70,0 and 100% ($p < 0.001$), respectively, compared to the beginning (Fig. 10).

Such an increase in sialoglycoproteins indicates the deepening of pathological processes and the development of the clinical stage of osteodystrophy, in which the destruction of organic components of bone tissue increases.

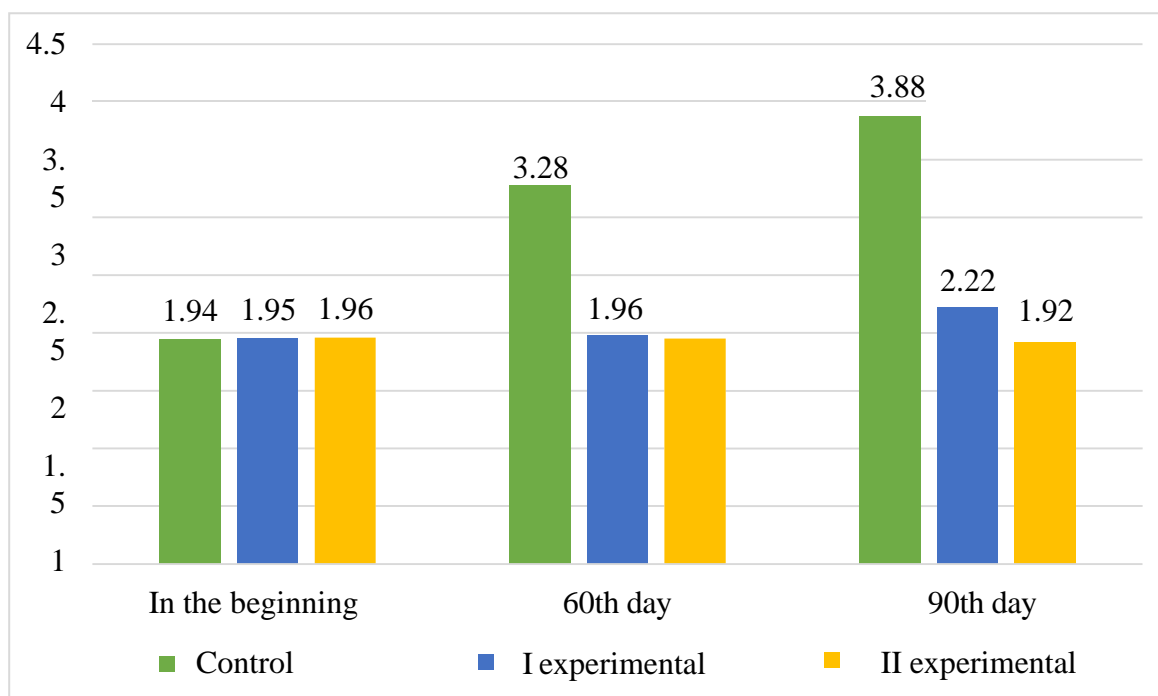


Fig. 10 – Content of sialoglycoproteins in blood serum of cows, mmol/l

In the first experimental group, the sialoglycoproteins concentration at the beginning of the experiment was, on average, 1.95 ± 0.05 mmol/l, and the 90th day of the experiment – 2.22 ± 0.03 mmol/l ($p_1 < 0.001$; Fig. 10). Such indicators were typical for cows with a subclinical stage of osteodystrophy. It should be noted that the concentration of sialoglycoproteins in the blood serum of cows of the 1st experimental group, which were fed inorganic salts of trace elements, was lower by 42.8% ($p_3 < 0.001$) compared to their level in the control group of animals (Fig. 10).

In the cows of the second experimental group, the concentration of sialoglycoproteins in blood serum, which received chelated compounds of microelements, did not change ($p_2 < 0.5$). However, compared to the control group of animals, on the 90th day of the experiment, their concentration was lower by 50.5% ($p_4 < 0.001$) and by 13.5% ($p_5 < 0.001$) compared to the first experimental group (Fig. 10).

Oxyproline is a component of collagen and reflects its catabolism. Its content in the blood serum of cows of the control group during the experiment increased from 65.0 ± 1.72 $\mu\text{mol/l}$ to 107.4 ± 2.48 and 127.7 ± 0.92 $\mu\text{mol/l}$. On the 60th and 90th days, which were 1.65 and 1.97 ($p < 0.001$) times higher, respectively, compared to the

beginning of the study (Fig. 11). An increase in the concentration of oxyproline indicates the destruction of collagen in bone tissue.

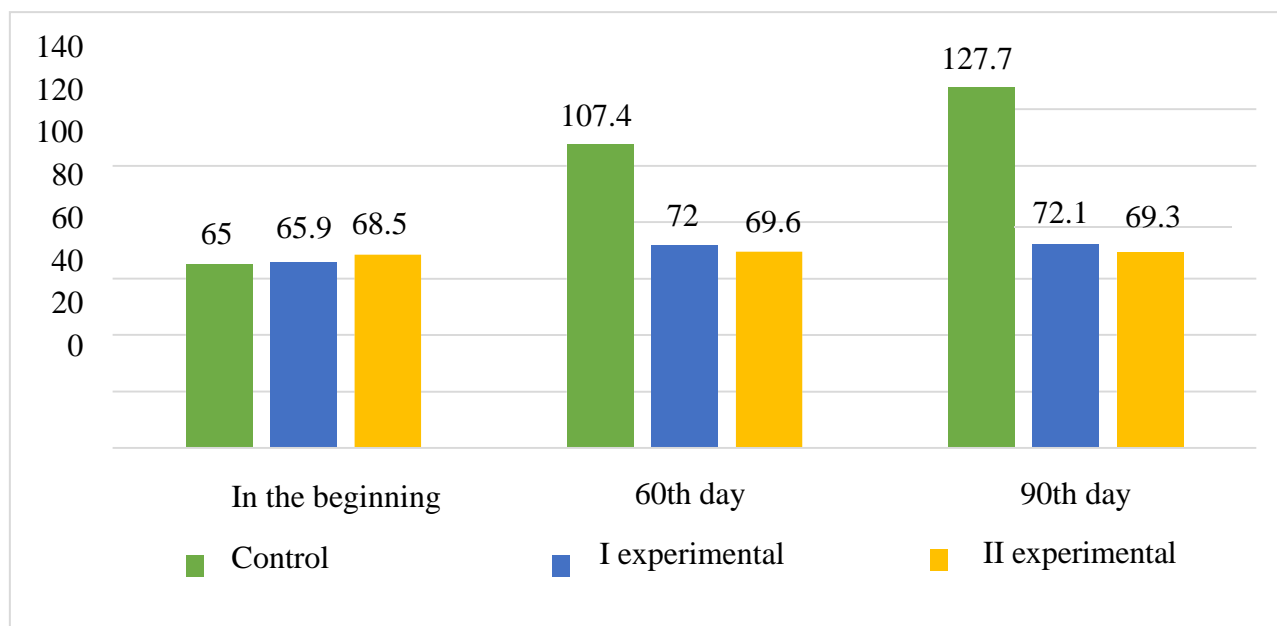


Fig. 11 – Oxyproline content in blood serum of cows, $\mu\text{mol/l}$

The oxyproline concentration in the blood serum of cows of the 1st group at the beginning of the experiment was, on average, $65.9 \pm 1.45 \mu\text{mol/l}$. However, on the 90th day – $72.1 \pm 0.86 \mu\text{mol/l}$ ($p_1 < 0.01$) was smaller by 43.5% ($p_3 < 0.001$) than in cows of the control group (Fig. 11).

In cows of the II experimental group, the results of the determination of oxyproline in blood serum were characterized by constant values (Fig. 11). On the 90th day of the experiment, the content of oxyproline in the blood serum of cows of the II group was lower by 45.7% ($p_4 < 0.001$) compared to the control group, and compared to the I group by 4.0% ($p_5 < 0.05$).

The central part of oxyproline is excreted in the urine due to the breakdown of collagen. Thus, the concentration of oxyproline in the urine of cows of the control group at the beginning of the experiment was $444.8 \pm 7.87 \mu\text{mol/l}$. However, in the end, it increased on average to $777.5 \pm 7.15 \mu\text{mol/l}$ ($p_1 < 0.001$) and was higher by 74.8% compared to the beginning of the experiment (Fig. 12).

In the urine of cows of the 1st experimental group, the concentration of

oxyproline also increased ($p_1 < 0.001$), but fluctuated within the reference values corresponding to the indicators of clinically healthy cows.

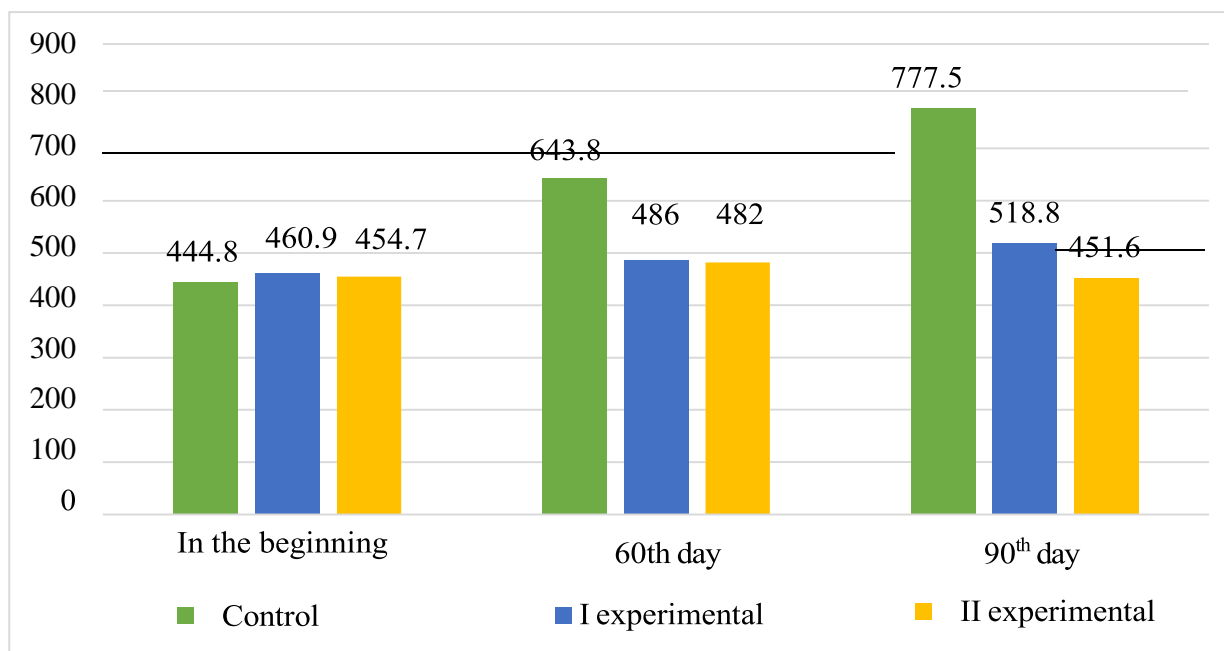


Fig. 12 – Concentration of oxyproline in the urine of cows, $\mu\text{mol/l}$

On the 60th and 90th days of the study, the average concentration of oxyproline in the urine of cows of the 1st experimental group, which were fed inorganic salts of trace elements, was lower by 24.5 and 33.3% ($p_3 < 0.001$), respectively, compared to the control group of cows (Fig. 12).

When feeding chelated compounds of trace elements in the cows' urine, the oxyproline concentration increased slightly on the 60th day. After the end of the experiment, it decreased and did not differ from the initial level ($p_2 < 0.5$; Fig. 12). At the same time, on the 60th and 90th days, the concentration of the marker in the urine was probably lower by 25.1 and 42.0%, respectively, compared to the control group of cows ($p_4 < 0.001$), and the difference with the indicators in cows of the 1st group was smaller by 13.0% ($p_5 < 0.001$) only on the 90th day. Thus, in cows fed inorganic salts of microelements, an increase in total GAG, chondroitin sulfates, and sialoglycoproteins in blood serum and oxyproline in urine was increased established. Stabilization (sialoglycoproteins) and reduction (total GAG, chondroitin sulfates, and oxyproline) of markers of connective tissue metabolism were established in cows

receiving chelated compounds of microelements, which indicates the suspension of destructive processes in the bone tissue of the studied cows.

Therefore, using chelated compounds of microelements gave a better therapeutic and preventive effect than inorganic salts of minerals ³⁶.

5. The concentration of citric acid in the blood serum of cows when using trace elements

We studied the dynamics of citric acid in the blood serum of cows with the comparative use of inorganic salts and chelated compounds of microelements.

At the beginning of the study, the citric acid content in the blood serum of cows of all experimental groups ranged from 227.2 to 262.4 $\mu\text{mol/l}$, corresponding to the indicators of clinically healthy cows.

The citric acid content is a highly informative indicator of the state of bone tissue. After 60 and 90 days from the beginning of the study, the blood serum of cows of the control group decreased to 210.4 ± 6.06 and 193.4 ± 3.10 $\mu\text{mol/l}$ ($p < 0.001$), respectively (Fig. 13), which is a sign of bone tissue resorption and disruption of its mineralization processes, which occur as a compensatory phenomenon for maintaining calcium homeostasis in the blood. Therefore, the subclinical course of osteodystrophy deepens in cows of the control group.

Sixty days after the start of the application of inorganic salts of trace elements in the cows of the 1st experimental group, the concentration of citric acid in blood serum was, on average, 238.2 ± 1.44 $\mu\text{mol/l}$, and on the 90th day - 230.7 ± 1.14 $\mu\text{mol/l}$, and was less by 5.7 and 8.7% ($p_1 < 0.001$) compared to the beginning of the experiment (Fig.13).

It should be noted that the concentration of citric acid decreased in the experimental group of cows, but the average values corresponded to the indicators obtained in clinically healthy cows. In addition, the concentration of citric acid in the blood serum of cows of the 1st research group on the 60th and 90th day of the study remained higher by 13.2 and 19.3% ($p_3 < 0.001$), respectively, compared to the control.

³⁶ Федорович В.Л. Профілактика остеодистрофії корів в умовах біогеохімічної зони регіону / В.Л. Федорович // Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького. 2011. Том. 13. №4 (50). Ч. 1. С. 472–476.

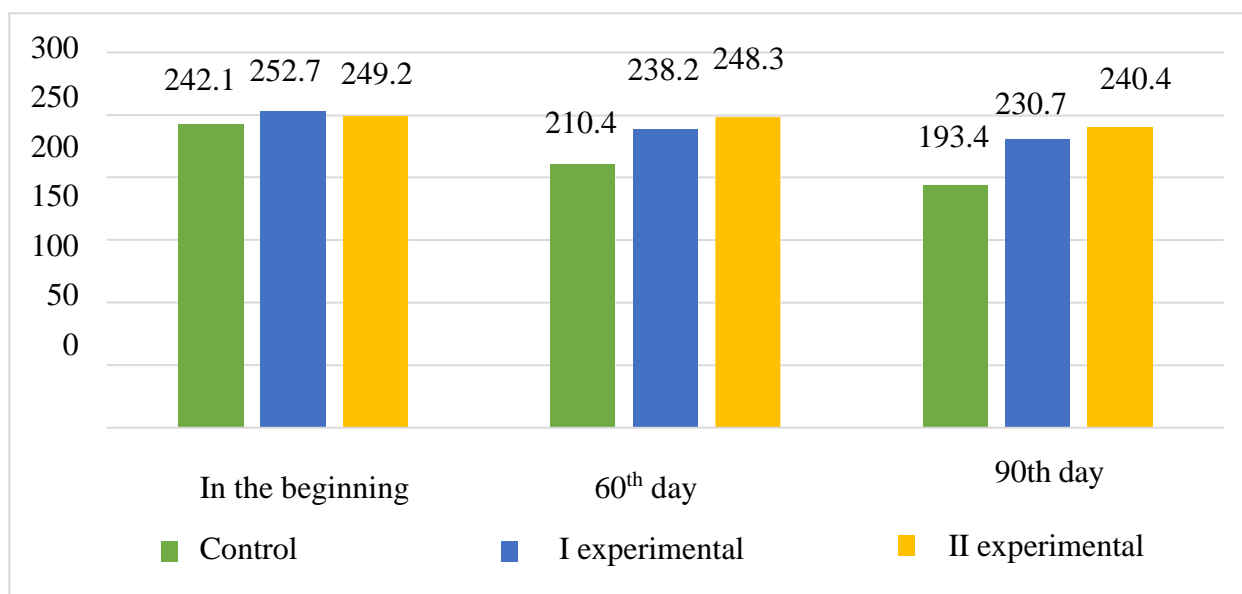


Fig. 13 – Concentration of citric acid in blood serum studied cows, µmol/l

The concentration of citric acid in the blood serum of cows of the II research group receiving chelated compounds of microelements during the study period was within limits corresponding to the indicators of clinically healthy cows.

Throughout the experiment, the concentration of citric acid in the II group of cows did not differ among themselves, compared to the beginning of the study, but on the 90th day, it decreased (3.5%), compared to the beginning (249.2 ± 1.85 µmol/l; $p < 0.001$). After comparing the II and I research groups, we found that on the 60th and 90th day, the concentration of citric acid in the first was probably ($p < 0.05$ and $p < 0.001$;) higher. Therefore, analyzing the obtained research results, we established a positive (stabilizing) effect of mineral feeding on the concentration of citric acid in the blood serum of the cows of the experimental groups.

Although the average concentration of citric acid in the cows of the 1st experimental group corresponded to the content of clinically healthy animals, it still tended to decrease. Its stable concentration in the blood serum of cows of the II research group is connected with the fact that chelated compounds of microelements have a higher degree of assimilation than inorganic salts.

6. The vitamins A, E, and 25OHD₃ content in blood serum for osteodystrophy of cows in the case of the use of trace elements and vitamins

Analysis of vitamin D metabolite content (25OHD₃) in cows of all groups established that its concentration at the beginning of the study was below standard.

In the blood serum of cows of the control group at the beginning, the content of 25OHD₃ ranged from 16.3 to 18.5 nmol/l (17.5±0.16). At the end of the experiment, its average concentration remained unchanged and averaged 17.7±0.13 nmol/l (p<0.5; Fig. 14).

Low-level 25OHD₃ in the blood serum of cows of the control group during the study indicates the development of D-hypovitaminosis and osteodystrophy^{37, 38, 39}. The reason for its low concentration is, first of all, an insufficient amount in the diet, which cannot provide the proper level in the blood serum of the cows of the control group.

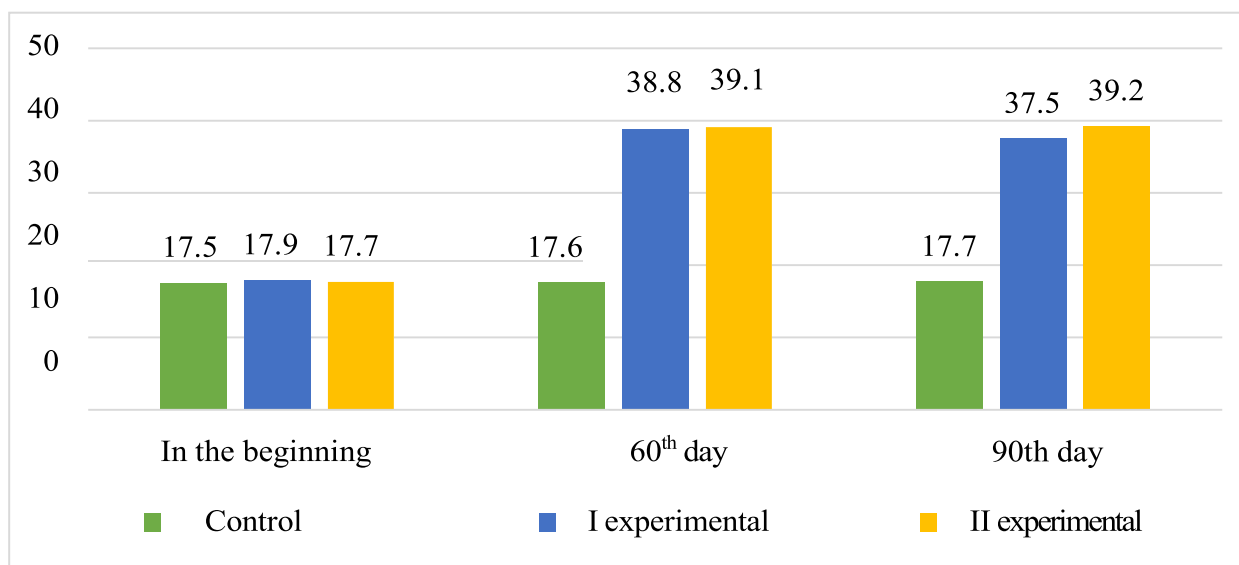


Fig. 14 – Content of 25(OH)D₃, in blood serum of cows, nmol/l

Application of mineral feeding in combination with vitamins I and II to

³⁷ Тишківська Н.В. Інформативність показників вуглеводно-білкових сполук під час діагностики порушень D-вітамінного і фосфорно-кальцієвого обміну у бичків на відгодівлі. *Вісник Білоцерківського державного аграрного університету*. 2008. № 51. С.94–98.

³⁸ В.О. Chernushkin, V.V. Vlizlo, L.G. Slivinska, B.V. Gutyj, A.R. Shcherbatyy, I.A. Maksymovych, M.I. Leno, V.I. Rusyn, M.H. Lychuk, V.L. Fedorovych, B.O. Lukashchuk, H.O. Zinko, O.I. Prystupa. Treatment strategies for sheep with acute yellow atrophy of the liver caused by the fasciolosis. *Ukrainian Journal of Ecology*. 2020. №10(2). P.294-301. doi: 10.15421/2020_100.

³⁹ Шарандак П.В., Левченко В.І. Гепатоостеодистрофічний синдром у вівцематок Сходу України. *Вісник аграрної науки*. 2017. №9. С. 35-39.

experimental groups of cows on the 60th day of the study led to an increase in 25OHD₃ from 17.9±0.07 to 38.8±1.38 nmol/l ($p_1 < 0.001$) and from 17.7±0.14 to 39.1±1.18 nmol/l ($p_2 < 0.001$), which is 2.2 times higher, respectively, with the beginning (Table 15).

The increase in the concentration of 25OHD₃ in the blood serum of the same animals continued in the subsequent period of the study. On the 90th day of the experiment, it was higher by 109.5 and 121.5%, respectively, compared to the beginning. At the same time, in experimental groups I and II, already on the 60th day of the study, the concentration of 25OHD₃ was 2.2 times higher ($p_3 < 0.001$; $p_4 < 0.001$) compared to the control. The vitamin A content at the beginning in the blood serum of the control and experimental groups of cows was practically the same. It was within the physiological range (Fig. 15).

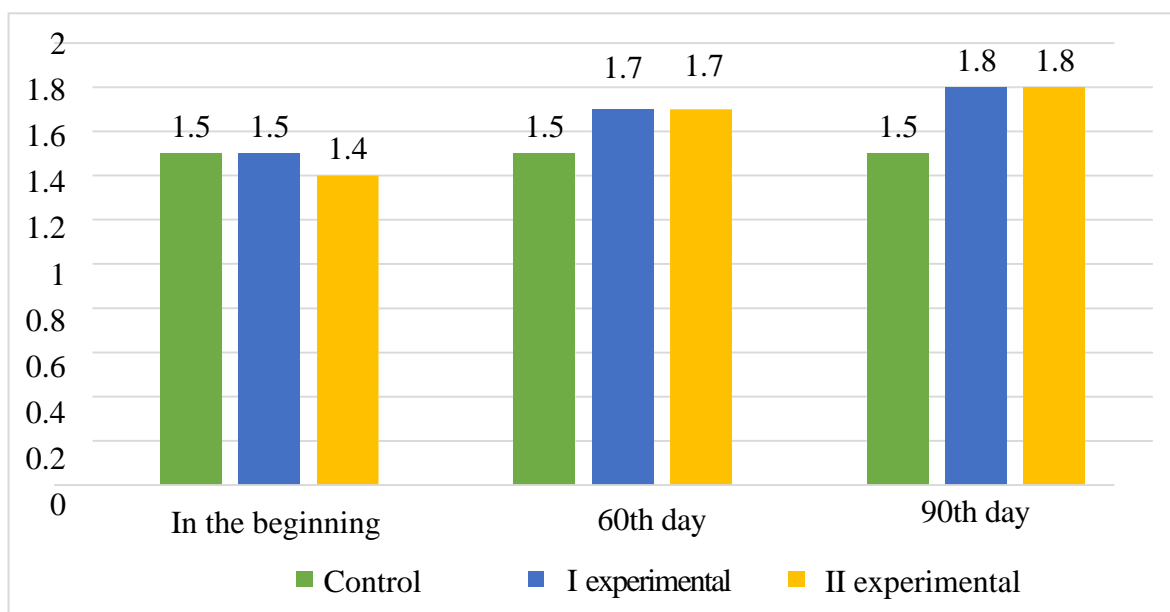


Fig. 15 – Vitamin A content in blood serum of cows, µmol/l

In the blood serum of cows of the control group, the content of vitamin A during the study remained at the initial values (1.5±0.01 µmol/l) and was within the physiological range (Fig. 15). It should be noted that despite the insufficient provision of the diet with its provitamin - carotene (70.4% of the need), the amount of retinol in

the blood serum was adequate due to the depot organ (liver)^{40, 41}.

After 60 and especially after 90 days, an increase in the content of vitamin A in the blood serum of cows of the I and II groups was noted by 13.3 and 21.4% ($p_1 < 0.01$ and 0.001) and 20.0 and 28.6% ($p_2 < 0.001$), respectively, compared to the beginning (Fig. 15).

Similar results were obtained when compared with the control group of cows ($p_3 < 0.001$; $p_4 < 0.001$), but there was no difference in vitamin A content between the indicators of cows in both experimental groups.

The content of vitamin E in the blood serum of cows of the control and experimental groups at the beginning of the experiment was within the physiological range (Table 3). In the blood serum of cows of the control group, its average amount was $9.7 \pm 0.13 \mu\text{mol/l}$. At the end of the research, changes in the concentration of vitamin E ($9.5 \pm 0.11 \mu\text{mol/l}$) were not established, but it was normal (Fig. 16).

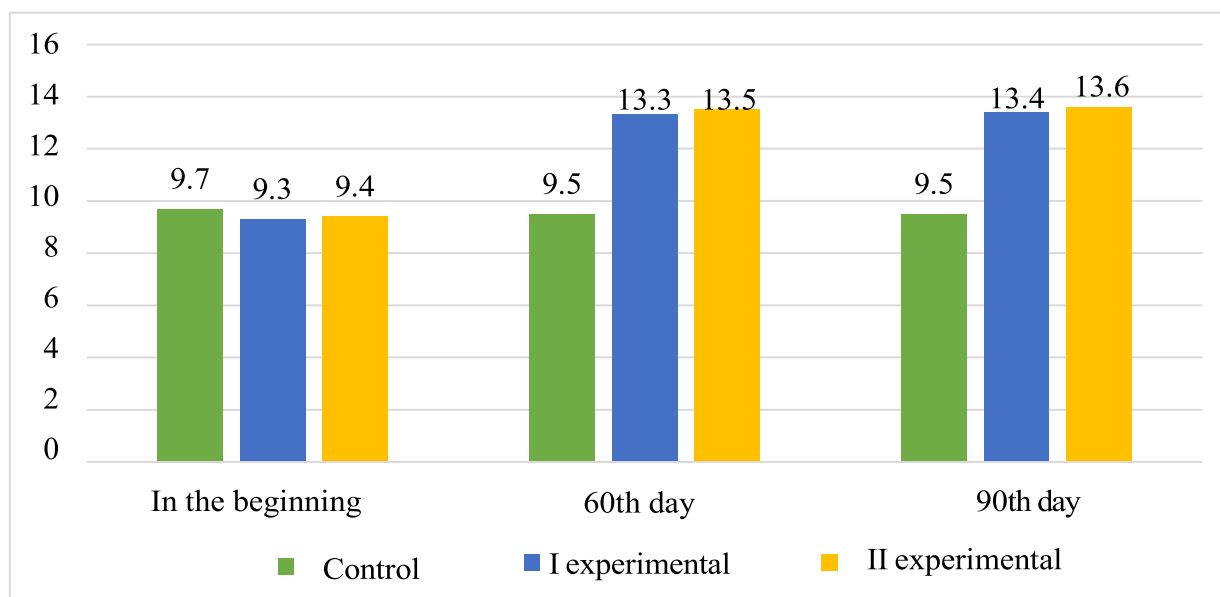


Fig. 16 – Vitamin E content in blood serum of cows, $\mu\text{mol/l}$

In the blood serum of cows of the I and II experimental groups on the 60th day, an increase in the concentration of vitamin E was established by 43.0 ($p_1 < 0.001$) and

⁴⁰ Стадник А.М., Федорович В.Л. Вітаміни А, D, E і остеокальцин у крові корів за ензоотичної остеодистрофії та в процесі комплексної терапії. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2008. Том. 10. №3 (38). Ч. 1. С. 234–239.

⁴¹ Щербатий А.Р. Вміст вітамінів А і Е у крові жеребних кобил. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій імені С.З. Гжицького*. 2010. Том 12. №2 (44). Ч.1. С. 350-354.

43.6% ($p_2 < 0.001$), respectively, compared to the beginning (Fig. 16).

Table 3

The content of vitamin E in the blood serum of cows, $\mu\text{mol/l}$

Groups of cows	Biometric indicator	Research period		
		In the beginning	60 th day	90 th day
Control, n=15	Lim	8,7–10,5	8,8–10,3	8,9–10,0
	M±m	9,7±0,13	9,5±0,22	9,5±0,11
	p<		0,5	0,5
I experimental, n=15	Lim	8,85–9,95	10,5–15,6	10,9–16,6
	M±m	9,3±0,08	13,3±0,7	13,4±0,42
	p ₁ <		0,001	0,001
	p ₃ <		0,001	0,001
II experimental, n=15	Lim	8,75–10,1	11,1–15,5	11,7–16,9
	M±m	9,4±0,09	13,5±0,6	13,6±0,38
	p ₂ <		0,001	0,001
	p ₄ <		0,001	0,001
	p ₅ <		0,5	0,5

At the experiment's end, the vitamin E concentration did not change and remained constant. Compared with the control group of cows, on the 60th and 90th days of the study, the concentration of vitamin E in the same animals was higher by 40.0 and 43.1% ($p < 0.001$), respectively (Fig. 16). It should be noted that the vitamin E content of the cows of the second experimental group was the same as that of the animals of the first experimental group ($p_5 < 0.5$). The data we obtained show that using inorganic salts and chelated compounds of microelements together with vitamins A, D, and E for osteodystrophy contributes to a complete normalization of the content of the studied vitamins in the blood of cows.

CONCLUSIONS

1. The administration of trace elements in chelated and inorganic forms for 90 days caused the disappearance of trace element deficiency symptoms and the stabilization of connective tissue metabolites in the blood serum of cows with osteodystrophy. The total glycosaminoglycans concentration in the cows of the first and second experimental groups was 32.0 ± 0.40 ($p < 0.001$) and 28.1 ± 0.34 mg/100ml

($p < 0.001$), sialoglycoproteins – 2.22 ± 0.13 and 1.92 ± 0.14 mmol/l ($p < 0.001$), chondroitin sulfates – 0.34 ± 0.07 and 0.30 ± 0.01 g/l ($p < 0.001$), urinary oxyproline – 518.8 ± 2.82 and 451.6 ± 1.86 $\mu\text{mol/l}$ ($p < 0.001$). In contrast, in the control group, these indicators were probably higher. In the cows of the first experimental group, the concentration of total calcium in blood serum on the 90th day was higher by 18.9% ($p < 0.001$), inorganic phosphorus – by 42.0%, compared to the control, and in the cows of the second experimental group, who received chelated compounds of microelements, by 28.8 and 55.5% ($p < 0.001$), respectively. Compared to the beginning of the experiment, the level of 25OHD_3 increased by 2.2 times - from 17.9 ± 0.07 to 37.5 ± 0.75 nmol/l ($p < 0.001$) and from 17.7 ± 0.14 to 39.2 ± 0.63 nmol/l ($p < 0.001$), vitamin A and E – by 21.4 and 40.0 and 28.6 and 43.1%, respectively ($p < 0.001$).

2. The concentration of citric acid in the blood serum of the cows of the experimental groups increased by 19.2 and 24.3% ($p < 0.01$), respectively, compared to the control. In the control group, it decreased to 193.4 ± 7.81 $\mu\text{mol/l}$ for norms of 210–250 $\mu\text{mol/l}$, which indicates the development of destructive processes in bone tissue.

3. Supplementing the cows of the research group with inorganic salts of microelements helped to increase the content of zinc, cobalt, manganese, and copper in the blood by 17.1; 65.2; 29.6 and 29.7%, and the use of chelating compounds, respectively, by 19.1%; 100; 40.4 and 38.7% compared to the control group.

SUMMARY

With the modern method of dairy farming, the significant spread of diseases caused by metabolic disorders remains an urgent problem. Osteodystrophy is particularly common among the latter. The relationship between the content of trace elements in the soil, fodder, and blood of cows with osteodystrophy in an area depleted of biogenic trace elements was established. Early diagnostic indicators of the subclinical course of osteodystrophy in cows are a low serum level of citric acid (147.6–178.5 $\mu\text{mol/l}$), osteocalcin (0.93–1.28 ng/l), $25(\text{OH})\text{D}_3$ (22.1–24.3 nmol/l), retinol (1.10–2.02 $\mu\text{mol/l}$), tocopherol (7.0–10.0 $\mu\text{mol/l}$), an increase in total glycosaminoglycans (32.4–41, 2 mg/100 ml), chondroitin sulfates (0.29–0.37 g/l),

sialoglycoproteins (2.29–2.97 mmol/l). The use of chelated compounds in cows restores the clinical status and indicators of macro- and microelement exchanges and stabilizes the content of markers of connective tissue metabolism.

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Information about the authors:

Slivinska Lyubov Grygorivna,

Doctor of Veterinary Sciences, Professor,
Head of the department of animal internal diseases
and clinical diagnostics

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

Fedorovych Vitalii Leonidovych,

Candidate of Veterinary Sciences,
Associate Professor at the Department of Animal Internal Diseases
and Clinical Diagnostics

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

Shcherbatyi Andrii Romanovych,

Candidate of Veterinary Sciences,
Associate Professor at the Department of Animal Internal Diseases
and Clinical Diagnostics

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