

Diagnostic informativeness of markers of bone-tissue metabolism and bone resorption in cows with osteodystrophy

L. G. Slivinska, V. L. Fedorovych, A. R. Shcherbatyy, N. M. Fedorovych,
B. V. Gutyj, V. V. Vlizlo, M. G. Lychuk, I. A. Maksymovych, H. O. Zinko

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine

Article info

Received 02.06.2023

Received in revised form

04.07.2023

Accepted 18.07.2023

*Stepan Gzhytskyi National
University of Veterinary
Medicine and Biotechnologies,
Pekarska, 50,
Lviv, 79010, Ukraine.
Tel.: +38-097-758-07-19.
E-mail: ua-andrea@ukr.net*

Slivinska, L. G., Fedorovych, V. L., Shcherbatyy, A. R., Fedorovych, N. M., Gutyj, B. V., Vlizlo, V. V., Lychuk, M. G., Maksymovych, I. A., & Zinko, H. O. (2023). Diagnostic informativeness of markers of bone-tissue metabolism and bone resorption in cows with osteodystrophy. Regulatory Mechanisms in Biosystems, 14(3), 349–353. doi:10.15421/10.15421/022351

Osteodystrophy is caused by polyetiological factors, the main being disturbance of metabolism of macroelements and D and A vitamins. The disease continues to impose great economic losses on animal husbandry, reduces cattle productivity, and hinders effective work in this sphere. To study the pathogenesis and early diagnostics of bovine osteodystrophy, we examined the markers of metabolism of the connective tissue: macro- and microelements, general glycosaminoglycans and their fractions, chondroitin sulfate, sialoglycoproteins, oxoproline, citric acid, and osteocalcin. In the cows with subclinical course of osteodystrophy, the content of total calcium was decreased by 18.9% and such of phosphorus by 5.7%. In 95% of the clinically ill animals, we diagnosed hypocalcemia, combined with hypophosphatemia, in 35.0% of the cows. In blood serum of the cows with subclinical course of osteodystrophy, we found decrease in the concentration of citric acid down to 157.7 ± 2.1 mmol/L, in the clinically ill – 146.8 ± 1.2 mmol/L and osteocalcin – to 1.12 ± 0.04 and 0.72 ± 0.04 ng/mL, respectively. We determined increases in concentrations of chondroitin sulfates, sialoglycoproteins, total glycosaminoglycans and their fractions: chondroitin-6-sulfate, chondroitine-4-sulfate, keratan- and heparin- and dermatan sulfates, and heparine. The most informative criteria for diagnostics of subclinical course of osteodystrophy were citric acid, total glycosaminoglycans and their first and third fractions, chondroitin sulfates and sialoglycoproteins, because they were above the physiological limits in 100% of the cows with subclinical course of the disease, whereas in the clinically healthy animals, osteocalcin decreased in 60 and 100% of the cases, respectively.

Keywords: blood serum; macroelements; microelements; citric acid; total glycosaminoglycans; chondroitin sulfates; sialoglycoproteins, osteocalcin.

Introduction

Mineralization of the bone tissue supports the main physiological functions of the locomotor system and is a “depot” of inorganic compounds, particularly calcium and phosphorus (Vlizlo et al., 2006; Slivinska et al., 2017). Studies of the content of macroelements in blood serum are the generally accepted indicators used for osteodystrophy diagnostics in animals (Vlizlo et al., 2012).

The main causes of osteodystrophy in animals are imbalanced nutrition and lack of physical activity, while the key mechanisms through which the disease onsets are dysbalances between the formation and breakdown of the bone tissue. The alimentary factor plays an important role in this context, because irregular and incomplete nutrition of animals affects their condition. Especially important factors are insufficient amounts of calcium and phosphorus in diets and dysbalance between those elements. Deficits of cobalt, zinc, copper, and manganese have been observed to have a certain role in the development of osteodystrophy. Microelements are known to be actively involved in peroxidation processes, tissue respiration, formation of the bone tissue, and mechanisms of regeneration after bone injuries.

Bovine osteodystrophy clinically manifests only in case of substantial changes in the bone tissue. Cattle undergo the pathology without a pronounced advantage of one of the three stages of pathological processes (osteomalacia, osteoporosis, and osteofibrosis) (Levchenko et al., 2010; Uhl, 2018; Mylostyyvi et al., 2021). Metabolism of the bone tissue can be evaluated using specific diagnostic markers, i.e. components of its matrix,

which changes in cases of different forms of osteodystrophy. The report presents the results of studies of biochemical blood parameters that characterize the mechanisms of bone-tissue resorption during bovine osteodystrophy.

Bone functioning is supported by continuous energy influx, protein components, mineral elements, vitamins, and other biologically active compounds involved in the mechanism of the bone-tissue formation. It contains around 98.5% of the body’s calcium, 83.0% of phosphorus, 70.0% of manganese, and 40.0% of sodium, and over 30 micro- and ultramicroelements. If they are in deficit, mineralization of organic matrix of the bone tissue malfunctions, resulting in osteodystrophy (Wilkens et al., 2013; Sakhniuk et al., 2015).

The objective of our study was to identify the informativeness of the markers of the bone-tissue metabolism in blood serum and cow urine for purposes of diagnostics of subclinical course of osteodystrophy.

Material and methods

During the experimental studies on cows, we adhered to all the bioethical norms regarding animals, which correspond to the requirements of Law of Ukraine No. 3447-4 On Protection of Animals from Abuse, positions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the position On Use of Vertebrate Animals for Research and Other Scientific Purposes at the S. Z. Gzhytskyi Lviv National University of Veterinary Medicine.

We chose such a direction of studies that consisted in identifying markers of the bone-tissue metabolism so as to determine their informativeness for early diagnosis of osteodystrophy on farms in Lviv Oblast. The material for the study was Black-Spotted Cows aged 3–10 years, weighing 450–550 kg, with 7,000 L milk productivity. To study pathogenesis and early diagnostics of bovine osteodystrophy, we examined the markers of the connective-tissue metabolism. To determine diagnostic information of the said parameters, depending on their content in blood serum, the cows were divided into three groups: I – clinically healthy cows; II – cows with subclinical course of osteodystrophy; III – cows with osteodystrophy.

In the blood, we measured the content of microelements (manganese, zinc, cuprum, and cobalt); in blood serum, we measured content of calcium (Arsenazo III complex), magnesium (by staining reaction with titanium yellow), inorganic phosphorus (by reduction of phosphorus-molybdenum acid), and oxyproline (Vlizo et al., 2012). Content of chondroitin sulfates in blood serum was identified using the Nemeth – Csoka method, modified by L. I. Slutskiy; total glycosaminoglycans were measured with alcian blue (B6) according to E.W. Gold, and their fractions (chondroitin-6- and chondroitin-4-sulfate, keratan-, heparin-, dermatan sulfates and heparine) according to M. R. Schtern and co-authors; concentration of citric acid was identified according to the study (Kartashov et al., 2010); oxyproline in the blood and urine was determined using periodic acid (Blumenkrantz & Asboe-Hansen, 1973), the chemiluminescence method was employed to measure osteocalcin, and sialoglycoprotein was evaluated after oxidation by iodic acid with resorption reagent (Jourdian et al., 1971).

The results of biochemical studies are given according to the International System of Units, recommended for the use in clinical laboratory practice. The results were analyzed using the Statistica 7.0 software pack (StatSoft Inc., USA). The data are presented as $\bar{x} \pm SD$ (mean \pm standard deviation). To compare the difference in the parameters between the control and experimental groups, we used the Tukey test, where the differences were considered statistically significant at $P < 0.05$ for all the data.

Results

The laboratory studies of blood serum of the cows with subclinical course of osteodystrophy revealed that the mean content of total calcium was lower than in the clinically healthy animals, but was within the physiological norms (Table 1). Ten cows of this group of 53 (18.8%) were diagnosed with hypocalcemia. In the group of cows that were clinically ill with osteodystrophy, the concentration of total calcium in blood serum was 15.4% ($P < 0.05$) lower than in the clinically healthy animals (Fig. 1). In the cows with subclinical course of osteodystrophy and clinically ill cows, the concentration of inorganic phosphorus in blood serum was 10.5% and 15.8% ($P < 0.05$) lower compared with the clinically healthy animals (Fig. 1). Hypophosphatemia was diagnosed in 3 (5.7%) and 7 (35.0%) of the cows.

The reason for hypocalcemia in the subclinical-osteodystrophy cows was insufficient amount of calcium in the diet (62.0–78.2% of the need), deficit of phosphorus (49.4–57.4% of the need) and vitamin D, which had been provided only in the amount of 8.9–14.1% of the need, and also elevated calcium-phosphorus ratio (2.23–2.45:1).

Concentration of magnesium in blood serum of the subclinical-osteodystrophy cows was at the lower boundary of physical fluctuations (Fig. 1). Hypomagnesemia in the cows of this group was diagnosed in 13 animals (24.5%). In the clinically ill cows, the average concentration of magnesium did not differ from the cows with subclinical course of osteodystrophy. Based on the results of laboratory studies of blood serum of 65 cows, we found deviations of the content of citric acid and formed three groups of animals: 10 clinically healthy, 10 with subclinical course of osteodystrophy, and 10 ill cows with symptoms of the disease.

Results of the studies revealed (Fig. 2a) that the concentration of citric acid in blood of the cows with subclinical course of osteodystrophy was 37.6% lower ($P < 0.001$), compared with the clinically healthy cows. In the clinically ill cows, the content of citric acid in blood serum was 41.9% ($P < 0.001$) lower than in the clinically healthy cows.

According to our studies, the cows with subclinical course of osteodystrophy underwent decrease in citric acid in the blood serum, as com-

pared with the clinically healthy cows. On average, the amount of osteocalcin in the blood serum of the sick animals was 19.7% and 49.0% ($P < 0.001$) lower than in the clinically healthy cows (Fig. 2b). Concentration of osteocalcin was lower than in the clinically healthy cows, in 60% of the subclinical-osteodystrophy cows and 100% of the clinically ill cows.

By content of total glycosaminoglycans (GAGs), one can identify presence of disorganizing processes in the organic matrix of the bone tissue, because those biopolymers, together with collagen, are necessary components of the bone, which promote its ossification and structural organization.

In the cows with subclinical course of osteodystrophy, the content of total GAGs in blood serum was 40.1% ($P < 0.001$) higher than in the clinically healthy animals (Table 1). In the blood serum of the clinically ill osteodystrophy cows, the concentration of total GAGs was 63.7% greater than in the clinically healthy animals. Besides identifying total GAGs, we studied their fractions, which provided us with more detailed data on their metabolism, because they localize in individual tissues.

Analysis of individual fractions of serum total glycosaminoglycans revealed significant ($P < 0.001$) increase in the content of fraction I – chondroitin-6-sulfate in the cows with subclinical course of osteodystrophy and in the sick animals.

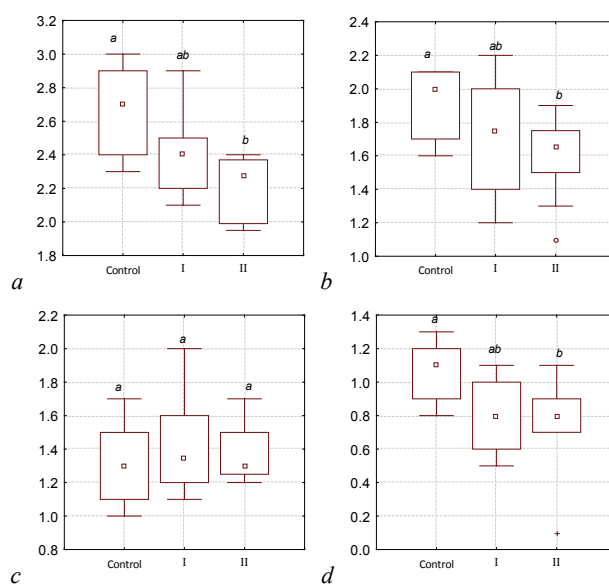


Fig. 1. Contents of macroelements in blood serum of cows ($n = 10$): *a* – concentration of total calcium (mmol/L); *b* – concentration of inorganic phosphorus, (mmol/L); *c* – Ca ratio : P; *d* – concentration of magnesium (mmol/L); different letters indicate that data sets are significantly ($P < 0.05$) different one from another according to the Tukey test with the Bonferroni correction

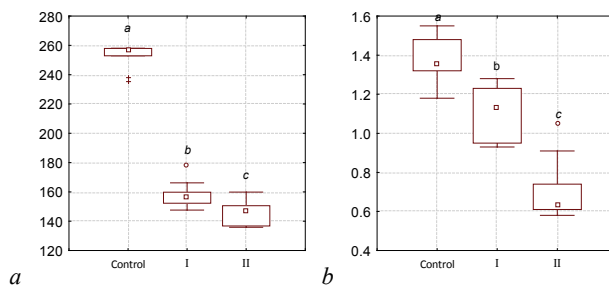


Fig. 2. Content of citric acid and osteocalcin in blood of clinically healthy cows, cows with subclinical course and cows with osteodystrophy ($n = 10$): *a* – content of citric acid ($\mu\text{mol/L}$); *b* – content of osteocalcin (ng/mL)

In the blood serum of the cows with subclinical course of osteodystrophy, content of fraction II of total glycosaminoglycans, which includes chondroitin-4-sulfate, was 8.3% ($P < 0.05$) higher than in the clinically healthy animals (Table 1). Concentration of this fraction in the blood

serum of the clinically ill cows was 30.0% ($P < 0.001$) greater compared with the clinically healthy animals. Chondroitin-4-sulfate is present in collagen of the bone tissue, and also the cartilage, cornea, skin, and aorta.

Table 1

Content of total glycosaminoglycans and their fractions in blood serum of clinically healthy, subclinical-course and osteodystrophy cows (mg/mL, $\bar{x} \pm SD$, $n = 10$)

| Parameters | Clinically healthy cows | Cows with subclinical course of osteodystrophy | Cows with osteodystrophy |
|--|-------------------------|--|--------------------------|
| Total glycosaminoglycans | 2.67 ± 0.82^a | 3.74 ± 1.14^b | 4.37 ± 1.08^b |
| Chondroitin-6-sulfate | 1.44 ± 0.23^a | 1.92 ± 0.64^{ab} | 2.28 ± 0.43^b |
| Chondroitin-4-sulfate | 0.60 ± 0.19^a | 0.65 ± 0.16^a | 0.78 ± 0.24^a |
| Keratan- and heparin- and dermatan sulfates, and heparin | 0.63 ± 0.15^a | 1.17 ± 0.24^b | 1.31 ± 0.30^b |

Note: different letters in a row indicate that data samplings are significantly ($P < 0.05$) different one from another according to the Tukey test with the Bonferroni correction.

Fraction III contains keratan-, heparin-, dermatin-sulfates and heparin. We detected increase in fraction III of general GACs in the cows with subclinical osteodystrophy. Increase in this parameter in the sick cows measured 108.0% ($P < 0.001$), compared with the healthy cows. This fraction of GACs was localized in the largest amount in the liver and was indirectly involved in the pathogenesis of osteodystrophy.

During the subclinical course of bovine osteodystrophy, the concentration of sialoglycoproteins in blood was higher by 28.5% ($P < 0.001$) compared with the clinically healthy cows (Fig. 3). In 100% of the cows, their content was above the threshold of the maximum norm. In the blood serum of the clinically ill osteodystrophy cows, the concentration of sialoglycoproteins was 80.2% ($P < 0.001$) higher than in the healthy animals (Fig. 3).

Informativeness of sialoglycoproteins was 100%, not only compared with the norm but also with the results from the cows with subclinical course of osteodystrophy ($P < 0.001$), in which the maximal level accounted for 2.97 mmol/L.

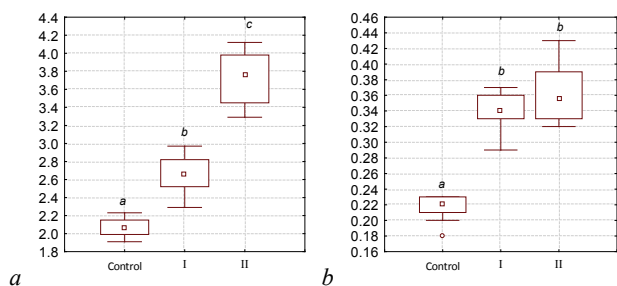


Fig. 3. Concentration of connective-tissue metabolites in blood serum of clinically healthy, subclinical-course, and clinically osteodystrophy-sick cows: *a* – content of sialoglycoproteins (mmol/L); *b* – concentration of chondroitin-sulfate (g/L); $\bar{x} \pm SD$

The studies of concentration of chondroitin sulfates revealed their increase in blood of 100% of the cows with subclinical course of osteodystrophy, being 54.5% ($P < 0.001$) higher than in the clinically healthy cows. Informativeness of the parameter was 100% (Fig. 3).

In the clinically ill osteodystrophy cows, the concentration of chondroitin sulfates was significantly higher by 63.6% ($P < 0.001$) than in the clinically healthy cows, though the difference with the content of chondroitin sulfates during subclinical course was insignificant.

Analysis of the data we obtained revealed that oxoproline content in blood serum of the cows with subclinical osteodystrophy was 6.4% higher compared with the clinically healthy animals (Fig. 4). Oxoproline in the blood serum of the clinically ill cows was 19.3% ($P < 0.001$) higher than in the clinically healthy. Compared with subclinical course, its concentration was 12.2% ($P < 0.001$) higher.

Increased excretion of oxoproline with urine occurred much more intensively than its accumulation in blood serum, because metabolites of collagen breakdown stockpile and are released mostly with urine. In the cows with subclinical course of osteodystrophy, the oxoproline concentra-

tion in the urine was higher by 8% ($P < 0.001$) than in the clinically healthy animals, which may indicate destruction of bone collagen (Fig. 4). Concentration of oxoproline in urine of the clinically osteodystrophic-ill cows was 47.3% ($P < 0.001$) higher than in the clinically healthy animals and 36.6% ($P < 0.001$) compared with the subclinical-course cows.

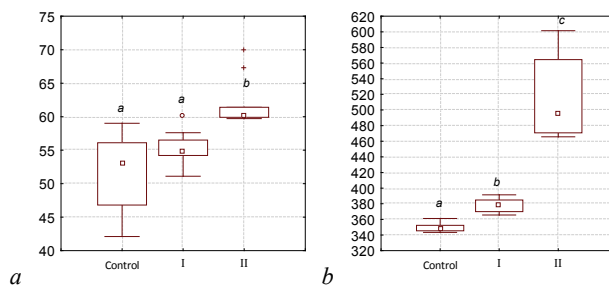


Fig. 4. Concentration of oxoproline in blood serum and urine of clinically healthy, subclinical-course, and clinically osteodystrophy-ill cows: *a* – content of oxoproline in blood serum ($\mu\text{mol/L}$); *b* – content of oxoproline in urine ($\mu\text{mol/L}$); $\bar{x} \pm SD$

Discussion

Despite deficit of calcium and phosphorus in the diets of dry cows, concentration of calcium and its ratio to phosphorus in blood serum were different from the parameters in the clinically healthy animals. Because the content of calcium in blood serum of cows is stable, while the bone tissue is a source of homeostasis support, it does not always reflect clinical condition of diseased animals during subclinical course of osteodystrophy.

According to the results of our studies, changes in the total calcium in the cows having subclinical osteodystrophy were not significantly different from the parameters of the clinically healthy cows. Therefore, study of the total calcium during osteodystrophy is not a pathognomonic test for the disease and cannot be used for early diagnostics of osteodystrophy.

As with magnesium concentration, its decrease in blood serum can indicate reduced magnesium absorption in the intestines as a result of high calcium level in the diets (186.9–188.3% of the need) (Čepelak & Čvorišćec, 2009; Gutjy, 2017).

The literature data about the role of citric acid in bovine-osteodystrophy pathogenesis remain a subject of discussion. There are some reports on metabolism of citric acid in cows (Allen et al., 1986), lambs (Allen et al., 1991), swine (Falkowski & Aheme, 1984), and poultry. Therefore, to analyze the process of remodeling of the bone tissue of cows having osteodystrophy, particularly its resorption, we carried out the laboratory analysis of blood serum for citric acid.

According to the literature data (Taylor et al., 2008), the amount of osteocalcin in blood of the cows during pre-labour period was 25.4 ng/mL. After labour, its concentration increased up to 32.4 ng/mL, and returned to the initial values over month. Increase in the osteocalcin concentration in cows after labour has been associated with enhanced bone metabolism (Moreira et al., 2009). Also, those authors observed increase in osteocalcin concentration before labor, which they attributed to its placenta synthesis. The same study mentions that during the dry period, the osteocalcin concentration was much lower than initially, ranging 19.7–21.4 ng/mL.

It has to be noted that our data are different from other reports (Taylor et al., 2008), according to which the osteocalcin in the cows' blood prior to labour accounted for 25.4 ng/mL (Liesegang et al., 2000). In our study, it equaled 34.6 ng/mL. The authors have noted that its concentration depended on content of phosphorus in the diet, increase of which in fodders heightened the osteocalcin concentration two weeks earlier than the diets with low phosphorus. According to other authors (Moreira et al., 2009) who studied the influence of various levels of phosphorus in the diet on productivity, calcium metabolism, and predictability of hypocalcemia development, osteocalcin concentration depended on phosphorus content in the diet. The authors found that the less the cows were provided with phosphorus, the lower was the osteocalcin concentration in the blood. Therefore, phosphorus deficit was assumed to be one of the reasons of bone resorption because it is mobilized from the bone tissues to blood in

order to support phosphatemia and calcemia. However, the authors have mentioned that the data they obtained were insignificant, assuming that more time is needed to identify a clear correlation.

In the cows with subclinical course of osteodystrophy, decrease in the osteocalcin concentration in blood serum is explained by the fact that processes of bone-tissue resorption dominate in the bone remodeling. Furthermore, the cows' diets lacked phosphorus, this element accounting for 49.4–57.4% of the need. Thus, our studies are coherent with the data given in other reports (Moreira et al., 2009). Therefore, to generalize the results of our studies of osteocalcin concentration in blood of cows with osteodystrophy, we may conclude that this marker is a highly sensitive and specific indicator of bone-tissue resorption, since it allowed us to detect changes in the bones of cows with subclinical course of the disease, while calcium and phosphorus levels remained unchanged.

In the available literature, we found no studies dealing with the content of citric acid. However, there are single literature sources (Oshima & Fuse, 1981) mentioning the concentration of citric acid in cows suffering mastitis. The cows having subclinical course of osteodystrophy were found to have changes in metabolism of citric acid and osteocalcin, which are markers of bone-tissue resorption, and the detected changes are typical for diagnosing osteodystrophy.

Some studies (Kibkalo, 2008) have substantiated the possible use of constituents of glycoconjugates as early diagnostic criteria of osteodystrophy. Some researchers (Kartashov et al., 2005) have reported their informativeness and specificity for the disease diagnostics. However, the views of researchers on their diagnostic value during bovine osteodystrophy vary. In particular, a study (Levchenko et al., 2010) has reported that diagnostic informativeness of total calcium in cows is significantly lower than the parameters of the connective tissue.

Such discrepancies in views could be related to many reasons, one of which is that components of the glycoconjugate group are easily subjected to degradation, and therefore they are hard to isolate and examine. Moreover, there are no unified methods of identifying glycoconjugates in biological substrates. Usually, their measuring is limited to dilution and isolation in pure form using methods of chromatography, electrophoresis, ultracentrifugation, or fractional precipitation, and analysis of components after hydrolysis (Morozenko & Leontieva 2016).

According to Kartashov et al. (2005), during osteodystrophy, fractions of total GAGs in blood redistribute. In particular, their total amount increases because of GAG fractions I and III – chondroitin-4-sulfate. Because fraction I localizes in the largest amounts in the bone tissue, such an increase suggests destructive changes in bones driven by osteodystrophy (Kartashov et al., 2005). According to the data (Stepanenko, 2013; Morozenko & Leontieva, 2016), GAG fraction I localizes in the cartilage and bone tissue, umbilical cord and aorta. Increase in content of GAG fraction I in the bone tissue of cows suffering osteodystrophy is clearly a result of reparative processes.

Chondroitin sulfates, together with keratan sulfate and hyaluronic acid, comprise a basis of extracellular organic matrix of the bone and cartilage tissues. It is assumed that chondroitin sulfates modify the structure of collagen fibrils. Their content can be used as a diagnostic test in case of a bone-joint pathology. Increases in concentrations of sialoglycoproteins and chondroitin sulfates in blood of cows with subclinical course of osteodystrophy indicate initial development of depolymerization of proteoglycans and catabolism of bone-tissue glycoproteins, as a result of which GAGs and sialic acids enter the general blood circulation. Heightened concentrations of those biopolymers suggest activation of mechanisms of bone-tissue resorption (Morozenko & Leontieva 2016).

Complete formation of the bone tissue requires synthesis of its protein components, particularly collagen proteins. One of the main indicators of collagen metabolism is oxoproline concentration in the blood. By its excretion with body fluids, one can assess the intensity of collagen breakdown, which reflects restructuring of the bone tissue. Oxoproline is considered a criterion for diagnostics of pathological conditions of the bone tissue, including osteodystrophy. However, it has to be noted that normally 90% of blood oxoproline reabsorbs in the kidneys and oxidates in the liver and only 10% are released in unaltered form with urine. That is why its level can also rise during pathology of the kidneys and dystrophic processes in the liver. The main amount of GAGs degrades in the liver,

and therefore increase in their concentration in blood serum is associated with loss or decrease in the hepatocytes' ability to breakdown GAG, which leads to their accumulation in the blood. Similar changes in GAG fraction III have been detected (Kibkalo, 2008).

Glycoproteins also include sialoglycoproteins – proteins that are attached to sialic acid. They are included in the connective tissue, including the bone tissue, and therefore their concentration in blood serum can be used to indirectly assess its condition.

It is believed that increase in sialoglycoproteins in the blood reflects only the processes of destruction of the connective tissue. However, as revealed, pathological changes in the liver can provoke increased synthesis of many glycoproteins that partake in reparation processes. Therefore, biologically active groups of glycoproteins, including sialoglycoproteins, are involved in inactivation of biological agents, and thereby exert defensive functions, inhibiting the development of pathological process (compensatory reaction).

An interesting research topic (Martin et al., 2001) is the content of sialoglycoproteins in young cows, because those compounds can be present not only in blood but also in other body fluids. The study allows us to identify sialoglycoproteins in milk produced by cows with osteodystrophy, and using this non-invasive method of assessing the bone-tissue condition is coherent with the concept of the Five Freedoms (Kozii & Kozii, 2011) of animals in complex diagnostics of high-productivity diseases (Levchenko et al., 2011).

Therefore, taking into account the results of the conducted studies, we should note that the cows with subclinical course of dystrophy were seen to have growing concentrations of general GAGs, their fractions I and III, sialoglycoproteins, and chondroitin sulfates. Such changes have been a result of breakdown of organic part of bone in case of subclinical course of osteodystrophy.

Complex study of their content can be an objective test for early diagnostics of the disease. Clinically ill cows – along with increase in the content of the said biopolymers – had hyperexcretion of oxoproline with urine. Such a pattern of metabolic alterations in blood of cows having subclinical course of osteodystrophy allows us to assess the condition of metabolism of connective-tissue polymers without using invasive methods of diagnostics (X-ray study). Those studies are also important for differential diagnostics of initial processes during osteodystrophy and secondary lesions of the liver and kidneys.

Conclusion

Based on identification of organic components of the bone tissue, we determined the informative criteria of osteodystrophy diagnostics. In blood serum of the cows with subclinical course of osteodystrophy, we saw decrease in the concentrations of total calcium, inorganic phosphorus, concentration of citric acid, osteocalcin, and increases in the concentrations of chondroitin sulfates, sialoglycoproteins, total glycosaminoglycans and their fractions: chondroitin-6-sulfate, chondroitin-4-sulfate, keratan-, heparin-, dermatan sulfates, and heparine. We found that the most informative criteria for diagnostics of subclinical course of osteodystrophy are concentrations of citric acid, total glycosaminoglycans, and their first and third fractions, chondroitin sulfates, sialoglycoproteins, and osteocalcin in blood serum.

The studies were financed by the Ministry of Education and Science of Ukraine within framework Development and Introduction of a Complex System of Diagnostics, Treatment, and Prophylaxis of Metabolic Pathology in Highly Productive Cows in the Context of Food Safety of Ukraine (0123U102256).

References

- Allen, V. G., Fontenot, J. P., & Rahnama, S. H. (1991). Influence of aluminum-citrate and citric acid on tissue mineral composition in wether sheep. *Journal of Animal Science*, 69(2), 792–800.
- Allen, V. G., Hom, F. P., & Fontenot, J. P. (1986). Influence of ingestion of aluminum, citric acid and soil on mineral metabolism of lactating beef cows. *Journal of Animal Science*, 62(5), 1396–1403.

- Blumenkrantz, N., & Asboe-Hansen, G. (1973). A quick and specific assay for hydroxyproline. *Analytical Biochemistry*, 55(1), 288–291.
- Čepelak, I., & Čvorišćec, D. (2009). Biochemical markers of bone remodeling – review. *Biochemia Medica*, 19(1), 17–35.
- Falkowski, J. F., & Ahern, F. X. (1984). Fumaric and citric acid as feed additives in starter pig nutrition. *Journal of Animal Science*, 58(4), 935–938.
- Gold, E. W. (1979). A simple spectrophotometric method for estimating glycosaminoglycan concentration. *Analytical Biochemistry*, 99(1), 183–188.
- Gorres, K. L., & Raines, R. T. (2010). Prolyl 4-hydroxylase. *Critical Reviews in Biochemistry and Molecular Biology*, 45(2), 106–124.
- Gutyj, B., Nazaruk, N., Levkivska, N., Shcherbatyj, A., Sobolev, A., Vavrysevych, J., Hachak, Y., Bilyk, O., Vishchur, V., & Guta, Z. (2017). The influence of nitrate and cadmium load on protein and nitric metabolism in young cattle. *Ukrainian Journal of Ecology*, 7(2), 9–13.
- Gutyj, B., Stybel, V., Darmohray, L., Lavryshyn, Y., Turko, I., Hachak, Y., Shcherbatyj, A., Bushueva, I., Parchenko, V., Kaplaushenko, A., & Krushelnyska, O. (2017). Prooxidant-antioxidant balance in the organism of bulls (young cattle) after using cadmium load. *Ukrainian Journal of Ecology*, 7(4), 589–596.
- Jourdian, W., Lawrence, D., & Roseman, S. (1971). A periodate-resorption method for the quantitative estimation of free sialic acids and their glycosides. *The Journal of Biological Chemistry*, 246(2), 430–435.
- Kartashov, M. I., Borovkov, S. B., & Kibkalo, D. V. (2005). Kliniko-biokhimichni aspekty diahnozyky osteodystrofi u koriv [Clinical and biochemical aspects of diagnosis of osteodystrophy in cows]. *Visnyk Bilotserkivskoho Derzhavnoho Ahramoho Universytetu*, 33, 69–75 (in Ukrainian).
- Kartashov, M. I., Tymoshenko, O. P., & Kibkalo, D. V. (2010). *Veterynarna klinichna biokhimiia [Veterinary clinical biochemistry]*. Espada, Kharkiv (in Ukrainian).
- Kibkalo, D. V. (2008). Perspektivy zastosuvannya biokhimichnykh pokaznykiv stanu spoluchnoi tkanyny v diahnozyky vnutrishnikh neinfektsiynykh khvorob tvaryn [Prospects for the use of biochemical indicators of the state of connective tissue in the diagnosis of internal non-infectious diseases of animals]. *Visnyk Bilotserkivskoho Derzhavnoho Ahramoho Universytetu*, 56, 75–78 (in Ukrainian).
- Kozii, V. I., & Kozii, N. V. (2011). Dobrobut tvaryn yak osnova preventyvoi veterynarnoi medytsyny [Animal welfare as the basis of preventive veterinary medicine]. *Naukovyj Visnyk Veterynarnoi Medytsyny*, 87, 65–68 (in Ukrainian).
- Levchenko, V. I., Sakhniuk, V. V., & Chub, O. V. (2010). Poshyrennia, etiologija, osoblyvosti perebihu ta diahnozyky mnozhyhnoji vnutrishnoji patolohiji u vysokoproduktyvnykh koriv [Common, etiology, features of the course and diagnostics of multiple internal pathologies in high-yielding cows]. *Visnyk Bilotserkivskoho Derzhavnoho Ahramoho Universytetu*, 56, 97–102 (in Ukrainian).
- Levchenko, V. I., Vovkotrub, N. V., & Tyshkivska, N. V. (2011). Doslidzhennia moloka koriv yak nevidjemna skladova kompleksnoji diahnozyky ta profilaktyky “khvorob vysokoi produktyvnosti” [Studies of cow's milk as an integral component of complex diagnostics and prevention of “diseases of high productivity”]. *Naukovyj Visnyk Veterynarnoi Medytsyny*, 87, 86–92.
- Liesegang, A., Eicher, R., Sassi, M. L., Risteli, J., Riond, J. L., & Wanner, M. (2000). Affiliations expand the course of selected bone resorption marker concentrations in response to short-term hypocalcemia experimentally induced with disodium EDTA infusions in dairy cows. *Journal of Veterinary Medicine Series A*, 47(8), 477–487.
- Martin, M. J., Martin-Sosa, S., Garcia-Pardo, L. A., & Hueso, P. (2001). Distribution of bovine milk sialo glycoconjugates during lactation. *Journal of Dairy Science*, 84(5), 995–1000.
- Moreira, V. R., Zeringue, L. K., Williams, C. C., Leonardi, C., & McCormick, M. E. (2009). Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows. *Journal of Dairy Science*, 92(10), 5189–5198.
- Morozenko, D. V., & Leontieva, F. S. (2016). Metody doslidzhennia markeriv metabolizmu spoluchnoi tkanyny u suchasnij klinichnij ta eksperymentalnij medytsyni [Methods of researching markers of connective tissue metabolism in modern clinical and experimental medicine]. *Young Scientist*, 29, 168–172 (in Ukrainian).
- Mylostyvyi, R., Lesnovskay, O., Karlova, L., Khmeleva, O., Kalinichenko, O., Orishchuk, O., Tsap, S., Begma, N., Cherniy, N., Gutyj, B., & Izhboldina, O. (2021). Brown Swiss cows are more heat resistant than Holstein cows under hot summer conditions of the continental climate of Ukraine. *Journal of Animal Behaviour and Biometeorology*, 9(4), 2134.
- Mylostyvyi, R., Sejian, V., Izhboldina, O., Kalinichenko, O., Karlova, L., Lesnovskay, O., Begma, N., Marenkov, O., Lykhach, V., Midyk, S., Cherniy, N., Gutyj, B., & Hoffmann, G. (2021). Changes in the spectrum of free fatty acids in blood serum of dairy cows during a prolonged summer heat wave. *Animals*, 11(12), 3391.
- Oshima, M., & Fuse, H. (1981). Citric acid concentration in subclinical mastitic milk. *Journal of Dairy Research*, 48(3), 387–392.
- Sakhniuk, V. V., Levchenko, V. I., & Chub, O. V. (2015). Profilaktyka mnozhyhnoji vnutrishnoji patolohiji u vysokoproduktyvnykh koriv [Prevention of multiple internal pathology in high-yielding cows]. *Naukovyj Visnyk Veterynarnoi Medytsyny*, 118, 30–37 (in Ukrainian).
- Shiqemura, Y., Kubomura, D., Sato, Y., & Sato, K. (2014). Dose-dependent changes in the levels of free and peptide forms of hydroxyproline in human plasma after collagen hydrolysate ingestion. *Food Chemistry*, 159(15), 328–332.
- Slivinska, L. G., Shcherbatyj, A. R., Lukashchuk, B. O., Zinko, H. O., Gutyj, B. V., Lychuk, M. G., Chemushkin, B. O., Leno, M. I., Prystupa, O. I., Leskiv, K. Y., Slepokura, O. I., Sobolev, O. I., Shkromada, O. I., Kystema, O. S., & Usienko, O. V. (2019). Correction of indicators of erythropoiesis and microelement blood levels in cows under conditions of technogenic pollution. *Ukrainian Journal of Ecology*, 9(2), 127–135.
- Slivinska, L. G., Vlizlo, V. V., Shcherbatyj, A. R., Lukashchuk, B. O., Gutyj, B. V., Drach, M. P., Lychuk, M. G., Maksymovych, I. A., Leno, M. I., Rusyn, V. I., Chemushkin, B. O., Fedorovych, V. L., Zinko, H. O., Prystupa, O. I., & Yaremchuk, V. Y. (2021). Influence of heavy metals on metabolic processes in cows. *Ukrainian Journal of Ecology*, 11(2), 284–291.
- Slivinska, L., Demydjuk, S., Shcherbatyj, A., Fedorovich, V., & Tyndyk, I. (2017). Etiology and clinical biochemical parameters of blood for nutritional osteodystrophy cows. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences*, 19(73), 79–83.
- Slivinska, L., Fedorovych, V., Gutyj, B., Lychuk, M., Shcherbatyj, A., Gudyma, T., Chemushkin, B., & Fedorovych, N. (2018). The occurrence of osteodystrophy in cows with chronic micronutrients deficiency. *Ukrainian Journal of Ecology*, 8(2), 24–32.
- Sobolev, O., Gutyj, B., Petryshak, R., Pivtorak, J., Kovalskyi, Y., Naumyuk, A., Petryshak, O., Semchuk, I., Mateusz, V., Shcherbatyj, A., & Semeniv, B. (2019). Biological role of selenium in the organism of animals and humans. *Ukrainian Journal of Ecology*, 8(1), 654–665.
- Stepanenko, H. O. (2013). Vykorystannia hlikozaminohlikaniv u diahnozyky ta likuvanni metabolichnykh osteopatiji u reptylji [The use of glycosaminoglycans in the diagnosis and treatment of metabolic osteopathy in reptiles]. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences*, 15(57), 305–311 (in Ukrainian).
- Taylor, M. S., Knowlton, K. F., McGilliard, M. L., Seymour, W. M., & Herbein, J. H. (2008). Blood mineral, hormone, and osteocalcin responses of multiparous jersey cows to an oral dose of 25-hydroxyvitamin D₃ or vitamin D₃ before parturition. *Journal of Dairy Science*, 91(6), 2408–2416.
- Uhl, E. W. (2018). The pathology of vitamin D deficiency in domesticated animals: An evolutionary and comparative overview. *International Journal of Paleopathology*, 23, 100–109.
- Vlizlo, V. V., Colohub, L. I., Yanovych, V. H., Antoniak, H. L., & Yanovych, D. O. (2006). Biokhimichni osnovy nomuvannya mineralnoho zhyvlennia velykoi rohatoji khudoby. 2. Mikroelementy [Biochemical bases of rationing of mineral nutrition of cattle. 2. Microelements]. *Biologija Tvaryn*, 8, 41–62 (in Ukrainian).
- Vlizlo, V. V., Fedoruk, R. S., & Ratyck, I. B. (2012). Laboratomi metody doslidzhenn' u biolohiji, tvarynnytsvi ta veterynarnij medytsyni [Laboratory methods up to biology, animal husbandry and veterinary medicine]. Lviv, Spolom (in Ukrainian).
- Vlizlo, V. V., Prystupa, O. I., Slivinska, L. G., Lukashchuk, B. O., Hu, S., Gutyj, B. V., Maksymovych, I. A., Shcherbatyj, A. R., Lychuk, M. G., Chemushkin, B. O., Leno, M. I., Rusyn, V. I., Drach, M. P., Fedorovych, V. L., Zinko, H. O., & Yaremchuk, V. Y. (2021). Functional state of the liver in cows with fatty liver disease. *Ukrainian Journal of Ecology*, 11(3), 168–173.
- Wilkens, M. R., Cohrs, I., Lifschitz, A. L., Fraser, D. R., Olszewski, K., Schröder, B., & Breves, G. (2013). Is the metabolism of 25-hydroxyvitamin D₃ age-dependent in dairy cows? *Journal of Steroid Biochemistry and Molecular Biology*, 136, 44–46.
- Zaitseva, O. V., Shandrenko, S. G., & Veliky, M. M. (2015). Biochemical markers of bone collagen type I metabolism. *Ukrainian Biochemical Journal*, 87(1), 21–32.