



УДК 619:616.071:636.2

L.H. SLIVINSKA, doctor of veterinary science, professor
Lviv national university of veterinary medicine and biotechnologies
named after S.Z. Gzhytskyi

V.I. LEVCHENKO, doctor of veterinary science, academic
Bilotserskiy national agricultural university

V.L. FEDOROVYCH, candidate of veterinary science, assistant
I.A. MAKSYMOVYCH, candidate of veterinary science, docent
Lviv national university of veterinary medicine and biotechnologies
named after S.Z. Gzhytskyi

DIAGNOSTICAL INFORMATIONALITY OF A CERTAIN BLOOD INDICATORS IN COWS WITH OSTEODYSTROPHY

The results of separate biochemical blood markers and metabolites of connecting tissue determination in cows with osteodystrophy are shown. The most informative tests for osteodystrophy and early diagnostics of it's subclinical course are described.

Diseases that run with the violation of metabolism, especially mineral in cattle breeding, remain the most spread and cause considerable economic losses, decrease cattle productivity and obstruct an effective work of the sphere [3, 4, 6, 10].

This problem especially arises in the Western part of Ukraine, which is biogeochemical zone. Osteodystrophy in cows takes one of the first places. The disease often has subclinical course [3–6, 10, 12].

Hematological and biochemical tests of urine and blood, according to which the concentration of general calcium, non-organic phosphorus, general alkaline phosphatase and alkaline reserve are determined in cows in order to diagnose preclinical course of osteodystrophy. Diagnostic indicators of the early stages of disease are not always enough informative due to the ability of homeostasis balance. The ineffectiveness of these tests leads to necessity of more sensitive biochemical criteria of bone metabolism disturbances. Thus, today it is important to determine metabolites, which are specific components of mineral and organic bone matrix or ingredients that perform a certain role in the process of bone forming or destruction [9]. Unfortunately their usage in national veterinary diagnostics is limited, because of high price of reagents, necessity of special equipment, qualified personnel. There are a number of markers, which can be a reliable criteria for bone tissue status evaluation and do not demand expensive spends and sophisticated manipulations. [4, 5, 10, 11].

Aim of the research – to determine metabolites of bone tissue metabolism in blood of cows with osteodystrophy and establish their diagnostic value depending on different types of its course.

MATERIAL AND METHODS

The research was carried out on three farms of Lviv region on cows of black-and-white breed, aged 3–10 years. Our previous works have already shown that the ration of cows is not balanced in contents of macro, microelements and vitamins [8].

For biochemical tests blood was extracted from cows and the following parameters were determined: general calcium – was determined using arsenazo III (Bauer P., 1981), non-organic phosphorus – in test with ammonium molybdate according to O.H. Lopez, J.A. Lowry method (1946), magnesium – by reaction with kal-malhit, lemon acid – according to E. Beitler and M.K. Veh (1959) method in the modification of At. Karakashov, E. Vichev (1968) [2], osteocalcin – by hemiluminiscent method, serum glycosaminoglycans levels using alcianic blue (B6) according to E.W. Gold (1979) and their fractional content [7], sialicglycoproteins – according to A. Gottschalk (1972), G.W. Jourdian et al. (1971), chondroitinsulphate – according to the methods of M. Nemeth-Csoka (1961) [12].

RESULTS OF THE RESEARCH

The clinical examination of cows established that their average body temperature was $38,2 \pm 0,3$ °C, breath frequency $29,0 \pm 0,9$ and heart rate $62 \pm 1,4$ per minute.

The following clinical features of osteodystrophy were observed: disfigurement of an appetite, detention of molt, excessive expansion and deformation of the hoofs, thinning and partial lysis of the last pair of ribs, resorption of the last 2–3 caudal vertebrae, partial spinal deformity, fragility of teeth, decline in nutritional status and productivity.

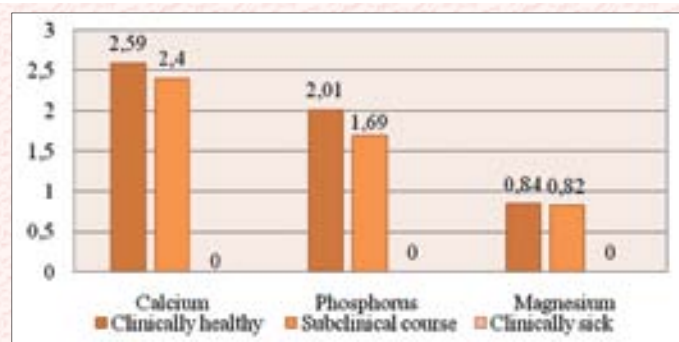
As far as symptoms of osteodystrophy do not always reflect status of bone tissue, biochemical blood tests are necessary for determining of pathogenesis and identification of metabolic disturbances. Basing on the revealed symptoms and laboratory tests results three groups of cows were formed: 10 – clinically healthy, 10 – with subclinical course of disease without obvious pathognomonic symptoms and 10 – sick cows with clinical signs of osteodystrophy.

The content of calcium and phosphorus (pic. 1) in clinically sick cows was 16 and 22,4% lower comparing to healthy cows; the same time in cows with subclinical course their level was 7,7 and 16% lower ($P < 0,05$). This indicates disturbances of calcium-phosphorus ratio, hypocalcemia and partial hypophosphatemia.

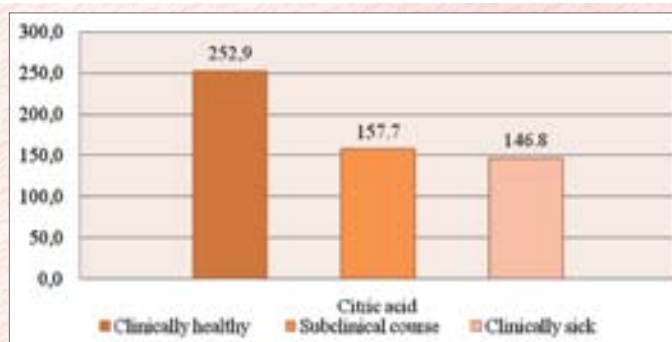
Serum magnesium level was normal in cows of all groups, though there is a tendency to it's lowering in clinically sick cows.

Citric acid – highly informative indicator of bone tissue status; key metabolite in the processes of mineralization, that creates complex compounds with phosphorus and calcium. It promotes elevation of their content in bone to the level, at which the processes of crystallization and mineralization starts [11].

Citric acid serum level was 58 and 37,6% lower in sick cows and with subclinical course comparing to healthy. It's level



Pic. 1. Serum macroelements levels in cows with osteodystrophy, mmol/l



Pic. 2. Serum citric acid levels in cows with osteodystrophy, mkmol/l

was critically low, accordingly: 157.7 ± 6.21 and 146.8 ± 5.89 mkmol/l ($P < 0.001$; pic. 2).

Lowering of citric acid serum level in sick cows is explained by the fact that normally vitamin D contributes to the transforming of pyruvic acid in to citric acid. Its lack can cause the accumulation of interim metabolism products – pyruvic acid and lactate, then acidosis is developed that slows citric acid creation [1, 13].

Osteocalcin – recently discovered new non collagen protein, synthesis of which is indicated by vitamin D [13]. Besides the connection between osteocalcin and citric acid with crystals of hydroxyapatite is viewed and they probably play important role in its forming and in bone resorption.

Our research established that the concentration of osteocalcin in blood serum of cows with the subclinical course of osteodystrophy was $0.93\text{--}1.28$ ng/ml and in clinically sick cows – $0.58\text{--}1.05$ ng/ml. In average its amount was 1.10 ± 0.03 and 0.7 ± 0.04 ng/ml that was lower for 19.7 and 49.0% comparing to clinically healthy animals (pic. 3).

Lowering of osteocalcin in blood serum of cows with subclinical course of

osteodystrophy is explained by the fact that tissue resorption prevails in the process of bone remodeling.

Determination of osteocalcin blood levels in cows with osteodystrophy can be a special marker of bone tissue resorption, since its levels are changed even in cows with subclinical course of the disease, when the levels of calcium and phosphorus remain unchanged.

Our research shows that blood levels of connective tissue metabolites in cows go beyond the frames of physiological fluctuation in both, clinically sick animals and those with subclinical course.

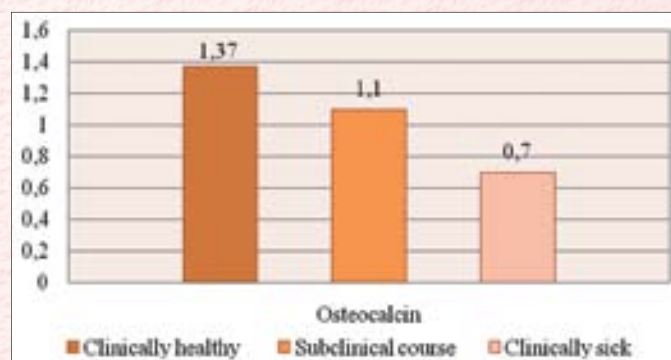
The levels of general glycosaminoglycans and sialoglycoprotein indicate the presence of destructive and disorganization processes in organic matrix of bone tissue. These biopolymers together with collagen are necessary components of bone organic matrix and promote its ossification and structural organization. Elevation of general glycosaminoglycans and sialoglycoprotein blood levels was observed in both sick cows and those with subclinical course comparing to healthy animals [11]. The blood levels of glycosaminoglycans was

35.0 and 58.0% (difference less than 0.01) and sialoglycoprotein – 28.5 and 80.0% increased comparing to healthy cows (difference less than 0.05; pic. 4). The increase of general glycosaminoglycans and sialoglycoprotein in blood indicates destruction of organic parts of the bone.

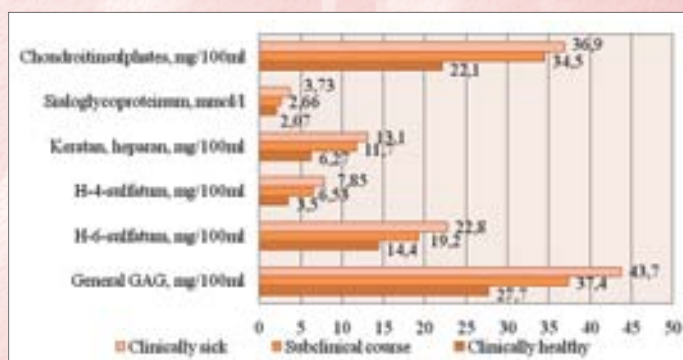
Fractional analysis of general glycosaminoglycans revealed the increase of chondroitin-4-sulfate sick to 20.0% in cows comparing to clinically healthy cows. Since this fraction is localized in bone tissue, its elevation indicates destructive changes in the bone [7]. The rise of chondroitin-6-sulfate in sick cows was 58.0% and the content of heparin, heparan-, dermatan-, keratan sulfate two times higher comparing to clinically healthy animals.

Serum concentration of general calcium and non-organic phosphorus in clinically healthy animals was normal ($2.2\text{--}3.12$ mmol/l), in cows with subclinical course – general calcium was 18.9% lowered.

In 94.3% of cows with subclinical course of osteodystrophy, the concentration of phosphorus was normal and in 5.7% – hypophosphatemia was observed.



Pic. 3. Content of osteocalcin in blood serum in cows with osteodystrophy, ng/ml



Pic. 4. Blood levels of connective tissue metabolites in cows with osteodystrophy



In 5,0% of clinically sick cows general calcium was normal and in 95,0% – hypocalcemia was revealed, with hypophosphatemia in 35,0% of sick animals. During this period the calcium and phosphorus homeostasis can not be supported by bone tissue and other mechanisms.

The blood serum concentration of citric acid was lowered in 100% cows with subclinical course and those with osteodystrophy. Its average content was $157,7 \pm 3,30$ and $146,8 \pm 1,20$ mkmol/l. Lowering of citric acid concentration was due to the oppression of Krebs cycle in osteoblasts under the influence of parathyroid hormone as a main regulator of calcium-phosphorus metabolism, which at first launches bone resorption and later oppresses its mineralization through the lowering of citric acid level in bone tissue. Generally the lowering of its serum blood concentration develops faster than the hypocalcemia.

The lowering citric acid serum levels in cows with subclinical course of osteodystrophy is connected with hypocalcemia and hypophosphatemia in 18,9 and 5,7% of cows relatively.

According to the literary data osteocalcin is a marker of bone tissue forming, reflects the activity of osteoblasts. Deficit of calcium ratio is accompanied by the lowering of its concentration in serum. When the optimal level of calcium is renewed, the amount of osteocalcin in blood rises indicating the lowering of calcium supply from bone to blood. The level of osteocalcin was lowered in 60,0% of cows with subclinical course and in 100,0% of sick animals, that combines with the lowering of concentration of citric acid.

The levels of general glycosaminoglycans, sialoglycoproteins and chondroitin-sulphate were increased in both cows with subclinical course and sick animals. Their number was increased in 100,0% of two researched groups of cows.

Diagnostic value of these parameters determination is connected with the fact that they constitute the major part of organic matrix of bone tissue. During bone resorption they appear in blood and thus are informative criteria for osteodystrophy preclinical diagnostics.

CONCLUSION

The results of research show violation of phosphorus-calcium metabolism in cows with osteodystrophy. The carried out research established that during osteodystrophy in cows the phosphorus-calcium metabolism, the metabolism of collagen and connective tissue metabolites is violated. Determination of citric acid, osteocalcin, glycosaminoglycans and their fractional content and also general calcium and non-organic phosphorus can be informative tests for early diagnostics of osteodystrophy and its subclinical course.

THE LIST OF LITERATURE

1. **Витамин D** и его роль в обеспечении здоровья детей и беременных женщин: Монография / Е.М. Лукьянова, Ю.Г. Антипин, Л.И. Омельченко, Л.И. Апуховская. – К.: Эксперт, 2005. – 230 с.
2. **Каракашов Ат.** Микрометоды в клинической лаборатории / Ат. Каракашов, Е. Вичев. – София: Медицина и физкультура, 1966. – 256 с.
3. **Клінічна діагностика внутрішніх хвороб тварин** / В.І. Левченко, В.В. Влізло, І.П. Кондрахін та ін.; за ред. В.І. Левченка і В.Л. Галюса. – Біла Церква, 2004. – 608 с.
4. **Ковзов В.В.** Диагностика нарушений обмена веществ у высокопродуктивных коров / В.В. Ковзов // Ученые записки Витеб. гос. акад. вет. медицины. – 2007. – Т. 43. – Вып. 1. – С. 109–111.
5. **Морозенко Д.В.** Біохімічні показники метаболізму сполучної тканини у діагностиці захворювань дрібних домашніх тварин: Монографія / Д.В. Морозенко. – Харків, 2011. – 120 с.
6. **Слівінська Л.Г.** Уміст у крові кісткових маркерів метаболізму за остеодистрофії корів / Л.Г. Слівінська, В.Л. Федорович // Наук. вісник вет. медицини: Зб. наук. праць. – Біла Церква, 2011. – Вип. 8 (87). – С. 151–155.
7. **Способ** определения гликозамингликансульфатов в сыворотке крови: А. С. № 960626 СССР, М. кл. 3. G 01 № 33148 / М.П. Штерн, О.П. Тимошенко, Ф.С. Леонтьева, Г.Ф. Ключева (СССР). – № 2998857128-13; Заявлено 23.10.80. Опубл. 23.09.82. Бюл. № 35 – С. 163.
8. **Стадник А.М.** Мікроелементний статус корів і крові корів та метаболічні зміни за ензоотичної остеодистрофії / А.М. Стадник, В.Л. Федорович, Г.О. Биць // Наука та прак-

тика. – 2007: 36. матеріалів міжнар. наук.-практ. конф. – Полтава, 2007. – С. 250–253.

9. **Стадник А.М.** Современные направления доклинической молекулярной диагностики остеодистрофии / А.М. Стадник, В.Л. Федорович // Ученые записки Витеб. гос. акад. вет. медицины. – 2007. – Т. 43. – Вып. 1. – С. 228–230.
10. **Федорович В.Л.** Стан кісткового метаболізму за остеодистрофії корів / В.Л. Федорович, Л.Г. Слівінська // Наук. вісник Луган. нац. аграр. ун-ту. – Луганськ, 2011. – № 31. – С. 223–226.
11. **Biochemical** markers of bone formation and resorption around parturition and during lactation in dairy cows with high and low standard milk yields / A. Liesegang, R. Eicher, M.-L. Sassi [et al.] // J. Dairy Sci. – 2000. – Vol. 83. – P. 1773–1781.
12. **Bovine** osteodystrophies / G. Caldow, B. Wain, A. Grant [et al.] // Veterinary Record. – 1995. – Vol. 136. – № 3. – P. 80–84.
13. **Plasma** osteocalcin in periparturient and postparturient cows: correlation with plasma 1,25-dihydroxyvitamin D, calcium, and inorganic phosphorus / Y. Naito, N. Shindo, R. Sato [et al.] // J. Dairy Sci. – 1990. – Vol. 73. – P. 3481–3484.

Одержано 8.09.2014

Діагностична інформативність окремих показників крові корів за остеодистрофії. Л.Г. Слівінська, В.І. Левченко, В.Л. Федорович, І.А. Максимович

Наведено результати досліджень окремих біохімічних маркерів крові та метаболітів сполучної тканини за остеодистрофії корів. Обґрунтовано найбільш інформативні тести ранньої діагностики остеодистрофії та її субклінічного перебігу.

Диагностическая информативность отдельных показателей крови коров при остеодистрофии. Л.Г. Сливинская, В.И. Левченко, В.Л. Федорович, И.А. Максимович

Приведены результаты исследований отдельных биохимических маркеров крови и метаболитов соединительной ткани при остеодистрофии коров. Обоснованы наиболее информативные тесты ранней диагностики остеодистрофии и ее субклинического течения. ○

З версією статті українською мовою можна ознайомитися на web-сторінці журналу – <http://vnuu.org.ua>