Influence of mineral-protein feed additive on the characteristics of the bone tissue of the vertebrae of cattle in alimentary osteodystrophy

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Abstract. In the experiments, it was found that in case of alimentary osteodystrophy of dairy cattle the use of the mineral-protein additive causes an increase in dimming during X-ray examination of the marginal epiphysic zone of the 5th caudal vertebrae by 9.4% (p <0.05); there was an increase in the mass and density of the 5th caudal vertebrae by 7.1% and by 9.6%, respectively; the stability of the bone structure of the spinal canal of the vertebrae to mechanical pressure increased by 7.9%, and the stability of the body of the caudal vertebrae increased by 25.1% (p <0.05); the level of magnesium and calcium in the bone substance of the vertebrae increased significantly by 40.2% (p <0.05) and 6.0% (p <0.01), respectively; histological examination revealed an increase in the density of the structure of bone tissue by 48% and the number of osteocytes - by 42.2%.

1. Introduction

In dairy cattle, the problem of disturbed mineral metabolism is the most acute because being in a state of intense lactation cows use three times more calcium compounds than in other physiological periods of life. In such cases, livestock breeders resort to more economically acceptable actions – introducing additives containing easily digestible calcium into the animal’s diet [1, 2].

Bone tissue is a unique indicator that first responds to calcium deficiency in the animal organism. Having a specific amino acid composition, which is enclosed in an organic matrix consisting of hydroxyapatite crystals (biomineral Ca2+ and P), skeleton bones are one of the main organs that exchange phosphorus and calcium in the system of general homeostasis. Calcium compounds form the basis of bone tissue; calcium ions provide muscle contractions, participate in a nerve impulse, being a part of various enzyme systems that catalyze biochemical reactions in the body, stimulate the contractile function of the heart and increases non-specific resistance [3].

In violation of the mechanisms of mineral metabolism regulation under the action of parathyroid hormone, calcium resorption from bone tissue occurs, which leads to the development of osteodystrophy of the alimentary type [4-6]. The pathology of mineral metabolism creates conditions for the violation of immune defense mechanisms, which contributes to the emergence of various non-communicable,
infectious and invasive diseases. In such animals, increases the percentage of development of infertility and abortion [7, 8]. Violation of mineral metabolism significantly affects the development of seasonal diseases of the gastrointestinal tract, as well as gynecological pathologies (endometritis, mastitis, etc.). In animals with alimentary osteodystrophy, decreases the percentage of pregnancy that occurs only with 3–4 insemination. Low levels of calcium in the blood lead to a decrease in the insulin concentration, increased mobilization of fatty acids, which leads to the accumulation of the endogenous fat [9, 10].

The aim of the study is the improvement of therapeutic and preventive measures for alimentary osteodystrophy in dairy cows. To achieve this aim, the following task was set: to assess the effect of the mineral feed additives on the histological, biochemical and biophysical parameters of the vertebrae of the base of the tail during alimentary osteodystrophy in lactating cows.

2. Materials and methods

The experimental part of the scientific work was carried out on the Russian Black Pied cows during intensive lactation with a diagnosis of alimentary osteodystrophy, which was confirmed by clinical and laboratory research methods. To study the effectiveness of the mineral-protein additive, two groups of animals with 20 cows each were formed. The experiment was carried out for two months from February to April.

An experimental group of cows got a complex mineral-protein feed additive into the diet, consisting of a mixture of yeast autolysate, monocalcium phosphate, chalk feed and natural mineral supplements – bentonite at a dose of 1.5 g / kg of body weight.

Animals of the control group got a treatment used in the livestock enterprise. They received 100 g of monocalcium phosphate with feed, as well as an intramuscular injection of tetramag (a complex of fat-soluble vitamins A, D, E, F). The preparation was administered once every 10 days by the method of «vitamin impulse» in a dose of 10 ml. Similar injections were received by animals of the experimental group.

At the end of the experimental period, five animals from the both groups were slaughtered. The 5th caudal vertebrae were extracted from them for histological, biochemical, radiological and physical studies.

The fragments of the axial skeleton (the 5th vertebrae of the caudal segment of the spine) were studied. To assess the mineral composition of the bone tissue, chemical studies of axial skeleton fragments were performed using atomic emission spectrometry and inductively coupled plasma mass spectrometry (Optima 2000 V, Perkin Elmer, USA) and mass spectrometry (Elan 9000, Perkin Elmer, USA) in the laboratory of the ANO “Center for Biotic Medicine” (RU.0001.22PYA05, dated 12.24.10), in accordance with the GOST 3178-96, GOST 26570-95 and GOST 26657.

X-ray examination of the vertebrae of the caudal spine was performed using a Dina-2 mobile X-ray apparatus with an exposure of 1 second at a distance of 60 cm and a Fire CR + veterinary 20 X-ray digitizer. The electronically obtained images were subjected to the biological analysis.

After the slaughter of the experimental animals, the vertebrae were secreted and cleared from soft tissues, after which their studies were conducted.

Vertebrae were weighed on the laboratory scales. The volume of the vertebrae was determined by the method of accounting for the volume of displaced water (according to the law of Archimedes). The density of the caudal vertebrae was determined by dividing the mass by volume and was expressed in grams per centimeter cubic (g / cm³). After that, the vertebrae were subjected to mechanical compression on a measuring hydraulic unit UIM-50, thereby determining their stability until the moment of fixed destruction. The vertebra was located under the hydraulic piston in the transverse position with the vertebral body down and the dorsal edge up. As a result of mechanical compression of the vertebrae, two results were recorded: the first is the destruction of the vertebra in the area of the backbone canal; the second is the destruction of the vertebral body.

When assessing histological characteristics, the relative bone density and the relative number of osteocytes were measured. The vertebrae were fixed in 10% formalin, decalcified, carried out by standard wiring, followed by pouring in paraffin. On a rotary type microtome, histological sections were
prepared with a thickness of 7 μm and stained with hematoxylin and eosin. Optical microscopy of the preparations was carried out using an imaging system based on an OlympusBX 41 microscope, followed by photographing of the preparations. Morphometric study of the obtained images was performed using the “Morphology 5.2” program (VideoTesT St. Petersburg, Russia), during which the state of bone tissue was evaluated.

The received information was statistically processed by the method of standard variation statistics using the computer application Microsoft Office Excel 2010.

3. Results and discussion

During the clinical evaluation of dairy cows in a livestock enterprise, it was found out that 53% of the animals had pathological processes in the area of the axial skeleton (49% - lordosis, 4% - kyphosis). A subsequent study of the sick animals revealed osteomalacia and lysing of the vertebrae in the final segments of the tail, the transverse processes of the lumbar vertebrae sagged under pressure, the incisor teeth easily loosened in the dental alveoli, the last ribs were reduced in diameter and easily bent. In most sick animals, inhibition of the motor function of the scar was noted.

During X-ray evaluation, the obtained images were used to measure the area of X-ray darkening of the bone epiphyses and the marginal sections of the diaphysis of the caudal vertebrae. After that, the mathematical ratio of the zone of maximum X-ray dimming to the length of the whole vertebra was derived.

It was determined that in animals of the control group, the marginal episodiaphyseal zone was 19.1 ± 2.72%, and in animals of the experimental group after course administration of the preparation it was 28.5 ± 3.12%. Differences in this trait between animals were 9.4% (p <0.05). In this case, a decrease in the area of radiographic dimming in the vertebrae in animals of the control group is evidence of demineralization of the inert substance, which is one of the first signs of osteomalacia.

Thus, the data of the X-ray examination indicate an increase in the density of the bones of the axial skeleton when using mineral-protein additives in the general complex of therapeutic measures.

The study of the physical parameters of the 5th caudal vertebrae of cows using a mineral-protein additive is presented in Table 1.

| Investigated parameters                | Groups of animals          |
|---------------------------------------|---------------------------|
|                                       | control     | experimental |
| Bone volume, cm³                      | 54.2±6.48   | 53.9±5.31    |
| Bone mass, g                          | 57.3±6.32   | 61.4±5.32    |
| Density of bone tissue, g / cm³       | 1.057±0.0531 | 1.159±0.0566 |
| The load required to destroy the caudal vertebra in the transverse position, kg |                       |
| Destruction of the matrix of the spinal canal, kg | 778.3±56.74 | 840.0±61.42 |
| Complete destruction of the vertebral body, kg | 884.0±54.49 | 1106.2±53.80* |

* – p <0.05; ** in relation to control.

The volume of the vertebrae in animals of the experimental and control groups was comparable to each other. The mass of the vertebrae of cows of the experimental group was 7.1% higher, and the density was 9.6% higher than control analogues.

When assessing the resistance of the vertebrae to mechanical pressure, the destruction of the bone structure of the spinal canal was recorded in animals of the experimental group with the load 7.9% higher, and the destruction of the body of the caudal vertebra with the load 25.1% higher (p <0.05).

Thus, alimentary osteodystrophy and its concomitant disturbance of mineral metabolism in cattle lead to a decrease in mass, density and resistance to mechanical compression of the vertebrae of the caudal area. The use of mineral feed additives leads to an increase in the quantitative characteristics of these biophysical parameters.
To evaluate the mineralization of skeleton bones using a mineral-protein additive, a biochemical analysis of the vertebral bone was performed, as a result of which it was determined that the calcium concentration in the bone tissue of the vertebrae of the tail base of the control group of cows was 27.9 ± 0.60%, and in animals, receiving the additive it was 33.9 ± 0.99%, the differences in the values of the experimental and control groups amounted to 6.0% (p <0.01). The magnesium concentration was 214.8 ± 32.60 mg / kg and 301.2 ± 21.67 mg / kg, respectively, with a difference of 40.2 mg (p <0.05) in favor of the experimental group. The phosphorus concentration in the bone tissue of the vertebrae in the experimental group was 2.62 ± 0.035%, and in the control group it was 2.77 ± 0.025%. There were no significant differences between the groups.

The data of a quantitative assessment of the mineralization of the vertebrae of animals from the experimental and control groups indicate an increase in the concentration of calcium and magnesium compounds in bone tissue when using a mineral-protein additive in the diet of sick cows.

The results of the histological study of the morphological structure of the caudal vertebrae are presented in figures 1-4.

Histological examination of micropreparations obtained from the caudal vertebrae in the first group showed a significant dissociation of bone trabeculae (figure 1), relative to the second group. The bone density was 316536 ± 10165 μm. Intertrabecular spaces were significantly expanded (figure 2). The number of empty gaps was 48%, and osteocytes – 52%.

On micropreparations of the experimental group of cows, their normal structure was noted. Bone trabeculae as a whole retained their structure (figure 3), the relative density of bone tissue was 469743 ± 13593 μm. The number of osteocytes was 72%, while in 28% of the lacunae the cells were not visualized (figure 4).

Morphological analysis of the cancellous bone tissue of the caudal vertebrae of the control group demonstrated the process of osteoresorption. This is evidenced by the greater number of dead osteocytes and bone fracture. We observe this picture due to prolonged osteodystrophic processes in sick animals.

In the second group of animals treated with the preparation, signs of osteoresorption were also observed, such as a slight increase in the intertrabecular space, a single expansion of the Haversian canals. However, bone density in the experimental group was 48% higher, and the number of osteocytes was 42.2% higher than in the control group.

Thus, the use of mineral-protein additives in therapeutic doses for alimentary osteodystrophy of cows contributes to an increase in the structural density of bone tissue and an increase in the number of osteocytes.

![Figure 1](image1.png) **Figure 1.** Group 1, fragmented bone trabeculae. Hematoxylin and eosin stain, 100x magnification.

![Figure 2](image2.png) **Figure 2.** Group 1, 1 – destruction of bone trabeculae and 2 – expanded Haversian channel. Hematoxylin and eosin stain, 400x magnification.
Summarizing the results of the study, we can say that all the methods used to assess the bone tissue of the vertebrae indicate the destructive effect of osteodystrophic processes associated primarily with the demineralization of the bone matrix. Obviously, skeletal bone demineralization during osteodystrophy of animals is a specific process that leads to the development of successive changes in the bones such as osteomalacia, osteoporosis and osteofibrosis associated with thinning, and subsequently with deformation of the bone matrix and the violation of its structure.

Our study confirms a significant decrease in the mass fraction of calcium as the main macroelement in the bones of the axial skeleton of diseased animals, which reliably causes the changes in all biophysical parameters of the vertebrae of the base of the tail – a decrease in the level of radiopaque contrast of the vertebrae, a decrease in their mass, density and mechanical fracture strength. Characteristic changes in the histological picture associated with the decrease in the structural density of bone tissue and the decrease in the number of osteocytes are noted.

All the used research methods make it possible to obtain informative criteria for assessing the state of bone tissue during subsequent pharmacological correction of the disease. The use of mineral–protein additives in the diet of sick cows made it possible to objectively determine an increase in all the studied parameters. The positive effect of the additive on the state of mineral metabolism as a result of the complex effect of the components of the test substance contributed to the restoration of mineralization, structure and function of the bone matrix. In turn, this led to an increase in the physical characteristics of the bone.

4. Conclusion

X-ray studies showed that when using a mineral-protein feed additive for alimentary osteodystrophy, the dimming of the marginal epidiaphyseal zone of the 5th caudal vertebrae of dairy cows increased by 9.4% (p <0.05); and there was an increase in the mass and density of the 5th caudal vertebrae by 7.1% and 9.6%, respectively.

The use of feed additives has increased the resistance of the vertebrae to mechanical pressure. So, in the experimental group, the mechanical load during the destruction of the bone structure of the spinal canal was higher by 7.9%, and during the destruction of the body of the caudal vertebra it was higher by 25.1% (p <0.05).

The additive had an influence on the mineralization of bones of cows, which was manifested by an increase in the level of magnesium by 40.2% (p <0.05) and calcium by 6.0% (p <0.01), as well as an increase in bone density and structure tissue (48.0%) and the number of osteocytes (42.2%).

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