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Effectiveness of the use of complex biological and mineral compounds in alimentary osteodystrophy of lactating cows

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Abstract. According to the data of Samara Veterinary Laboratory, in serum of cattle in 40.7 % of studied samples the calcium indicators have low values while increasing the level of inorganic phosphorus by 18.2 %, which indicates a significant prevalence and severe form of nutritional osteodystrophy in animals of the examined farms. The therapeutic use of a protein-mineral additive based on yeast autolysate, fodder bentonite, mono calcium phosphate and chalk in the general complex of therapeutic measures for alimentary osteodystrophy of lactating cows stimulates mineral metabolism, normalizing the calcium-phosphorus ratio, and has a positive effect on the milk productivity, increasing milk yield by 41.9 %. The preparation helps to stimulate hemo- and erythropoiesis and has an anti-toxic effect on liver. The use of the additive allows increasing the economic efficiency of milk productivity during alimentary osteodystrophy of cows by 11.2 %.

1 Introduction

All the most important physical and chemical processes in the body occur with the participation of mineral elements. At full mineral starvation, despite of the intake of all nutrients and water in the body, there is a loss of appetite, emaciation and death. The lack of some mineral components leads to delayed growth and development of young animals, reduced productivity and the occurrence of various diseases (rickets, osteodystrophy, etc.) [1, 2]. At the same time, this problem does not cause confusion among practicing professionals, since it does not create emergency situations for veterinary well-being in livestock farms [3].

Pathology of mineral metabolism is reflected in all spheres of vital activity of the organism. At rickets and osteodystrophy, disorders of the nervous, muscular, respiratory, cardiovascular and digestive systems can develop. Such diseases are often complicated by anemia, decrease in natural and specific resistance factors, exhaustion, which significantly reduces the body's resistance to infectious diseases and contributes to the occurrence of massive cases of bronchopneumonia, gastroenteritis in young animals and gynecological pathology in adult animals [4].

Calcium is one of the main elements determining the quality of mineral metabolism. Calcium forms bone tissue, maintains muscle tone, prevents the premature development of osteodystrophy and osteomalacia, contributes to the proper formation of the musculoskeletal system [5, 6]. The reduction of blood calcium levels in addition to violation of mineral metabolism leads to impaired neutrophil function, increased risk of subclinical ketosis, metritis and subclinical endometritis, abomasum displacement, decreased milk productivity and decreased chances of pregnancy in primiparous cows [7–11]. Early lactation in cows is characterized by intense changes in bone metabolism. During this period, cows experience increased bone resorption and calcium loss through milk production [12].

The absence of clinical signs and the lack of diagnostic tools at the early stage of calcium metabolic disorders make prevention the only alternative mean of therapeutic measures [13, 14]. Reasonable use of calcium supplements in the period of the beginning of lactation has a positive effect in any case. In healthy cows, the level of calcium in blood does not change, but there is an increase in milk productivity. In cows with initially reduced levels of total calcium in blood, it increases and improves the general condition [15, 16].

In the general complex of therapeutic and prophylactic measures of disorders of mineral metabolism, the use of active forms of vitamin D is essential. At the same time, a combined diet with acidic components with calcidiol gives the best effect in calcium metabolism and thus improves energy metabolism and efficiency during lactation (D3) [17].

In the present time, the preparations containing proteinaceous and biologically active substances, such as yeast, deserve special attention. They are characterized by a high growth rate and by resistance to extraneous microflora. They are also able to assimilate many food sources and can be easily separated from the culture fluid [18].

The implementation in the diet of natural sorbents has a positive effect on the biochemical blood parameters of animals. Enterosorbents have a high

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adsorbing activity and detoxification effect. They normalize the microbiocenosis of the large intestine, bind in the intestine and remove pathogenic bacteria from the body, promote the elimination of toxic products, have a beneficial effect on the processes of digestion and absorption and normalization of intestinal peristalsis in animals [19, 20]. Bentonite (montmorillonite) clays belong to the group of clay minerals with properties of both a mineral additive and a mineral sorbent [4].

Practical experience shows that the solution of a complex problem is to use diverse means and methods. The treatment and prevention of the osteodystrophy of lactating cows require the use of a system of measures, including combination of supplements, the components of which would affect various key mechanisms of disease development.

The purpose of the research is the improvement of therapeutic and preventive measures at alimentary osteodystrophy of lactating cows.

To achieve this purpose, the following tasks were defined:

− to study the prevalence and causes of violations of calcium-phosphorus metabolism in livestock farms of the Samara region;

− to study the therapeutic efficiency of the mineral-protein complex in alimentary osteodystrophy in lactating cows.

2 Materials and methods

A production experiment in testing the protein-mineral preparation was carried out on the farm of OJSC Samarskoe, Kinel District, Samara Region, on lactating cows with laboratory-confirmed diagnoses: alimentary anemia and alimentary osteodystrophy. The experiment was carried out from the beginning of February to the beginning of April. Previously, in the regional veterinary laboratory a statistical analysis of data on the biochemistry of cattle blood in the farms of the Samara region was made for 3 years (from 2013 to 2015).

The therapeutic efficiency of the preparation was evaluated on animals with diseases. The selection was carried out on the principle of analogue pairs.

The animals, timely vaccinated and treated against invasive diseases, were selected for the research. Experimental animals were subjected to general clinical research. The animals were evaluated according to the general condition, fatness, condition of the joints, hair-covering, transverse processes of the lumbar vertebrae, caudal vertebrae, ribs. Animal health was monitored during the whole experiment.

In the experiment on the study of therapeutic efficiency, two groups of black-and-white cows, 20 animals each, were involved during lactation, (blood and milk for the study were taken from 10 animals), and the experiment was carried out for 60 days. The first group was used as a control. The animals of this group received 100 g of calcium phosphate per head per day as a mineral additive. The second experimental group received a protein-mineral additive. The preparation was used in the morning and the evening feeding at the rate of 1 g/kg body weight of the animal per day. Throughout the whole experiment, vitaminization of animals of both groups was carried out with the complex vitamin preparation tetramag containing fat-soluble vitamins A, D, E, F. The preparation was administered intramuscularly using the “vitamin push” method in a dose of 10 ml once in 10 days.

To study the effect of the preparation in the dynamics at the beginning of the experiment, blood from animals of each group was taken on 20, 40 and 60 days of research. Blood sampling was carried out from the jugular vein, two blood samples were taken from each animal. The volume of the first sample was 10 ml and it was used to obtain serum. The second sample was stabilized with heparin anticoagulant, its volume was at least 2 ml. Milk sampling was carried out in accordance with the current GOST on microbiological research methods.

Research work was performed using the following methods:

1. Hematological method – the number of leukocytes and erythrocytes, hemoglobin content, hematocrit value and erythrocyte indices were determined using a hematological veterinary analyzer Mindray BC-2800 Vet (quantitative counter of blood cells with leukocyte differentiation). ESR was determined by the method of Panchenkov. The calculation of leukocyte formula was carried out by a microscopic method according to the standard methods, blood smears were stained according to Papenheim.

2. Biochemical method – the concentration of total calcium, inorganic phosphorus, total protein, urea, creatinine, glucose, transaminase enzymes (alanine aminotransferase, aspartate aminotransferase) were determined on a Mindray BS-380 automatic biochemical analyzer (PRC) using commercial kits.

3. Protein fractions – the content of albumin, alpha-globulins, beta-globulins and gamma-globulins were determined in the blood serum using the turbidimetric method [21] on the spectro-photometer Fluorat-02 ABLF-T. According to the degree of turbidity of the solutions to be installed via photoelectric colorimeter we judged the concentration of proteins in the test sample.

4. Milk productivity was evaluated by the results of control milking conducted on the farm once a month. Qualitative indicators of milk – fat, protein, lactose, solids and urea – were analyzed on a Milkoscan Minor automatic milk analyzer (Foss, Denmark).

The statistical processing of the obtained data was performed by the method of standard variation statistics using Student's reliability criterion [22] on a PC using Microsoft Office Excel 2010.

3 Results

According to Samara Veterinary Laboratory of the Samara Region, laboratory tests of cattle blood serum, carried out in a number of farms in the Samara Region in 2013–2015, showed significant metabolic disturbances and, consequently, disturbances in the constancy of the internal environment of the organism. Thus, the concentration of total protein was reduced in 30.2 % of cases. Alkaline reserve was reduced in 18.3 % of cases. The disturbances in the metabolism of macroelements and the decrease in the concentration of calcium (in 39.5 % of cases) and phosphorus (in 9.9 % of cases) were found. The level of carotene was reduced in 75.9 % and the level of glucose was reduced in 88 % of cases. A similar trend was noted for the other indicators. The analysis showed that the main indicators characterizing the state of mineral metabolism in a sufficient number of cases was outside the lower limit of the norm. The situation has the same tendency for a number of years with a slight variability of changes. Taking into account that such indicators as calcium and phosphorus in the blood decrease beyond the minimum limit of the norm in cases when animals already have external clinical manifestations of osteodystrophy, it can be stated that there is a significant disturbance of mineral metabolism in dairy cattle breeding across farms, areas and the whole region.

One of the main reasons of this pathology is the violation of the technological process: the use of poor-quality feed, insufficient and/or inadequate feeding and the lack of mineral and protein supplements, the lack of physical exercise. The season of the year is also important. Clinical manifestations of osteodystrophy in most cases are registered in the winter-spring period of the year, which is associated with a deficit of sunlight. This prevents the natural synthesis of vitamin D in the body. The systematic use of canned acidic feed contributes to metabolic, which leads to acidosis and increased bone demineralization. The particular importance has the physiological status of the animal. During lactation, the cow loses a large amount of mineral substances for milk production and fetal development.

The treatment of osteodystrophy should be approached comprehensively, since this disease in the first stages has no pronounced signs, but affects all body systems. It reduces the immunity of animals, whereby they become vulnerable to infectious agents. Expenditures on care, medicinal products are increasing, while the productivity and the total income of farms decrease.

The complex feed additive consists of the following components: feed bentonite, feed chalk, yeast autolysate and monocalcium phosphate. All components of the preparation have the appropriate regulatory and technical documentation confirming the possibility of their use for animals.

The use of the protein-mineral complex fodder for cows with signs of alimentary osteodystrophy during lactation helps to increase the concentration of calcium and phosphorus in the diet, improve their absorption due to the systemic action of bentonite clay, because of the improved quality of digestion and absorption of mineral and biologically active substances from the ration. All of the above factors contribute to the restoration of metabolism and increase the productive qualities of lactating cows.

During the baseline studies, it was found out that animals have clinical signs of alimentary osteodystrophy, confirmed by anamnesis and laboratory data.

The preliminary data showed that animals in OJSC Samarskoe receive the following ration: *Bromus inermis* hay – 8 kg, brewer's grains – 20 kg, maltodextrin – 1.5 kg, bran – 2 kg, crushed corn grain – 2 kg, crushed barley grain – 3 kg. This ration is sufficient for nutritional, protein and carbohydrate components, but deficient in mineral components and vitamins. The development of osteodystrophy is aggravated by the presence of brewer's grains in the diet, which has milk expelling effect and promotes the active elimination of mineral elements from the body.

Preventive vitaminization in this farm is not practiced. Animals use passive exercise on the walking yard of the animal-breeding complex for several hours a day.

The clinical examination showed that the majority of cows had: softening and shakiness of the transverse processes of the lumbar vertebrae; softening and resorption of the last caudal vertebrae and the last rib; lordosis; in a number of animals, the X-shaped staging forelimbs against the background of an increase in carpal joints are observed.

In laboratory studies of blood, there was a decrease in the level of total calcium and an increase in the concentration of inorganic phosphorus, which leads to a significant decrease in the phosphorus-calcium ratio. There was also the decrease in hemoglobin and hematocrit, which is characteristic of nutritional anemia. Metabolic disorders affect the decrease in milk productivity of animals.

During the experiment, it was found out that the number of red blood cells did not have marked differences in groups and varied from 6.1 to $6.9*10^{12}$ l. The hemoglobin level and the average hemoglobin content in the erythrocyte initially had low values, which amounted to 87.6±2.01 g/l and 14.6±0.44 pC, respectively. The hemoglobin level in the experimental group reached standard values after 20 days, and in the control group – after 40 days of the experimental period. By the end of the experiment, the differences between the experimental and control groups were 9.1 % ($p < 0.05$). Indicators of the control and the experimental groups increased in relation to the background values by 10.2 % ($p < 0.05$) and 20.3 % ($p < 0.01$), respectively.

The level of hematocrit value at the beginning of the experiment was beyond the minimum limit of reference values (28.0±0.47 %). In the experimental group, it reached the regulatory indicators in 20 days, and in the control – in 40 days from the start of the experiment. By the end of the experiment, the differences between the control and experimental groups were 8.4 % ($p < 0.05$), while in relation to the background indicators, the hematocrit level in the experiment group increased by 24.6 % ($p < 0.01$), in the control group by 15.0 %. The average volume of red blood cells at the beginning of the experiment had a value of 47.7±2.12 fl. After 60 days of the experiment in the control group, the erythrocyte volume did not change, and in the experimental group it increased by 7.3 %.

In addition, there were positive changes in the dynamics of platelet count. At the beginning of the experiment, the content of platelets in blood was within the permissible limits and amounted to 382.8±21.69*109/ l. During the experiment, in both groups there was a tendency of increasing the number of blood platelets with a pronounced priority in animals treated with the complex preparation. At the end of the experiment, the platelet count in the experimental group increased by 13.3 % ($p < 0.05$) in relation to the control, and by 28.9 % ($p < 0.001$) in relation to the background, respectively.

The analysis of white blood indices revealed the absence of pronounced changes associated with the use of the feed additive.

Based on the performed analysis, it can be concluded that in animals, in the composition of the diet of which a complex mineral-protein preparation was used, a quantitative and qualitative improvement in indicators characterizing the state of red blood associated with the activation of hematopoiesis was observed. The latter became possible because of enhancing digestion, improving the absorption of nutrients and minerals.

During the assessment of biochemical parameters in dynamics, the mixed results were obtained. The level of total protein and glucose throughout the experiment in both groups was within acceptable limits.

The level of calcium in the initial period of the experiment was significantly reduced (2.05±0.085 mmol/ l), and the level of phosphorus, on the contrary, was overstated (3.05±0.183 mmol/ l). As a result, the phosphorus-calcium ratio was 1/0.67 (normal ratio is 1/1.5), which indicates its gross violation in connection with the pathology of mineral metabolism, proceeding by hyperphosphatemic type. In the dynamics of the experiment, the level of total calcium in the control group showed slight tendencies to increase, and by the end of the experiment the difference with the background was 7.3 %. Whereas in the experimental group there was a steady dynamics of an increase in the indicator, and by the end of the experiment its values corresponded to the average normal values (2.49±0.023 mmol / l). As a result, the concentration of total calcium became more than background values by 21.5 % ($p<0.01$) and more than control values by 13.2 % ($p < 0.05$).

When assessing the level of inorganic phosphorus, the opposite dynamics was observed. In the both groups, there was a decrease in this indicator. At the end of the experiment, the values in the control and experimental groups were 1.69±0.074 mmol/l and 1.60±0.092 mmol/l, respectively. The difference with the background indicators was 55.4 % and 52.5 % ($p<0.01$), respectively. The phosphorus-calcium ratio has also changed. At the end of the experiment in the control group, it was 1/1.3, and in the experimental group, it was 1/1.55.

Thus, the use of the complex mineral-protein additive in the diet of lactating cows using yeast autolysate and bentonite clay with alimentary osteodystrophy for two months helps to restore the characteristics of calcium-phosphorus metabolism. There is an increase in calcium level, a decrease and normalization of the level of phosphorus, as well as a restoration of the phosphorus-calcium ratio. In the control group, in which only calcium hydrophosphate and the standard vitaminization regimen were prescribed, positive dynamics in the restoration of phosphorus level and its ratio with calcium was also observed, but the total calcium content in blood remained at low values.

In clinical practice, the study of enzyme systems as biochemical markers of various pathological conditions is essential. In our case, the data on aspartate aminotransferase (AST) is of interest. Its activity was initially increased and amounted to 98.1±6.58 unit/l. During the experiment, in the experimental group there was a dynamic decrease in the indicator and, by the end of the experiment, the difference in relation to the initial values was 22.8 % ($p < 0.05$). In the control group, the activity of AST changed insignificantly and in the end the difference with the experimental group was 23.7 % ($p<0.05$), without any differences with the background values.

Aspartate aminotransferase serves as an indirect marker of pathology of liver and heart, its increase is associated with cytolytic phenomena in these organs. It is considered in combination with alanine aminotransferase, and their ratio is determined by the De Ritis coefficient. For background indicators, it amounted to 2.93, for control values at the end of the experiment it is 2.78, for experimental groups it is 2.42. In all cases, the values were more than one unit that indicates overstrain of the heart muscle. This condition is very typical for lactating cows during lactation on the background of mineral deficiency, because the heart has to work in an enhanced mode on the background of deficient pathology. As it can be seen from the results of our studies, the use of a feed additive contributed to the restoration of myocardial functionality.

The analysis of the ratio of whey protein fractions showed that they had interdependent changes. The changes were determined only in the dynamics of gamma globulins in the control group. On the 40th day in this group there was a significant decrease of the indicator relative to the values in the animals of the experimental group by 12.4 % ($p<0.05$). It can be seen that in this situation, the use of the complex mineral-protein additive has a stabilizing effect on the immune background, which is provided mainly by antibodies from the class of gamma globulins.

The changes in milk productivity and milk quality were very significant in our research. At the beginning of the study, the daily milk yield of the animals was 10.5±0.360 kg. During the study period in the control group, this value remained stable, while in the experimental group there was a dynamic increase of the indicator. A month later, in the experimental group, in relation to the background and control indicators, the daily milk yield increased by 3.5 kg, respectively, which amounted to 33.3 % ($p<0.05$). At the end of the experiment, the daily milk yield increased by 4.4 kg (41.9 % ($p < 0.05$)) in relation to the background, and by 4.2 kg (39.3 % ($p < 0.05$) in relation to the control.

The protein concentration in milk at the beginning of the experiment was 3.36±0.057 %. Throughout the experiment, the indicators in the groups did not change significantly, however, in the experimental group these values never dropped below the initial ones and were higher than the control indicators by 0.14 %. At the beginning of the study, the milk fat content of the experimental cows was 3.61±0.23 %. During the study, the fat content in milk in all groups was higher in the experimental group and the values did not fall below the background. The difference with control and background values was 0.16 %. Initially, the urea level was above the upper limits of the norm and amounted to 9.44±0.385 mmol/l.

Throughout the experiment, the urea level changed. In the end, in the experimental group, it was 5.36±0.404 mmol/l, in the control group it was 5.79±0.358 mmol/l, which is 44.3 and 38.7 % less than background values, respectively. The difference between the control and the experimental group was 7.4 %.

The integral indicator for the content of all milk dispersed and soluble components are milk solids. At the beginning of the study, this indicator corresponded to 12.75±0.28 %. As for other milk indicators, there was no pronounced dynamics of changes, but the experimental indicators were higher than the control ones. At the end of the experiment, the difference between the experimental group and control and background values was 0.24 and 0.18 %, respectively.

Thereby, the use of the complex mineral-protein additive, yeast and bentonite autolysate in rations of dairy cows with the diagnosis of alimentary osteodystrophy contributes to the increase in daily milk yields, milk fat, dry matter, and to the decrease in urea concentration. Despite the fact that, in some cases, the changes were not reliable, they had stable constancy for all series of experiment and for all indicators.

At the end of the analytical work on the obtained scientific material, an economic calculation was carried out, which showed that the use of complex protein-mineral additive can increase the efficiency of milk productivity by 11.2 % compared with the use of the main diet for alimentary osteodystrophy of cows.

4 Conclusion

According to the data of Samara Veterinary Laboratory, in 40.7 % of the studied samples, the calcium values in cattle serum were low, while the level of inorganic phosphorus increases by 18.2 % that indicates the significant prevalence and severe form of alimentary osteodystrophy in animals of the examined farms.

As a result of the studies, it was found out that the use of the protein-mineral additive based on yeast autolysate, fodder bentonite, monacalcium phosphate and chalk in the general complex of therapeutic measures for alimentary osteodystrophy of lactating cows has the antianemic effect, normalizing red blood counts and platelet values; stabilizes the phosphorus-calcium metabolism, increasing calcium in hypocalcemia and reducing phosphorus in hyperphosphatemia, normalizing the phosphorus-calcium ratio; provides a constant ratio of protein fractions, especially gamma globulins; helps to reduce the elevated level of aspartate aminotransferase and, as a result, indirectly helps to improve the conditions for the functionality of the heart muscle; causes an increase in milk productivity and an improvement in the basic biochemical characteristics of milk. Therefore, the use of the protein-mineral complex provides an improvement in the basic physiological and metabolic parameters of the body, as well as contributes to the recovery of animals and is economically reasonable.

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