Pharmacological therapy of white muscle disease in lambs with selenium preparations in a comparative aspect

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Abstract. In the course of the experiment on the comparative assessment of selenium-containing preparations selephlan and plexomin Se-2000 in the treatment of white muscle disease in young sheep, it was determined that selephlan has a more pronounced effect on the clinical and physiological state and intestinal biocenosis of lambs, their morphological and biochemical blood parameters, as well as the level of antioxidant protection of the body of animals. Under the influence of the preparation, the erythro- and hematopoiesis is activated in lambs, as well as the normalization of biochemical blood homeostasis. The level of enzymatic activity (AST, ALT, ALP, LDH) significantly decreases in 1.36 (p≤0.05); 1.71 (p≤0.001); 1.21 (p≤0.05) and 1.3 (p≤0.01) times, while the concentration of carotene increases in 3.1 times, vitamin E increases in 1.73 times and vitamin A increases by 26.8%. Selephlan helps to reduce the concentration of DC by 21.8%, KD – by 28.7%, MDA – in 1.61 (p <0.05) times.

1. Introduction
One of the most important tasks in animal husbandry is to ensure a high productivity of animals, including the qualitative improvement of the feed base and control of the usefulness of diets not only for the main nutrients such as proteins, carbohydrates, lipids, but also for a number of biologically active substances, a special place of which is occupied by a group of mineral elements containing in feed in very small quantities, but playing an important role in the body – the so-called micro- and ultramicrolelements, the imbalance of which in soil and vegetation often leads to the development of enzootics [1, 2, 3].

These microelements include selenium, which is contained in almost all body tissues and involved in many biological processes, possessing pronounced antioxidant properties. Together with vitamin E, selenium destroys peroxides in glutathione peroxidase, ensuring the activity of redox reactions and the antioxidant system of the body. Selenium deficiency in the body disrupts carbohydrate, lipid and fat metabolism, under-oxidized products accumulate in tissues and organs, liver infiltration and dystrophy occurs, as well as the destructive changes arise in skeletal and cardiac muscles, leading to the development of white muscle disease [4, 5, 6, 7].

The white muscle disease is a serious disease of young animals and poultry that occurs in the first days and weeks of life, characterized by a violation of all types of metabolism and accompanied by
functional, dystrophic and necrobiotic changes in skeletal muscles, heart muscle, organs and tissues. Young animals of all types can get sick. The damage is determined by a high percentage of death, a sharp decrease in the reproductive capacity of the breeding stock and a decrease in the hereditary qualities of animals [8, 9].

In connection with the above mentioned, there is a need to develop the new effective therapeutic and prophylactic veterinary preparations aimed at correcting selenium deficiency in young animals [10].

2. Materials and methods

An experiment to study the therapeutic efficiency of the preparation selephlan was carried out on young sheep in the conditions of the peasant farm in Krasnodar region. The reason for the appeal of the herd owner was the high morbidity and mortality of lambs at the age from 1 to 5 months.

The main clinical signs of the disease were characterized by decreased appetite, stunted growth, weakness, rapid fatigue, inactivity, shaky gait, in some cases lameness and muscle tremor. As a concomitant pathology, the lambs were diagnosed with the violation of the gastrointestinal tract, manifested by diarrheal syndrome.

Postmortem examination of the dead animals showed swelling of the subcutaneous tissue, signs of the focal dystrophic or atrophic lesions of muscle tissue, especially in the area of the posterior zone and diaphragm. On the cut muscle had gray-white and flabby consistency. The heart was enlarged, on the epicardium and endocardium were visible whitish and yellowish stripes (tigroid). The liver had a pale gray-red color with an icteric tinge and dense consistency with dystrophic areas of various sizes.

Based on the clinical picture of the disease and pathomorphological changes in organs and tissues of lambs, a preliminary diagnosis of white muscle disease was made. To confirm the diagnostic hypothesis, samples of feed and blood were taken from the farm for laboratory research.

As an additional nutrition, the farm uses only salt without the inclusion of biologically active substances in the diets such as vitamins and microelements, the deficiency of which inhibits the normal development of animals and fetuses in ewe at lambing.

In the course of mycological, toxicological and biological as well as enzyme immunoassay of feed samples taken from the farm, the concentration of fungal spores of Fusarium sp. and Mucor sp was revealed, which amounted to $2.2 \times 10^4$ and $2.8 \times 10^4$, and to $0.8 \times 10^4$ and $1.0 \times 10^4$, respectively. Mycotoxins in the samples were represented by aflatoxin at the concentration of 0.01 mg / kg, T-2 toxin at the concentration of 0.04 mg / kg and zearalenone at the concentration of 0.2 mg / kg, which was confirmed by a bioassay on laboratory animals (white mice), making it possible to classify the feed samples as slightly toxic.

General analysis of the blood of lamb cows revealed the most significant changes in the level of erythrocytes, the indicators of which were lower than the reference values of the species norm in 1.27 times on the average. The concentration of hemoglobin was reduced or was at the lower limit of the norm in 40% of the samples.

The results of the biochemical study of blood serum revealed significant deviations in lamb homeostasis, manifested by hyperproteinemia due to an increase of the level of $\gamma$-globulins, hypocholesterolemia and low triglyceride values (in 50% of samples).

A specific violation of mineral and vitamin metabolism was expressed, manifested by a deficiency in the number of macro- and essential microelements on the background of the significant decrease in vitamins (carotene, retinol and tocopherol). The total calcium level was reduced in 100% of the examined blood samples. Copper concentration was low or at the lower reference limit in 40% of samples. The selenium concentration in the serum of 70% of the examined animals did not reach the lower limits of the species norm, remaining at the level of 0.06-0.075 μg / ml.

The level of vitamin A was low in 60% of blood samples, and the level of vitamin E was low in 100% of cases. The carotene concentration was recorded in trace concentrations in all examined animals.

An increase in destructive processes in liver cells on the background of a low antioxidant potential of lambs led to the activation of lipid peroxidation, leading to the destruction of hepatocyte membranes, which caused the release into the bloodstream of transamination enzymes – transaminases with the
priority on alanine aminotransferase. Thus, the level of ALT was increased in 60% of blood serum samples (on average, by 14.5-50.0%), the level of AST was increased in 40% of samples.

Significant changes in the skeletal muscles of lambs with disease influenced the concentration of lactate dehydrogenase (LDH), the level of which was significantly higher (in 1.9-2.47 times from the upper limits of the norm). In this case, LDH is an important marker of tissue destruction of muscle cells, confirming the earlier diagnosis.

The concentration of alkaline phosphatase (ALP) in some cases exceeded the upper values of the norm (in 1.6-2.2 times), which can be associated with the development of pathological processes in liver, accompanied by the damage to its parenchyma and cell necrosis.

Thus, based on the complex results of clinical and laboratory studies, the diagnosis of lamb myopathy (white muscle disease) was confirmed.

The effectiveness of pharmacological therapy of the disease in young lambs was evaluated in the comparative aspect of the two preparations. To conduct the research, two groups of lambs (experimental and control) with 20 animals in each were formed from animals with disease.

The experimental group received the preparation selplan in the feed rations at the dose of 3% of the preparation (300 g per 10 kg of feed) for three weeks. The control analogs received the preparation plexomin Se-2000 with the feed, 1 kg of which contains at least 2000 mg/kg of selenium in the composition of selenium-containing inactivated yeast culture Saccharomyces cerevisiae.

During the experiment, daily clinical control was carried out, the symptoms of the disease, as well as the assessment of the physiological state and metabolic status of animals by morphological and biochemical blood parameters at the beginning and at the end of the experiment were taken into account.

A comprehensive hematological analysis was performed on an automated analyzer “Mythic 18 vet” (Switzerland). Blood biochemical parameters were determined on an automatic chemical analyzer Vitalab Selectra Junior with software version 1.0. (open system for photometric tests, manufactured by VitalScientific N.V. Netherlands) using reagents from ELITech Clinical Systems (France) and Analyticon biotechnologies AG (Germany). Serum protein fractions were determined by nephelometric method.

The level of lipid peroxidation processes was determined in accordance with the methodological recommendations of All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology and Therapy (2010).

Statistical processing of the results was carried out using statistical software packages ARCADA, Microsoft Excel XP and Statistical for Windows. The study of quantitative characteristics was assessed by comparing the average values of the two sample sets with the determination of the Student’s criterion and the level of significance (p).

3. Results and discussion
The results of the research have determined that pharmacotherapy with selenium preparations had a pronounced effect on the clinical and physiological state of the body of the sheep participating in the experiment. Already after the first week of therapy, the animals showed an improvement in appetite and overall physical activity. Starting from the 8th (experimental group) and 11th (control group) day such signs as muscle tremor, lameness and shaky gait in lambs in some cases stopped. Cardiac arrhythmias and / or tachycardia were not revealed.

It should be noted that in the experimental group of young sheep already on the 7th day of using the preparation the symptoms of digestive disorders completely stopped, while in the group of control analogs cases of lesions of the gastrointestinal tract were frequent and were manifested by an increase in the frequency of defecation.

When assessing the morphological parameters of the blood of animals of both groups, the following features were determined. In sheep, receiving plexomin Se-2000 as a therapeutic agent, a relative leukocytosis was determined according to the level of leukocytes due to the redistribution of their morphological composition because of the increase in segmented neutrophils (the difference between the experimental and control groups was 15.48%). In this case, drug therapy, aimed only at eliminating
the signs of myopathy, did not allow leveling the syndrome of intestinal inflammation that occurs on the background of the weakening of the natural resistance of sick and weakened animals, while the multicomponent composition of selephlan had a normalizing effect on the intestinal biocenosis of lambs.

The rest of the determined parameters in the experimental group of animals, despite the fact that they were comparable with similar data obtained in the control group, being within the species norm, in terms of hemoglobin level and erythrocyte concentration were higher, exceeding the values of control analogues by 4.8% and 5.4% (at the trend level).

Analysis of biochemical parameters of animals of both groups participating in the experiment revealed certain differences in a number of constants of the metabolic status of sheep on the background of long-term use of preparations. On the background of selephlan therapy the animals showed a tendency for higher values of total protein, the level of which in the experimental group exceeded the values of the control analogues by 11.6%, which correlated with the values of the albumin fraction of the protein spectrum of blood serum (the increase in the experimental group was by 8.2% at a simultaneous decrease in the concentration of γ-globulins by 12.5%) and with a concentration of urea exceeding the control analogs by 6.8%.

Enzymatic activity of such enzymes as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase in the group with the use of selephlan significantly decreased in 1.36 (p≤0.05); 1.71 (p≤0.001); 1.21 (p≤0.05) and 1.3 (p≤0.01) times. In the positive control group, the level of enzyme activity remained pathologically high.

Selephlan promoted the activation of lipid metabolism, as indicated by an increase in the concentration of triacylglycerides. Intergroup differences for this indicator amounted to 22.2%, but there was no statistically significant increase.

In the vitamin metabolism, a significant increase in the concentration of carotene in 3.1 times was revealed, while there was a direct correlating dependence of carotene with vitamin A, the level of which increased by 26.8% on the background of the use of selephlan. Possessing provitamin activity, carotene acted not only as a component of antioxidant protection, but also promoted the synthesis of retinol in the body of experimental sheep.

By the end of the experimental period, the level of vitamin E increased in both experimental groups relative to the background values. However, the intergroup differences were significant and amounted to 1.73 times in favor of animals treated with selephlan. In the experimental group the assimilation of vitamin E turned out to be the most effective, which, in combination with high values of selenium (1.04 μg / ml), contributed to the strengthening of the antioxidant defense of the animal body.

The analysis of free radical oxidation in the blood serum of experimental animals revealed significant differences in indicators of lipid peroxidation (LPO), reflecting a decrease in the intensification of processes under the influence of the therapy (table 1).

| Indicators                        | Groups          | Experimental | Control       |
|-----------------------------------|-----------------|--------------|---------------|
| Diene conjugates (DC) (232), AU / mg lipids | 0.68±0.03 a     | 0.87±0.05    |
| Ketodienes (KD) (273), AU / mg lipids         | 0.73±0.08      | 0.94±0.07    |
| Malondialdehyde (MDA) (537), μM / l          | 1.28±0.12 a    | 2.06±0.16 a  |

a - p <0.05 in comparison with the control group.

Of all LPO products, the most reactive are primary products such as diene conjugates (DC), which act as inducers of peroxidation and contribute to cell membranes damage. In our case, the level of DC in the experimental group of sheep in comparison with the positive control group was reduced by 21.8% and was statistically significant. The concentration of LPO secondary products – ketodienes (KD) in animals of the experimental group decreased in comparison with the values of the control young animals by 28.7%, and the values of malondialdehyde decreased in 1.61 times (p <0.05). Thus, during the
experiment selenium contributed to a more pronounced suppression of the formation of free radicals on the background of strengthening antioxidant and antiradical protection due to the combination of the organic form of selenium with flavonoids exhibiting high antioxidant properties.

4. Conclusion
Selenium deficiency, associated with its low concentration in soils and feed, leads to the development of white muscle disease in young sheep, the decrease in productivity and resistance to infectious diseases.

The use of the complex preparation selephlan has a pronounced effect on the clinical and physiological state and intestinal biocenosis of lambs, improves the morphological and biochemical parameters of the blood and reduces the amount of lipid peroxidation products. The mechanism of action of the preparation depends on the synergistic components, which are the part of its composition; they protect cells from the effects of peroxides arising in the process of oxidation, protect liver cells from agents damaging hepatocytes, saturate the body with organic selenium and also increase the growth and development of young animals, which, ultimately, contributes to a more effective pharmacological therapy of the disease in comparison with the analogue preparation.

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