Effect of different doses of the tissue biostimulant on some indicators of the immune status of heifers at the age of 6 months

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Abstract. The article presents the results, the purpose of which was to study the effect of the tissue biostimulant obtained from the slaughterhouse tankage of antler deers on some indicators of the immune status of heifers at the age of 6 months. Experimental studies were carried out in 2020 on the basis of Prigorodnoye Uchkhoz JSC of the Industrial District of Barnaul, the Altai Territory. For the experiment, we selected one-month-old heifers with an average live weight of 51.0 kg. Throughout the experiment, the animals of the experimental groups were given 1 injection in 1, 2, 3, 4, 5 and 6 months of raising with an interval of 30 days. The study preparation was administered subcutaneously into the lower third of the neck. Animals in the control group were administered with saline from 1 to 5 months of raising at a dose of 3 ml/head, at 6 months. - 6 ml/head, to analogs of the I experimental group from 1 to 5 months - 2 ml/head. At 6 months. - 4 ml/head, in II - from 1 to 5 months. - 3 ml/head, at 6 months - 6 ml/head, in III - from 1 to 5 months. - 4 ml/head at 6 months - 8 ml/head. According to the results of the experiment, it was found that the heifers of experimental group II had the best immune status, in which the largest increase of the indicators of spontaneous Nitro Blue Tetrazolium Reduction Test (NBT-test) was noted - by 12.5% (p≤0.01), of stimulated NBT-test - by 10.0% (p ≤0.05), of phagocytic index - by 5.8% (p<0.05) in comparison with the control.

1. Introduction

The main goal of calves raising is to achieve good growth rates and low morbidity. High morbidity and mortality of young animals has a significant impact on the economic efficiency of dairy farming [1].

The morbidity of calves is largely dependent on the functional activity of the immune system. The immune system of young cattle in the initial period of postnatal ontogenesis is insufficiently formed, which makes calves susceptible to bacterial and viral pathogens [2].

Factors that have an adverse effect on the immune system of calves include various metabolic disorders of the body of mothers’ cows in late pregnancy [3, 4], low levels of immunoglobulins in colostrum [5, 6], errors in feeding and keeping young animals [7]. Various biologically active feed additives and preparations are used to maintain the natural defenses of the body of calves, reduce the incidence of diseases and increase the intensity of their growth in the dairy farming [8, 9].

One of these preparations is tissue biostimulants. The use of tissue biostimulants in the dairy farming is a great additional reserve for increasing the productivity of animals, increasing the profitability of the
animal husbandry industry. At present, on the basis of experimental studies, it has been established that under the influence of tissue preparations there is an increase in the general reactivity of the body, the functional state of the reticuloendotolial system, improvement of digestion processes by activating the work of the gastric glands, stimulation of regenerative processes, gas exchange, glycolysis, phosphorus metabolism, hematopoiesis, an increase in indicators of natural resistance and other vital functions of the animal body [10]. At the same time, the issues related to the influence of tissue preparations on the formation of nonspecific resistance of the blood of young animals remain insufficiently studied.

In this regard, the aim of our research was to study the effect of different doses of the tissue biostimulant on the immune status of heifers at the age of 6 months.

2. Material and research methods
Experimental studies were carried out in 2020 on the basis of JSC "Uchkhoz" Prigorodnoye "of the Industrial District of Barnaul, the Altai Territory, on replacement young cattle. The experiment scheme is presented in table 1.

Table 1. Experiment scheme.

| Group            | n   | Preparation name | Age of replacement heifers while preparation injection, months | Preparation dose ml/head. | Frequency of preparation administration |
|------------------|-----|------------------|---------------------------------------------------------------|----------------------------|----------------------------------------|
| Control          | 10  | Saline           | 1-5                                                          | 3.0                        | 1 time in 30 days                     |
|                  |     |                  | 6                                                            | 6.0                        |                                        |
| I experimental   | 10  | Tissue biostimulant | 1-5                                                        | 2.0                        | 1 time in 30 days                     |
|                  |     |                  | 6                                                            | 4.0                        |                                        |
| II experimental  | 10  | Tissue biostimulant | 1-5                                                        | 3.0                        | 1 time in 30 days                     |
|                  |     |                  | 6                                                            | 6.0                        |                                        |
| III experimental | 10  | Tissue biostimulant | 1-5                                                        | 4.0                        | 1 time in 30 days                     |
|                  |     |                  | 6                                                            | 6.0                        |                                        |

According to the principle of analogous pairs, 4 experimental groups of heifers, 10 heads each, were formed. When selecting animals, the age (1 month) and the body weight (51.0 kg) were taken into account. The experiment lasted 180 days.

During the experiment, the animals of the control and experimental groups received the same diet, balanced for all normalized nutrients. A sample batch of tissue biostimulant was made from the uterus with fetuses, placenta, lymph nodes, mediastinum, spleen and liver obtained by slaughtering antler deer. The resulting native material was placed in a refrigerator for 6 days at a temperature of +2 +4 °C. After the expiration of this period, all the material was crushed in equal parts and placed in an ultrasonic installation.

Quality control for toxicity and reactogenicity was carried out on white mice, GOST 31926-2013 "Medicines for veterinary use. Methods for safety determining " and methodological instructions "On bacteriological control of sterility of veterinary biological products " No. 115-6A dated 03.06.1980.

Blood sampling for immunological studies was carried out at the age of 6 months twice before the start of the preparation administration and on the 14th day after the injection. Blood was taken from the cervical vein into vacuum tubes (lithium heparin was preservative).

The indicator of the spontaneous NBT - test was determined by the number of phagocytes that absorbed nitro blue tetrazolium from the solution, the stimulated NBT- test was determined by the number of phagocytes incubated with a stimulator (zymosan solution) that absorbed nitro blue tetrazolium from the solution, and the phagocytic index was determined by counting the number of phagocytes that absorbed latex.

The data obtained during the experiment were biometrically processed using the Microsoft Excel 2016 software package. The reliability of the results of the experiment in relation to the control group
was calculated using the Student's t-test for independent samples, the differences were considered statistically significant at * p≤0.05; ** p≤0.01. *** p≤0.001. The reliability of the results of blood biochemical parameters on the 14th day after the injection of the preparation in comparison with the beginning of the experiment according to the Student's t-test for dependent samples, the values at (*) p≤0.05 were considered statistically significant; (**) p≤0.01; (***) p≤0.001.

3. Research results and their discussion

Indicators of nonspecific blood resistance of replacement young cattle are presented in table 2.

Table 2. Indicators of nonspecific resistance of blood of replacement young cattle at the age of 6 months.

| Indicator | control | I experimental | II experimental | III experimental |
|-----------|---------|----------------|-----------------|-----------------|
| NBT spon. conventional units | 0.41±0.008 | 0.42±0.005 | 0.43±0.003* | 0.44±0.007* |
| NBT stim. conventional units | 0.40±0.012 | 0.44±0.005*** | 0.45±0.005** | 0.46±0.006*** |
| Phagocyte index, % | 53.8±0.42 | 56.0±0.71* | 57.2±0.55** | 57.4±0.47*** |

Note: on the top line before the administration, on the bottom line on the 14th day after the injection.

Analysis of nonspecific blood resistance of replacement heifers at the age of 6 months (table 2) shows that 5-fold administration of the tissue biostimulator with an interval of 30 days into animals of experimental groups I, II and III contributed to an increase in spontaneous NBT- test, reflecting the metabolic potential of cells capable of phagocytosis, by 2.4%, 4.8% (p≤0.05) and 7.3% (p≤0.01), respectively, compared with the control. According to the value of the stimulated NBT- test, which characterizes the state of the oxygen-dependent mechanism of the bactericidal activity of phagocytes, the replacement young animals of the experimental groups tend to increase by 5.0-6.7%, respectively, in comparison with the animals of the intact group. The phagocytic blood index, which reflects the absorption capacity of phagocytes, increased in experimental group I by 2.2% (p≤0.05), in experimental group II by 3.4% (p≤0.01), in III by 3.6% (p≤0.001) compared with analogs of the intact group.

At the age of 6 months, on the 14th day after the injection of the tissue biostimulant, replacement young animals of the I, II and III experimental groups exceeded the control by 10.0% (p≤0.05), 12.5% (p≤0.01) and 15.0% (p≤0.01) respectively in terms of the value of the spontaneous NBT- test. Compared to the values of the spontaneous NBT test in the blood of heifers of the experimental groups before the preparation administration, an increase in this indicator was noted in the experimental group I by 4.7% (p≤0.001), in II - by 4.6% (p≤0.01), and in III - by 4.5% (p≤0.001). The considered value decreased by 2.5% in the control.

The highest value of the stimulated NBT- test was noted in the blood of heifers of the II experimental group, which is 10.0% (p≤0.01) more than in the control. In the animals of experimental groups, I and II, the index of the stimulated NBT- test in the blood tended to increase by 5.0 and 8.3%, respectively, in comparison with the control. When comparing the value of the stimulated NBT- test with a similar indicator before the administration of the preparation, an increase by 1.6-3.1% (p≤0.01), was noted in the considered value in the blood of heifers of the experimental groups. The indicator of the stimulated NBT- test in the control increased by 1.6%.

The phagocytic index of blood in the replacement young animals of the experimental groups after the injection of the tissue biostimulator increased in the I experimental group by 4.0% (p≤0.01), in II - by 5.8% (p≤0.001), in III - by 5%, 4% (p≤0.001) than in the control. In comparison with the initial data, the phagocytic blood index in heifers of the II experimental group increased by 1.0% (p≤0.05).
blood of replacement young animals of the I and II experimental groups, the considered indicator remained practically at the same level with a minimum discrepancy of 0.4%. In animals of the control group, the phagocytic index in the blood decreased by 1.4%.

The results obtained in our experiment are consistent with the researches of other authors who studied the effect of tissue preparations "Gamavit" and "Bio-TEK" on the immune system of young cattle [11, 12]

There are two main hypotheses explaining the effect of tissue preparations on the immune system of animals. Both hypotheses are not contradictory and complement each other. The first hypothesis is more conservative: the immune system of the animal body responds to the introduction of a foreign protein, mobilizing the body's defenses [13]. The second indicates that according to the teachings of academician V.P. Filatov special substances, called "biogenic stimulants" accumulate in the tissues of animals preserved in conditions unfavorable for their existence. Organic acids with high molecular weight are isolated from these tissues. Animal tissues in the preparation, when administered parenterally, slowly disintegrate with the formation of a large number of moderate irritants, which affect enzymes. With some they enter into a chemical bond, attaching to the enzyme protein molecules, in relation to others they are catalysts. And since the nervous tissue, as Academician V.P. Filatov believed, contains the necessary highly active enzyme systems, which are the most sensitive, they are the first to experience the influence of biogenic stimulants. This ensures the leading role of the nervous system and cerebral cortex when using tissue preparations. Under the influence of biogenic substances, the tone of the central nervous system and autonomic innervation increases, their regulatory effect on organs and tissues, including the organs of the immune system, improves [10].

The nervous and immune systems of the animal body are closely related. It has been proven that the nervous system innervates the central and peripheral immunocompetent organs. Biologically active substances produced by the nervous system are capable of influencing the functional state of the immune system. The hypothalamus is the key link in the apparatus of nervous regulation of immunity. It gives rise to a complex efferent pathway for the transmission of central neuroregulatory influences on immunocompetent cells, which possess the corresponding receptors for factors of the nervous regulation [14].

Thus, the administration of the tissue biostimulant to replacement young cattle in different doses contributes to the increase in the nonspecific resistance of the organism. The administration of one injection every 30 days in doses: from 1 to 5 months - 6 ml/head should be considered as the optimum scheme of the tissue biostimulant application. This provides an increase in the indicators of spontaneous NBT- test by 12.5% (p≤0.01), stimulated NBT- test -by 10.0% (p≤0.05), phagocytic index- by 5.8 % (p≤0.05) compared to the control.

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