**Dynamics and Number of T- And B-Lymphocytes in the Blood of Replacement Young Cattle
after Injection of Tissue Biostimulator**

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Abstract

The article presents the results of a study of the effect of a tissue preparation obtained from offal and slaughter reindeer by-products using a patented technology on the level and dynamics of blood T- and B-lymphocytes in young cattle of black-motley breed. Experimental studies were carried out in 2020 on the basis of "Uchkhoz Prigorodnoye" JSC in the Industrial District of Barnaul, Altai Territory on one month old heifers with an average live weight of 51,0 kg. Heifers of the experimental group (n = 10) got the tissue preparation injections once subcutaneously at a dose of 3.0 ml/head, and animals of the control group (n = 10) got saline in the same dose and multiplicity. Studies have shown that the introduction of the preparation increases the relative number of activated lymphocytes (bE-rosetting cell) by 1.5% ($p \leq 0.05$), helper-inductors (rE-rosetting cell) by 1.8% ($p \leq 0.05$), B-lymphocytes (EM-rosetting cell) by 1.7% ($p \leq 0.05$), the absolute number of activated lymphocytes (bE-rosetting cell) and B-lymphocytes (EM-rosetting cell) by 48.1% ($p \leq 0.05$) and 46.4% ($p \leq 0.05$) in comparison with the control. The relative number of killer-suppressors (cE-rosetting cell) in the blood of the heifers of the experimental group decreased by 2.6% ($p \leq 0.05$), and the ratio of the ratio of helper-inductors and killer-suppressors was increased by 18.1% ($p \leq 0.05$), in comparison with animals of the intact group.

Keywords: cattle; replacement young animals; tissue biostimulant; T-lymphocytes; B-lymphocytes.

Introduction

During the first two months after birth, young cattle undergo critical periods associated with the development of the immune system, the formation of which affects the disclosure of the genetic potential of productivity (Hernández-Castellano et al., 2015; Renaud et al., 2020).

Despite the great successes achieved in veterinary medicine during the rearing of young animals, high morbidity and mortality are often recorded, which is mainly due to the weakening of the body's natural defenses (Batista et al., 2015; Aliev et al., 2020; Abuelo et al., 2019).

Factors that adversely affect the immune system of calves include various metabolic disorders of the body of mother cows in late pregnancy (Ling et al., 2018; Hussain et al., 2018), a low content of immunoglobulins in colostrum (Lora et al., 2018; Aydogdu & Guzelbektes, 2018; McGee, Earley, 2019), errors in feeding and keeping young animals (Gerbert et al., 2018; Jacometo et al., 2018). To maintain the body's natural defenses of calves, reduce the frequency of cases of diseases and increase the intensity of their growth, various biologically active feed additives and preparations are used in the practice of dairy cattle breeding (Hussain et al., 2018; Zinchenko, 2014).

In this regard, the purpose of our research was to study the effect of tissue biostimulant on the level and dynamics of blood T- and B-lymphocytes of replacement young cattle.

Methods

Experimental studies were carried out in 2020 on the basis of “Uchkhoz Prigorodnoye” JSC in the Industrial District of Barnaul, Altai Territory on replacement young cattle. The experimental scheme is presented in Table 1.

Table 1. Scheme of the experience

Group	n	Preparation name	Age of replacement heifers at the time of the preparation introduction, months	Dose of subcutaneous injection of the preparation, ml/head.
Control	10	Saline solution	1	3.0
Experimental	10	Tissue Biostimulator	1	3.0

According to the principle of analogue pairs, 2 experimental groups of heifers of 10 heads in each were formed. When selecting animals, age (1 month) and live weight (47.0 kg) were taken into account. The experiment lasted for 14 days.

During the experiment, the animals of the control and experimental groups received the same diet, balanced for all normalized nutrients. An experimental preparation of tissue biostimulant was made from offal and slaughter reindeer by-products using a patented technology. Quality control for toxicity and reactogenicity was carried out on white mice, GOST 31926-2013 “Medicines for veterinary use. Methods for determining harmlessness” and guidelines “On bacteriological control of the sterility of veterinary biological preparations” No.115-6A dated 06/03/1980.

Blood sampling for immunological studies was performed twice before the start of preparation administration and on day 14 after injection. Blood was taken from the jugular vein into vacuum tubes (preservative lithium heparin).

The relative amount of T-lymphocytes (%) was determined by spontaneous rosette formation with sheep erythrocytes under different incubation modes, and B-lymphocytes - rosette formation with mouse red blood cells. The absolute number of T- and B-lymphocytes (cells/mm³) and the ratio of “helper-inductors” and “killer- suppressor” (%) - by calculation method.

The article presents reliable values indicated in relation to the control at * $p \leq 0.05$. Reliable value in relation to the beginning of the experiment with (*) $p \leq 0.05$. The tabular material in the numerator presents the values before the introduction of the preparation in the denominator on the 14th day after the injection of the preparation.

Results

The results of studies of the relative content of T- and B-lymphocytes in the blood of replacement young animals after injections of a new tissue biostimulator are shown in Table 2.

Table 2. Relative content of T- and B-lymphocytes in blood of dry cows, %

Indicator	Group	
	Control	Experimental
tE-rosetting cell	<u>31.8±4.26</u> 30.7 ±0.48	<u>30.1±1.25</u> 33.2±0.77
bE-rosetting cell	<u>11.1±3.99</u> 11.2±0.17	<u>11.8±0.25</u> 12.7±0.51*
rE-rosetting cell	<u>15.4±0.72</u> 15.6±0.22	<u>16.6±1.01</u> 17.4±0.44*
cE- rosetting cell	<u>15.1±5.77</u> 15.8±0.53	<u>16.2±1.26</u> 13.2±0.22*
EM-rosetting cell	<u>26.6±1.10</u> 26.4±0.30	<u>26.3±0.30</u> 28.1±0.35*(*)

According to the data presented in Table 2, before the start of the experiment, there were detected no significant differences in the number of the studied lymphocyte subpopulations between the heifers of the control and experimental groups. Subcutaneous administration of tissue biostimulant at a dose of 3.0 ml/goal of replacement young animals at the age of 1 month contributes to an increase in the number of tE-rosetting cell by 2.7% in comparison with the same indicators in the control. In comparison with the beginning of the experiment, the number of tE- rosetting cell in the blood of heifers of the experimental group increased by 3.1%, in the control the same indicator decreased by 1.1%. On the 14th day after the injection of the tissue biostimulator, the proliferative activity of the subpopulations of lymphocytes bE-rosetting cell and rE-rosetting cell in heifers of the experimental group increased by 1.5% ($p \leq 0.05$) and 1.8% ($p \leq 0.05$) in comparison with control. Relative to the initial values, the proliferation of bE-rosetting cell and rE-rosetting cell in the blood of young animals of the experimental group increased by 0.8-0.9%, in the control the analyzed values remained almost unchanged with a difference of 0.2%. The highest amount of bE-rosetting cell was noted in the blood of young control group, which is 2.6% ($p \leq 0.05$) more than in animals of the experimental group. When assessing B-lymphocytic immunity, an increase in the number of EM-rosetting cell in the blood of calves of the experimental group was found to be 1,7% ($p \leq 0.05$) compared with the control. When comparing the number of EM-rosetting cell on the 14th day of the

experiment with the initial data, an increase of the considered subpopulation of lymphocytes in the blood of the heifers of the experimental group by 1.8% ($p \leq 0.05$) was revealed, in the control the similar value became lower by 0.2%.

The absolute number of T- and B-lymphocytes of blood replacement young animals are presented in Table 3.

Table 3. Absolute content of blood T- and B-lymphocytes of replacement young animals, cells/mm³

Indicator	Group	
	Control	Experimental
tE-rosetting cell	<u>0.82±0.024</u>	<u>1.04±0.321</u>
	0.93±0.108	1.10±0.120
bE-rosetting cell	<u>0.22±0.067</u>	<u>0.34±0.104</u>
	0.27±0.030	0.40±0.017*
rE-rosetting cell	<u>0.41±0.059</u>	<u>0.45±0.152</u>
	0.41±0.045	0.52±0.040
cE-rosetting cell	<u>0.48±0.234</u>	<u>0.41±0.082</u>
	0.42±0.032	0.40±0.049
EM-rosetting cell	<u>0.94±0.085</u>	<u>0.81±0.179</u>
	0.71±0.075	1.04±0.037*(*)

Immunological screening of the results presented in Table 3, allows concluding that the absolute number of the considered lymphocyte subpopulations before the experiment did not have significant differences. After the introduction of a tissue biostimulator, the absolute amount of tE-rosetting cell in the blood of heifers of the experimental group increased by 18.2% in comparison with the control, and by 5.7% in relation to the initial values. The absolute amount of tE-rosetting cell in the blood of animals of the control group compared with the beginning of the experiment increased by 13.4%. The number of bE-rosetting cell in the blood of repair young animals of the experimental group is 48.1% higher ($p \leq 0.05$), and rE-rosetting cell is 26.8% higher than in the control. In comparison with the beginning of the experiment, the proliferative activity of the sub-populations of bE-rosetting cell and rE-rosetting cell in the blood of heifers of the experimental group increased by 17.6% and 15.5%, respectively. In the control, the amount of bE-rosetting in the blood increased by 22.0%, and rE-rosetting cells remained at the same level. The number of lymphocyte subpopulations of bE-rosetting in the blood of repair young animals of the experimental group is 4.8% lower than in intact animals. In comparison with the beginning of the experiment, the amount of bE-rosetting cell in the blood of animals from the control and experimental groups decreased by 12.5% and 2.5%, respectively. The number of B-lymphocytes in the blood of heifers of the experimental group as a result of the introduction of tissue biostimulant increased by 46.4% ($p \leq 0.05$) in comparison with the control. A comparison of the number of EM-rosetting with the

beginning of the experiment revealed an increase in the considered subpopulation of lymphocytes in the blood of heifers of the experimental group by 28.3% ($p \leq 0.05$). In the control, a similar indicator became lower by 24.5%.

The absolute ratio of helper-inductors and killer-suppressors in the blood of replacement young experimental groups is presented in Figure 1.

Figure 1. The absolute ratio of helper-inductors and killer-suppressors in the blood of replacement young animals, units

According to the data presented in Figure 1, it follows that the introduction of a tissue biostimulator to heifers at the age of 1 month makes it possible to increase the absolute ratio of helper-inductors and killer-suppressors in their blood by 18.1% ($p \leq 0.05$), in comparison with the control and by 30.0% in comparison with the beginning of the experiment, which indicates an increase in the functional activity of the immune system of replacement young animals due to the use of the studied preparation.

Discussion

The use of tissue biostimulants in the practice of conducting dairy cattle breeding is a large additional reserve for increasing the productive indicators of animals, increasing the profitability of the livestock industry. Currently, on the basis of experimental studies, it has been established that under the influence of tissue preparations, an increase in the general reactivity of the body, the functional state of the reticuloendotolial system, an improvement in digestion by activation of the gastric glands, stimulation of regenerative processes, gas exchange, glycolysis, phosphorus metabolism, hematopoiesis, an increase in the rate of natural resistance and other vital functions of the animal organism (Rubinskiy, Petrova, 2011). At the same time, issues related to the influence of

tissue preparations on the formation of blood T- and B-lymphocytes in young animals remain insufficiently studied. In this regard, the study of the effect of tissue biostimulant on the level of blood T- and B-lymphocytes in the repair of young cattle is relevant.

In the current studies, it was found that the introduction of a new tissue biostimulator contributed to an increase in the relative content of bE-rosetting cell by 1.5% ($p \leq 0.05$) and rE-rosetting cell by 1.8% ($p \leq 0.05$) in comparison with the control. The bE-rosetting cell subpopulation is activated lymphocytes, whose function is to synthesize cytokine molecules, in the case when they play the role of T-helpers or lysis of the affected cells, in the case when they perform the function of T-killers, the other part of the activated cells is transformed into T-cells memory. The subpopulation of rE-rosetting cell lymphocytes are helper-inducers, they stimulate the immune response; under their influence, the work of cytotoxic lymphocytes increases, information about the presence of a foreign protein is transmitted to B-lymphocytes, which secrete protective antibodies against them, and have a stimulating effect on the work of phagocytes, mainly monocytes. The low amount of pE-rosetting cell in the blood of heifers of the control group indicates an immunosuppressive state of their body in comparison with analogues of the experimental group.

This fact is also confirmed by a higher relative indicator of bE-rosetting cell in the blood of control animals by 2.6% ($p \leq 0.05$) compared with the heifers of the experimental group. The cE-rosetting cell subpopulation is killer-suppressors, which perform the function of regulating the strength of the body's immune response to foreign agents and their toxins. The higher their value in the blood, the lower the strength of the immune response. The established fact indicates a high immunological load in the body of repair young animals of the control group, associated with the destruction of foreign agents and their toxins. A significant increase in the relative and absolute number of B-lymphocytes in the blood of animals of the experimental group by 1,7% ($p \leq 0,05$) and 46,4% ($p \leq 0,05$) when using a tissue biostimulator, indicates the activation and proliferation of humoral cells link of the immune system in animals of the experimental group.

The data obtained in the experiment are consistent with the results of other researchers describing the stimulating effect of the preparation Gamavit (which includes denatured emulsified placenta) on the proliferative activity of cattle blood T- and B-lymphocytes (Topuriya, Topuriya, 2011).

Academician Filatov and his school followers considered biogenic stimulants to be the active principle of tissue preparations, which are substances that accumulate in tissues during their preservation in the cold. Organic acids with a high molecular weight were isolated from these tissues. Animal tissues in the preparation, when administered parenterally, slowly disintegrate with the formation of a large number of moderate irritants that act on enzymes. With some of them they

enter into a chemical bond, joining the molecules of the protein of the enzyme, in relation to others are catalysts. Since nervous tissue, as Academician Filatov believed, contains the necessary highly active enzyme systems, which are the most sensitive, they are the first to be affected by biogenic stimulants, which ensures the leading role of the nervous system and cerebral cortex in tissue therapy. Under the influence of nutrients, the tone of the central nervous system and autonomic innervation increase, their regulatory effect on organs and tissues, including the organs of the immune system, improves (Smolentsev, 2017).

The nervous and immune systems of animals are closely related (Hartenstein, Giangrande, 2018). It is proved that the nervous system innervates the central and peripheral immunocompetent organs. Biologically active substances produced by the nervous system can affect the functional state of the immune system. The key link in the apparatus of nervous regulation of immunity is the hypothalamus. The hypothalamus gives rise to a complex efferent pathway for the transmission of central neuro-regulatory effects on immunocompetent cells, which have corresponding receptors for factors of nervous regulation (neurotransmitters, neuropeptides). Hypothalamic neurosecretory cells are capable of producing interleukin-1 (Sozaeva, Berezhanskaya, 2014), which in turn stimulates the synthesis of interleukin-2 by T-helper cells (rE-rosetting cell) and also promotes the maturation of B-lymphocytes. Synthesized by T-helper cells, interleukin-2, activates the differentiation of T-killers, resulting in increased functional activity of the immune system, which is confirmed by the results of the experiment.

Conclusion

Subcutaneous administration of a tissue biostimulator to replacement young cattle at a dose of 3.0 ml/head promotes an increase in their blood relative content of bE-rosetting cell by 1.5% ($p \leq 0.05$), rE-rosetting cell by 1.8% ($p \leq 0.05$), EM-rosetting cell by 1.7% ($p \leq 0.05$), the absolute content

of bE-rosetting cell and EM-rosetting cell by 48.1% ($p \leq 0.05$) and 46.4% ($p \leq 0.05$), respectively, in comparison with the control. The relative amount of bE-rosetting cell in the blood of heifers of the experimental group is 2.6% less ($p \leq 0.05$) than in the control, and the ratio of the ratio of helper inductors and killer suppressors is 18.1% more ($p \leq 0.05$), in comparison with animals of the intact group.

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References

- Abuelo, A., Havrlant, P., Wood, N., Hernandez-Jover, M. (2019). An investigation of dairy calf management practices, colostrum quality, failure of transfer of passive immunity, and occurrence of enteropathogens among Australian dairy farms. *Journal of Dairy Science*, 102, 8352-8366. <https://doi.org/10.3168/jds.2019-16578>
- Aliev, A.A., Khalipaev, M.G., Gadzhiev, B.M., Dibirov, S.S., Azizov, I.M., Dzhamaludinov N.M. (2020). Treatment of dyspepsy in calves with an innovative drug – iodpropionix. *EurAsian Journal of Bio Sciences*, 14(1), 173-176.
- Aydogdu, U., Guzelbektes, H. (2018). Effect of colostrum composition on passive calf immunity in primiparous and multiparous dairy cows. *Veterinari Medicina*, 63(1), 1-11.
- Batista, C.F., Blagitz, M.G., Bertagnon, H.G., Gomes, R.C., Santos, K.R., Della Libera, A.M.M.P. (2015). Evolution of phagocytic function in monocytes and neutrophils blood cells of healthy calves. *Journal of Dairy Science*, 98(12), 8882-8888. <https://doi.org/10.3168/jds.2015-9573>
- Gerbert, C., Frieten, D., Koch, C., Dusel, G., Eder, K., Stefaniak, T., Bajzert, J., Jawor, P., Tuchscherer, A., Hammon, H.M. (2018). Effects of ad libitum milk replacer feeding and butyrate supplementation on behavior, immune status, and health of Holstein calves in the postnatal period. *Journal of Dairy Science*, 101(8), 7348-7360. <https://doi.org/10.3168/jds.2018-14542>
- Hartenstein, V., Giangrande, A. (2018). Connecting the nervous and the immune systems in evolution. *Communications Biology*, 1(1). <https://doi.org/10.1038/s42003-018-0070-2>
- Hernández-Castellano, L.E., Morales-de la Nuez, A., Sánchez-Macías, D., Moreno-Indias, I., Torres, A., Capote, J., Argüello, A., Castro, N. (2015). The effect of colostrum source (goat vs. sheep) and timing of the first colostrum feeding (2 h vs. 14 h after birth) on body weight and immune status of artificially reared newborn lambs. *Journal of Dairy Science*, 98(1), 204-210. <https://doi.org/10.3168/jds.2014-8350>
- Hussain, D.A., Singh, S.K., Mondal, B.C., Palod, J., Kumar, A., Singh, V., Sharma, R.K., Khadda, B.S. (2018). Effect of probiotic and synbiotic on faecal microbial count and cell-mediated immunity in crossbred calves. *Indian Journal of Animal Research*, 52(10), 1452-1456. <https://doi.org/10.18805/ijar.B-3383>

- Jacometo, C.B., Alharthi, A.S., Zhou, Z., Luchini, D., Loor, J.J. (2018). Maternal supply of methionine during late pregnancy is associated with changes in immune function and abundance of micro RNA and mRNA in Holstein calf polymorphonuclear leukocytes. *Journal of Dairy Science*, 101(9), 8146-8158. <https://doi.org/10.3168/jds.2018-14428>
- Ling T., Hernandez-Jover, M., Sordillo, L.M., Abuelo, A. (2018). Maternal late-gestation metabolic stress is associated with changes in immune and metabolic responses of dairy calves. *Journal of Dairy Science*, 101(7), 6568-6580. <https://doi.org/10.3168/jds.2017-14038>
- Lora, I., Barberio, A., Contiero, B., Paparella, P., Bonfanti, L., Brscic, M., Stefani, A.L., Gottardo, F. (2018). Factors associated with passive immunity transfer in dairy calves: combined effect of delivery time, amount and quality of the first colostrum meal. *Animal*, 12(5), 1041-1049. <https://doi.org/10.1017/S1751731117002579>
- McGee, M., Earley, B. (2019). Review: passive immunity in beef-suckler calves. *Animal*, 13(4), 810-825. <https://doi.org/10.1017/S1751731118003026>
- Renaud, D.L., Waalderbos, K.M., Beavers, L., Duffield, T.F., Leslie, K.E., Windeyer, M.C. (2020). Risk factors associated with failed transfer of passive immunity in male and female dairy calves: A 2008 retrospective cross-sectional study. *Journal of Dairy Science*, 103(4), 3521-3528.
- Rubinskiy, I.A., Petrova, O.G. (2011). *Immune stimulants in veterinary medicine*. USAA. (in Russian)
- Smolentsev, S.Y. (2017). Improving the safety of calves when using immunostimulants in combination with a mineral feed supplement. *Scientific Life*, 2, 49-55. (in Russian)
- Sozaeva, D.I., Berezhanskaya, S.B. (2014). The main interaction mechanisms of the nervous and immune systems. Clinical and experimental data. *Kuban Scientific Medical Bulletin*, 3, 145-150. (in Russian)
- Topuriya, G.M., Topuriya, L.Y. (2011). The immune status of cattle when using gamavita. *Bulletin of the Orenburg State Agrarian University*, 1(29), 69-71. (in Russian)
- Zinchenko, D.A. (2014). The effect of tissue bio-stimulants on the animal organism. *International Journal of Applied and Basic Research*, 3, 124-125. (in Russian)