Assessment influence of recombinant interleukin-2 and polyoxidonium to physiological condition and formation of non-specific resistance of calves 30 days of age

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Abstract. The basis of health and the possibility of realizing productive potential of farm animals are high level of natural resistance and immune status of their body. One of the reserves for increasing the productivity of young cattle is increase their resistance, especially in conditions of unbalanced feeding of mother cows and violations of the animal housing. The study period of 20-30 days of age of the calves was chosen taking into account the fact that during this period they have reduced resistance to infectious diseases, because immunoglobulins received with maternal colostrum, breaking by this age, and their own are just beginning to be produced. Active immunity is formed only by 1.5-2 months of age. The calves organism needs at this time stimulation of the immune system and nonspecific resistance, and the effect of immunomodulating drugs is more pronounced. The objective of the study was to assess the effect of recombinant interleukin-2 and polyoxidonium on the physiological state, morphological composition of the blood and the formation of nonspecific resistance of calves. The calves of the first experimental group were parenterally injected with recombinant interleukin-2. The calves of the second experimental group were injected polyoxidonium. The calves of the control group were injected physiological saline. The experimental part was performed on calves at farming enterprise Dalnekonstantinovsky district of Nizhny Novgorod region, Russia. The experimental work was conducted in the spring.

1. Introduction
Agricultural enterprises often violate parameters of microclimate, animal feeding in consequence to the intensification of animal husbandry. These disorders affect to body as stress factors. They negatively affect to physiological state. Nonspecific resistance decreases in animals and various diseases arise. Animal productivity declines. Businesses lose profits or suffer losses. Scientists propose to use various biological products to ensure the health and productive potential of animals through immunoprophylaxis.

Semenov V.G. and Nikitin D.A. studied the indicators of nonspecific resistance of calves after the use of immunostimulating drugs of the PS series. It is proved that PS-6 and PS-7 promote more
intensive growth and development of calves, prevention of intestinal and respiratory diseases, activation of cellular and humoral factors of nonspecific resistance of an organism, besides their application is profitable [1, 2].

Sun P., Wang J. Q., Zhang H. T. (2010) studied the effects of Bacillus subtilis natto on performance and immune function of dairy calves during the preweaning phase. The results showed that Bacillus subtilis natto increased general performance by improving the average daily gain and feed efficiency and advanced the weaning age of the calves. No difference was observed in serum IgE, IgA, and IgM, whereas serum IgG was higher in the Bacillus subtilis natto-supplemented calves than in the control calves. Furthermore, calves fed with Bacillus subtilis natto were found to secrete more IFN-γ, but tended to produce less IL-4 than did the control calves, although serum IL-6 and IL-10 were not affected. This study demonstrated that Bacillus subtilis natto did not stimulate IgE-mediated allergic reactions, but increased serum IgG and IFN-γ levels in the probiotic fed calves. Authors propose that the viable probiotic characteristics of Bacillus subtilis natto benefit calf immune function [3].

Reddy P.G., Morrill J.L., Minocha H.C. et al. (1986) investigated the effect of vitamin E on immune responses of Holstein calves. Treatments were: 0, 1400, and 2800 mg of dl-α-tocopheryl acetate given orally at weekly intervals or 1400 mg of dl-α-tocopherol weekly by injection. Calves were on experiment until they were 12 wk of age. Lymphocyte stimulation indices were significantly higher for calves given the high amount of oral supplementation and for injected calves than for unsupplemented calves. There were no significant differences at any of the individual weeks between unsupplemented and orally supplemented calves. Injected calves showed significantly higher values than unsupplemented calves at wk 4 and than all other calves at wk 8. There were no significant differences in the concentrations of immunoglobulins G1 and G2 among treatments. Immunoglobulin M was significantly higher at wk 6 in calves given the high amount of oral supplementation than in all other calves. At wk 12, serum from calves given the high amount of oral supplementation and calves given injections inhibited infectious bovine rhinotracheitis viral replication in tissue cultures as compared with those of unsupplemented calves [4].

Hidiroglou M., Batra T.R., Ivan M. (1995) studied effects of supplemental vitamins E and C on the immune responses of calves. Concentrations of α-tocopherol in blood plasma were significantly higher for supplemented than for control calves. The concentrations increased from birth to wk 1, and then very little change occurred from wk 1 to 6. Differences among treatments in the concentrations of IgG1, IgG2, IgM, and titer to keyhole limpet hemocyanin were not significant; however, concentrations of IgM in calves supplemented with vitamins E and C generally tended to be higher than those of control calves. Antibodies to keyhole limpet hemocyanin were higher at 6 wk than at 4 wk of age [5].

Bordignon R., Volpato A., Glombowsky P. (2019) studied immune and antioxidant responses in dairy calves during pre- and post-weaning period after using injection vitamins A, E and minerals copper, selenium, zinc, and manganese. Levels of serum copper, selenium, zinc, and manganese were measured on day 1; and the results showed that calves were not deficient in these minerals. The TREAT group had greater BW gain during the final third of the experiment. There was an increase in total leukocyte numbers as a result of elevation in neutrophil counts (day 45) and monocytes (days 30 and 45) in the TREAT group. This group also had lower reactive oxygen species (ROS) content (days 15, 30 and 45) and lipid peroxidation (LPO; days 15 and 45). Furthermore, the TREAT group had greater antioxidant capacity against peroxyl radicals (ACAP; days 15 and 30), activities of the enzymes glutathione peroxidase (GPx; days 15, 30 and 45) and superoxide dismutase (SOD; day 15), concentrations of total serum proteins (day 30), serum globulin (days 15 and 30), ceruloplasmin (day 15), tumor necrosis factor-alpha (TNF-α), interleukin-1, (IL-1; days 30 and 45) and interferon gamma (IFNγ; day 45), compared to CON group. Taken together, the data suggest that mineral and vitamins injections increased the growth performance and boosted the antioxidant and immunological systems of dairy calves during the diet transition period in summer [6].
In previous experiments we studied influence of drugs thymogen, recombinant interleukin-2, polyoxidonium, sinoestrol-2% and combined action of sinoestrol-2% and recombinant interleukin-2 on the physiological state, non-specific resistance of newborn calves after used of these drugs to down-calving cows [7, 8].

The objective of the present research is to assessment influence recombinant interleukin-2 and polyoxidonium on the physiological state, morphological and immunobiochemical composition of calves blood 30 days age.

Interleukin-2 (IL-2) is a cytokine, the central regulator of the immune response, which, by controlling the proliferation, differentiation and survival of target cells, determines the type and duration of immune responses. The target cells for IL-2 are B- and T- (including NK-) lymphocytes, monocytes / macrophages, dendritic cells on which specific membrane receptors are expressed. In veterinary medicine there is a recombinant analogue of IL-2 - the drug Roncoleukin [9, 10].

Polyoxidonium - a Russian drug with a wide spectrum of pharmacological action. Polyoxidonium has an immunomodulatory effect, increases the body's resistance to local and generalized infections. The basis of mechanism immunostimulating effect of Polyoxidonium is a direct effect on phagocytic cells and natural killers, as well as stimulation of antibody formation. Polyoxidonium has a pronounced detoxification and antioxidant activity, has the ability to remove toxins from the body, salts of heavy metals, and inhibits lipid peroxidation. The indicated properties are determined by the structure and high molecular nature of the preparation Polyoxidonium [11-13].

2. Materials and methods
The experimental part was performed on calves 30 days age at farming enterprise Mir (Dalnekonstantinovsky district of Nizhny Novgorod region, Russia). The experimental work was conducted in the spring of 2018 year. Spring is a season when decrease non-specific resistance in animals body. The studying objects were 15 calves of black-motley breed, 30 day age, 44.5±0.85 kg. Selected based on the principle of analogs considered the breed, age, live weight and clinical and physiological state. Selected calves were divided into control and two experimental groups. Recombinant interleukin-2 was injected parenterally to calves of the 1-st experimental group at a dose of 0.2 mg 200 000 IU per animal, twice with an interval of 5 days. Polyoxidonium was injected parenterally to calves of the 2-nd experimental group at a dose of 6 mg per head. The animals from the control group were exposed of sodium chloride physiological solution.

Blood samples from calves were taken from the jugular vein before drug injection, as well as 10 and 30 days after drug injection.

We studied blood counts in calves during the experiment. Hematologic indicators were estimated on in vitro blood analyzer XT 2000, (fluorescence flow cytometry method), (Sysmex, GmbH). The excretion of leukogram was determined by counting in blood smears of different types of white blood cells stained by Romanovsky-Gimza.

Biochemical indicators were detected as the level of total protein in the serum of animals by means of a biochemical analyzer AU480 (Olympus, Japan) (spectrophotometry method), protein fraction of blood (albumin, alpha-, beta-, gamma-globulins) by means of analyzer Minicap (Sebia, France) (capillary electrophoresis method); the content of urea and glucose in the blood was determined by the methods described in the biochemical guide.

Immunologic indicators included determining bactericidal activity of blood serum was examined using *Escherichia coli* test culture (microbial strain O111). Lysozyme activity was measured using a *Micrococcus lysodeikticus* test culture in the modification of USRIEVM (Ukrainian Scientific-Research Institute of Experimental Veterinary Medicine), and neutrophil phagocytic rate was analyzed by a *Staphylococcus albus*, T-lymphocytes – by Erosette assay and B- lymphocytes – via EAC-rosette assay.

We got the results during the experiment. We subjected them statistical processing using conventional parametric methods. The degree of confidence was determined by Student’s t-test employing the Microsoft Excel (2007) software package and STAT 3 software program [14].
Animals were kept in the large calf house in groups of 10-12 animals. The floor area per animal is 1.5 m². Feeders and drinking bowl are located on the side of the feed passage. On the side of the building there are rooms for storage and preparation of feed, and inventory is also stored here.

The farm breeds cattle of black and motley breed. The animals are large, of proportional build, with a strong constitution and the correct form of the udder and nipples. For insemination of the breeding stock, sperm of high-value bull improvers for milk and fat of the leading lines of the Holstein breed from OJSC Nevskoye for breeding (St. Petersburg) is used.

The calf feeding ration was as follows: until 7 days of age, the calves were fed with colostrum (6 kg/day), after 7 days of life, the calves were fed with whole milk (6 kg/day). From 30 days of age, the calves were fed skim milk (started to give 1 kg) and gradually increased amount of skim milk (up to 4 kg by the end of the second month). From the second week of life, calves were given oatmeal (100-400 g) until the age of 1.5 months. From the second month of life, the calves began to give wheat bran (0.3 kg) and clover-timothy hay (0.2 kg). Salt (5 g) and chalk (5 g) were given to calves from the second week of life. Watering was carried out from automated drinkers.

All experiments were carried out in accord with the European Convention for the Protection of Vertebrate Animals Used for Experimentation and other Scientific Purposes, No. 123 of 18 March 1986, Strasbourg.

3. Results and discussion

Assessment of the physiological state calf's body was carried out according to objective morphological indicators of blood.

Analyzing the data obtained in an experiment conducted in agricultural production cooperative the number of erythrocytes and leucocytes of calves experimental groups has increased. Ten days after the injection of recombinant interleukin-2 the calves have shown increase of the number of leukocytes in the blood by 9.5% (P<0.05), mainly due to segmented neutrophils, their number was higher 24.3%, with a slight decrease in the level of lymphocytes, although the total number of lymphocytes (K/mcL) increased slightly. Calves of the 2nd experimental group have shown increase of the number of erythrocytes by 10.9% and leucocytes (by 13.2%). At that, the number of leucocytes was higher mainly due to segmented neutrophils (by 16%), while the relative content (%) of lymphocytes in the blood of calves of the 2nd experimental group was lower, and the absolute (K/mcL) was similar to the control group.

Tokar I.S. (1938), Nikitin V.N. (1946), Zhukov A.P. (2012) studied age-related changes cattle white blood cells. They note that changes in the content of basophils and monocytes with age are insignificant and do not have regularities. Changes in the content of eosinophils are pronounced. In the early period of ontogenesis, up to a year, is observed hypoeosinophilia. A common pattern for the ontogenesis of neutrophils is a rapid drop in their content at a very early age and a slow but continuous increase in their number in the future. Myelocytes and immature neutrophile can be found only at a very young age. The number of stab neutrophils is relatively large in very young calves. Age-related changes in the number of lymphocytes are an inverse reflection of changes in neutrophils [15]. The results of our studies are consistent with these data.

Apparently a change in the content of neutrophils and lymphocytes has led to a change of nonspecific reactivity indicators. Thus, the index lymphocytes/segmented neutrophils has decreased by 14.6% in animals of the 1st experimental group and by 26.1% in the 2nd experimental group, but the index neutrophils/lymphocytes has increased by 19.8 and 38.3% in the 1st and 2nd experimental groups respectively (table 1). The body has reserve segmented neutrophils. They can be used as needed. Recombinant interleukin-2 and polyoxidonium stimulated the release of segmented neutrophils into the blood-stream.

We recorded increase the relative and absolute number T- and B- lymphocytes of calves blood with age. Calves of the 1st experimental group have shown increase relative and absolute content T-lymphocytes by 8.5 and 12.1% respectively. Recombinant interleukin-2 activated T-cells in a body of calves. Recombinant interleukin-2 had a positive effect on the level of B-lymphocytes. Calves of the
1st experimental group have shown increase relative and absolute content of B-lymphocytes by 9.4 and 13.8% respectively, compared with the control group. Polyoxidonium had a predominant effect on B-lymphocytes, there increase in the relative and absolute content of these cells in calves blood of the 2nd experimental group, respectively, by 12.6 and 9.2%.

According to the data of Petryankin F.P., Semenov V.G., in newborns and calves of the early postnatal period of ontogenesis, cellular immunity factors mainly prevail. So, about 80% of the lymphocytes in the lymph nodes, spleen and blood are represented by T cells. However, there is a deficiency of T-helpers and T-suppressors, which affects the development of humoral immunity. The B-system of calves immunity is poorly developed, which is responsible for the synthesis of various classes of immunoglobulins [16, 17]. The results of our observations are according with the results of these scientists.

Table 1. The values of the morphological parameters of calves blood.

| Indicator                          | Group        | Before drug injection | after 10 days | after 30 days |
|------------------------------------|--------------|-----------------------|---------------|--------------|
| Erythrocytes, 10\(^12\)/l          | control      | 7.21±0.44             | 7.53±0.38     | 10.21±0.83   |
|                                    | experimental 1 | 7.30±0.39             | 7.84±0.31     | 9.33±0.69    |
|                                    | experimental 2 | 7.34±0.40             | 8.35±0.28\(^*\) | 10.3±0.87    |
| Hemoglobin, g/l                    | control      | 93.8±4.2              | 93.9±6.2      | 106.5±5.7    |
|                                    | experimental 1 | 92.1±3.8              | 91.7±2.4      | 91.8±4.8     |
|                                    | experimental 2 | 92.9±3.1              | 97.4±3.6      | 108.3±3.9    |
| Leukocytes, 10\(^9\)/l             | control      | 9.12±0.24             | 9.24±0.35     | 11.28±0.65   |
|                                    | experimental 1 | 9.54±0.31             | 10.12±0.17\(^*\) | 12.49±0.49 |
|                                    | experimental 2 | 9.72±0.29             | 10.46±0.42\(^*\) | 13.1±0.51 |
| Differential white blood cell count:| control      | 1.2±0.06              | 1.3±0.09      | 0.6±0.07     |
| Eosinophils, %                     | experimental 1 | 1.0±0.09              | 1.1±0.08      | 0.3±0.05     |
|                                    | experimental 2 | 0.9±0.14              | 0.8±0.06      | 0.7±0.05     |
| Basophils, %                       | control      | 2.2±0.11              | 2.9±0.11      | 1.1±0.09     |
|                                    | experimental 1 | 2.0±0.16              | 1.8±0.12      | 0.7±0.1     |
|                                    | experimental 2 | 2.5±0.19              | 3.1±0.09      | 1.3±0.1     |
| Stab neutrophils, %                | control      | 5.8±1.21              | 1.9±0.14      | 1.0±0.11     |
|                                    | experimental 1 | 6.2±1.11              | 3.2±0.11      | 0.8±0.09     |
|                                    | experimental 2 | 6.4±1.08              | 3.5±0.12      | 1.0±0.10     |
| Segmented neutrophils, %           | control      | 39.0±1.19             | 38.2±2.3      | 42.2±2.8     |
|                                    | experimental 1 | 43.0±1.16             | 42.2±3.1      | 38.6±2.5     |
|                                    | experimental 2 | 40.1±1.12             | 44.3±2.7\(^*\) | 36.0±3.2     |
| Monocytes, %                       | control      | 3.6±0.64              | 6.1±0.28      | 6.3±0.34     |
|                                    | experimental 1 | 4.4±0.37              | 4.9±0.31      | 6.8±0.38     |
|                                    | experimental 2 | 3.9±0.51              | 5.8±0.35      | 7.2±0.29     |
| %                                  | control      | 48.2±1.9              | 49.6±2.8      | 48.8±3.1     |
|                                    | experimental 1 | 43.4±1.84             | 46.8±3.7      | 52.8±3.9     |
|                                    | experimental 2 | 46.2±1.22             | 42.5±3.4      | 53.8±4.2     |
| Lymphocytes K/mcL                   | control      | 4.39±0.92             | 4.58±0.85     | 5.5±0.89     |
|                                    | experimental 1 | 4.14±0.95             | 4.74±0.79     | 6.59±0.81    |
|                                    | experimental 2 | 4.49±0.74             | 4.44±0.81     | 7.05±0.84    |
| White blood cell ratio:            | control      | 1.23±0.21             | 1.3±0.08      | 1.16±0.11    |
| lymphocytes / segmented neutrophils | experimental 1 | 1.02±0.14             | 1.1±0.07      | 1.37±0.12    |
|                  | control     | experimental 1 | experimental 2 |
|------------------|-------------|----------------|----------------|
| Neutrophils / lymphocytes | 0.92±0.08   | 1.13±0.1       | 1.0±0.09       |
| %                | 59.2±0.94   | 61.0±1.53      | 60.5±1.74      |
| B lymphocytes    | 2.59±0.15   | 2.79±0.33      | 3.30±0.28      |
| %                | 17.4±0.49   | 20.9±0.19      | 19.2±0.74      |
| K/mcL            | 0.72±0.12   | 0.99±0.09*     | 1.26±0.18*     |

*≤0.05 as compared to control.

The revealed differences in the morphological parameters of blood in calves of the experimental and control groups were reflected in the biochemical and immunological parameters of the blood of these animals (table 2). The level of total protein in the blood serum of calves gradually increased from the beginning of the experiment to its completion both in the control and in the 1st and 2nd experimental groups from 55.9±1.9 to 69.45±2.6; from 58.2±2.1 to 79.43±2.2 and from 56.9±3.7 to 73.35±2.7, respectively, which occurred in connection with the growth of animals, an increase in the intake of proteins and amino acids with food, as well as morphological and functional formation of the organs of the gastrointestinal tract. Calves of the 1st and 2nd experimental groups have shown significantly higher level of total protein 10 days after drug injection (by 18.4 and 15.0%), and 30 days after drug injection (by 14.4 and 5.6%) in comparison with control group. The level of total protein of calves experimental groups was higher due to immunoglobulins.

The level of α- and β-globulin protein fractions in the blood serum of animal experimental groups increased slightly, the difference in the corresponding values between the compared groups was insignificant.

Immunoglobulins are a type of protein compounds of the blood that are synthesized by plasma cells in animals and humans in response to getting into the organism of foreign or potentially dangerous substances. Immunoglobulins can perform an opsonizing function, enhancing phagocytosis. At the beginning of the study, calves of the experimental groups have shown a reduce content of immunoglobulins. Scientists have proved that calves with a blood content of less than 15 g/l of serum immunoglobulins can be susceptible to various infections (including those causing diarrhea and bronchopneumonia) [18-21]. Ten days after use of recombinant interleukin-2 and polyoxidonium experimental calves have shown increase the level of immunoglobulins by 65.4 and 61.2% respectively, in comparison with control group. This effect was preserved after 30 days. Therefore, interleukin-2 and polyoxidonium stimulated the production of immunoglobulins by plasma cells in the body of calves.

The bactericidal activity of blood serum, reflecting the total effect of cellular and humoral defense factors, was higher in calves of the 1st and 2nd experimental groups 10 days after the use of drugs by 6.2 and 7.7% (P <0.05) compared with a control group.

An important indicator of nonspecific resistance of the body is the phagocytic activity of segmented neutrophils. A low level of phagocytic activity at the age of 20-30 days is associated with a low level of immunoglobulins in blood serum. The increase in this indicator in calves of the experimental groups is associated with the activation of intracellular systems of phagocytes, an
increase in the opsonic abilities of immunoglobulins and an increase in the activity of the complement system. 10 and 30 days after birth, the indicator of this activity in calves of the 1st and 2nd experimental groups exceeded the control value by 13.3 and 18% and by 14.8 and 16.1%, respectively.

Table 2. Biochemical and immunological parameters of calves blood.

| Indicator                     | Group             | Blood sampling time             |
|-------------------------------|-------------------|---------------------------------|
|                               | Before drug injection | after 10 days | after 30 days |
| Total protein, g/l            | control           | 55.9±1.9 | 59.7±1.2 | 69.45±2.6 |
|                               | experimental 1    | 58.2±2.1 | 70.75±0.8 * | 79.43±2.2 * |
|                               | experimental 2    | 56.9±3.7 | 68.9±0.7 * | 73.35±2.7 * |
| Albumins, g/l                 | control           | 26.1±0.45 | 28.13±0.07 | 30.24±1.23 |
|                               | experimental 1    | 25.9±0.59 | 29.72±0.63 | 31.23±0.84 |
|                               | experimental 2    | 26.4±0.74 | 29.96±0.51 | 30.42±0.67 |
| Alpha-globulins, g/l          | control           | 9.98±0.39 | 10.61±0.46 | 12.3±0.34 |
|                               | experimental 1    | 10.9±0.65 | 12.3±0.71 | 14.52±0.71 |
|                               | experimental 2    | 10.1±0.54 | 11.17±0.58 | 12.25±0.48 |
| Beta-globulins, g/l           | control           | 9.85±0.51 | 10.3±0.43 | 11.54±0.35 |
|                               | experimental 1    | 9.54±0.39 | 9.73±0.26 | 10.76±0.39 |
|                               | experimental 2    | 9.65±0.41 | 9.83±0.35 | 10.18±0.72 |
| Gamma-globulins, g/l          | control           | 10.17±0.59 | 11.16±0.62 | 16.73±0.8 |
|                               | experimental 1    | 11.55±0.34 | 18.43±0.28 * | 22.44±0.71 * |
|                               | experimental 2    | 10.9±0.48 | 18.04±0.33 * | 19.92±0.52 * |
| Hematocrit, %                 | control           | 29.2±1.7 | 30.4±2.7 | 32.8±2.9 |
|                               | experimental 1    | 29.4±1.5 | 28.8±1.9 | 27.0±3.1 |
|                               | experimental 2    | 29.5±0.9 | 29.7±1.4 | 30.8±2.2 |
| Blood urea, mmol/L            | control           | 3.89±0.14 | 3.82±0.22 | 3.65±0.29 |
|                               | experimental 1    | 3.94±0.12 | 3.87±0.18 | 3.78±0.25 |
|                               | experimental 2    | 4.12±0.15 | 4.03±0.21 | 3.92±0.27 |
| Blood glucose, mmol/L         | control           | 3.95±0.18 | 4.25±0.31 | 4.07±0.3 |
|                               | experimental 1    | 3.83±0.15 | 3.92±0.21 | 3.97±0.27 |
|                               | experimental 2    | 3.75±0.12 | 3.82±0.28 | 3.87±0.29 |
| Bactericidal activity, %      | control           | 40.0±1.8 | 54.5±4.3 | 58.7±5.1 |
|                               | experimental 1    | 40.9±1.1 | 57.9±4.8 * | 61.4±5.8 |
|                               | experimental 2    | 41.8±0.9 | 58.7±5.5 * | 60.5±6.2 |
| Lysozyme activity, %          | control           | 18.1±2.2 | 20.3±2.1 | 19.4±2.4 |
|                               | experimental 1    | 21.2±1.8 | 23.6±1.9 | 22.5±1.9 |
|                               | experimental 2    | 20.1±2.5 | 21.3±2.2 | 20.9±2.1 |
| Neutrophil phagocytic rate, % | control           | 42.1±3.4 | 47.1±3.2 | 48.5±3.8 |
|                               | experimental 1    | 40.5±0.9 | 53.4±4.3 * | 55.7±4.7 * |
|                               | experimental 2    | 41.9±4.2 | 55.6±5.1 * | 56.3±5.3 * |

* * ≤0.05 as compared to control.

The urea level of calves experimental groups as they matured slightly decreased in the control group from 3.89±0.14 to 3.78±0.25, in the 1st experimental group from 3.83±0.15 to 3.78±0.25, in the 2nd experimental group from 4.12±0.15 to 3.92±0.27 mmol/L, this is likely to be associated with an increase in the efficiency of use of feed nitrogenous substances. No significant differences were found in the blood urea level of calves experimental and control groups.
The blood glucose level with age in the calves of the experimental groups increased slightly in the control group from 3.95±0.18 to 4.07±0.3, in the 1st experimental group from 3.83±0.15 to 3.97±0.27, and in the 2nd experimental one from 3.75±0.12 to 3.87±0.29 mmol/L. An increase in blood glucose level in the calves of the experimental groups during the experiment indicates an increase in carbohydrate metabolism in their body. No significant differences in the blood glucose level of calves of the experimental groups with the control were found.

4. Conclusion
We investigated the effect of recombinant interleukin-2 and polyoxidonium on the physiological state, blood morphological composition, immunological and biochemical blood parameters of calves 20-30 days of age. As a result of the experiment, we found the stimulation of leukopoiesis in calves of the first and second experimental groups on the 10th day of the experiment. In the blood of calves of first and second experimental groups there was increase number of leukocytes by 9.5 and 13.2% compared with the control group. The increase in the number of leukocytes in the blood of calves experimental groups occurred mainly due to segmented neutrophils. The level of immunoglobulins in calves of the first and second experimental groups was higher by 65.4 and 61.2%, respectively, 10 days after the start of the experiment. The phagocytic activity of neutrophils and the bactericidal activity of blood serum were higher in the calves of the experimental groups compared to the control. Thus, recombinant interleukin-2 and polyoxidonium stimulated the formation of nonspecific resistance in calves. The data obtained allow to clarify some aspects of the regulation of the formation of immunity and the formation of nonspecific resistance in calves during the dairy period of growing, which should be taken into account when developing physiologically reasonable methods of immunostimulation during this period, often accompanied by immunodeficiencies and infectious diseases.

References
[1] Semenov V G 2005 Stimulation of adaptive processes and the biological potential of cattle Journal Veterinary pathology [In Russian] 1 87
[2] Nikitin D A and Semenov V G 2013 Growth, development and nonspecific resistance of calfs at application of new immunomodulators Scientific notes of the Kazan State Academy of Veterinary Medicine. N.E. Bauman 213 185
[3] Sun P, Wang J Q and. Zhang H T 2010 Effects of Bacillus subtilis natto on performance and immune function of preweaning calves Journal of Dairy Science 93 (12) 5851 doi: 10.3168/jds.2010-3263
[4] Reddy P G, Morrill J L and Minocha H C 1986 Effect of Supplemental Vitamin E on the Immune System of Calves Journal of Dairy Science 69 (1) 164 doi: 10.3168/jds.S0022-0302(86)80382-4
[5] Hidiroglo M, Batra T R, and Ivan M 1995 Effects of Supplemental Vitamins E and C on the Immune Responses of Calves 1995 Journal of Dairy Science 78 (7) 1578 doi: 10.3168/jds.S0022-0302(95)76781-9
[6] Bordignon R, Volpato A and Glombowsky P 2019 Nutraceutical effect of vitamins and minerals on performance and immune and antioxidant systems in dairy calves during the nutritional transition period in summer 2019 Journal of Thermal Biology 84 451 doi: 10.1016/j.jtherbio.2019.07.034
[7] Velikanov V I, Klyapnev A V and Kharitonov L V 2016 The level of metabolic and immunological status of newborn calves under the action of timogen on the body of down-calving cows Biosciences Biotechnology Research Asia 13 (2) 1247 doi: 10.13005/bbra/2159
[8] Kharitonova O V, Kharitonov L V and Velikanov V I 2018 Study of the efficiency of various methods of improving colostral immunity in newborn calves Problems of Productive Animal Biology [In Russian] 2 81 doi: 10.25687/1996-6733.prodanimbiol.2018.2.81-93
[9] Moiseev A N, Sakharova E D and Ostrovsky M V 2009 Infectious diseases: the effect of Roncoleukin on non-specific immunity factors Journal veterinary doctor [In Russian] 8 15
[10] Smith K A 1998 Interleukin-2: inception, impact and implication Science 240 1169 doi: 10.1126/science.3131876
[11] Petrov R V 2000 Polyoxidonium - a new generation of immunomodulators with a known structure and mechanism of action Journal Immunology [In Russian] 5 24
[12] Pinegin B V, Nekrasov A V 2006 Polyoxidonium: new data on its clinical use Journal Allergology and Immunology [In Russian] 3 434
[13] Pinegin B V, Nekrasov A V and Haitov R M 2004 Immunomodulator Polyoxidonium: mechanisms of action and aspects of clinical application Journal of Cytokines and Inflammation [In Russian] 3 (3) 41
[14] Cooke R, Costantini D 2003 Statistics in Science (Springer Netherlands) p 200
[15] Zhukov A P, Sharafutdinova E B and Datsky A P 2017 Age-related changes in integral hematological indices in cattle News of the Orenburg State Agrarian University [In Russian] 2 (64) 110
[16] Petryankin F P, semenov V G and Ivanov N G 2015 Immunostimulators in veterinary medicine practice Publishing and printing company "New time" (Cheboksary) [In Russian] p272
[17] semenov V G, Nikitin D A and petrov N S 2015 Ensuring the health and safety of calves with domestic biostimulants Russian Journal Problems of Veterinary Sanitation, Hygiene and Ecology [In Russian] 4 (16) 68
[18] Sidorov M A, Gushchin V N 1984 Prevention of colibacillosis of calves Veterinary science [In Russian] 3 41
[19] Furdui V F 1994 The formation of immune status in calves in the early postnatal period Proceedings of the Institute of Physiology, Academy of Sciences of the Republic of Moldova: Chisinau [In Russian] 4
[20] Telcov L P, Michaelievskaya E O and Muzyka I G 2011 Productivity and animal development laws Bulletin of the agricultural sector of the Upper Volga [In Russian] 2 (14) 22
[21] Shulga N N 2005 The effect of colostral immunity on the safety of newborn calves Reports of the Russian Academy of Agricultural Sciences [In Russian] 4 41