Effect of feeding probiotic "Rumit-V" in the Russian Black Pied dairy cows

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Abstract

The effect of the use of the enzymatic-probiotic preparation "Rumit-V" in feeding cows of the Holsteinized Russian Black Pied breed was studied. The objectives of the study included the analysis of biochemical parameters of animal blood, the study of the effect of the feed additive on the activity of rumen microflora, and the level of milk productivity, as well as the calculation of the economic efficiency of the use of the studied probiotic. In the experiment, during the lactation period, two groups of cows (each of 30 heads) were formed based on their blood quality, live weight, number of calvings and milk productivity. The cows of the control group received a household diet, and the cows of the experimental group additionally received the probiotic "Rumit-V" at 50 g per head each day during their daily feeding. The duration of feeding the probiotic was 90 days. At the end of the experiment, the hematological analysis of the animals' blood showed that the level of inflammatory processes in the cows in the control group increased, as evidenced by a higher content of leukocytes in the blood (by 40.2%, $p \leq 0.01$) compared to the experimental group, while in the experimental animals the content of this indicator decreased compared to the initial one. The use of the probiotic in cows of the experimental group contributed to an increase in the activity of rumen microflora by 18.6% ($p \leq 0.05$). At the end of feeding probiotics, there was a positive trend in some biochemical parameters of the blood. The study revealed that the addition of the probiotic to the diet of dairy cows led to metabolic processes normalization and improvement in the health of animals; as a result, the milk productivity of animals in the experimental group increased by 7%. The additional profit from the sale of milk in the experimental group amounted to 0.8 US dollars per head per day.

Keywords: blood; milk production; probiotic; cost-effectiveness

Introduction

Modern industrial animal husbandry, especially dairy cattle breeding, includes, as a rule, rigid modes of production processes that cause an increased load on the functional activity of animal body systems, which in turn significantly exacerbates the problem of proper feeding, animal husbandry and obtaining high-quality products. It is particularly difficult to optimize the nutrient content of dry matter and ensure a balanced diet in high-yielding animals (De Vries & Marcondes, 2020; Gruber et al., 2021).

In case of violation of the technology of feeding and keeping animals, unbalanced and inadequate rations, industrial stress, high concentration of livestock, all metabolic processes in the body are disrupted, in connection with which there is a violation of the intestinal microflora, a decrease in milk productivity and natural resistance of the body. A decrease in immunity and a violation of the composition of the microflora provoke susceptibility to infectious agents and disorders of digestive functions (Costa-Roura et al., 2022). In animal husbandry, a large number of different drugs are currently used for therapeutic and preventive purposes in diseases of both infectious and non-infectious etiology. For more than 50 years, antibiotics have been the preferred antimicrobial drug. In agriculture, antibiotics have commonly been used in animal husbandry to prevent bacterial infection and promote livestock growth (Burke et al., 2021). The widespread and not always justified use of these drugs is accompanied by the formation of drug-resistant strains of microorganisms in animals, as well as in humans (Wemette et al., 2021).

In addition, excessive or incorrect use of antibiotics in animal husbandry can pose a threat to human health. Antibiotics used for therapeutic purposes and to stimulate the growth and development of young animals accumulate in significant quantities in food products such as meat, milk, and eggs (Chen et al., 2019; Hassan et al., 2021). The free concentration of antibiotics is excreted from the animal's body for a brief period with waste products – feces, urine, products (milk, eggs), and the concentration associated with proteins and other components is stored in the body for a long time (Raykova et al., 2023). Antibiotics excreted from the body get into the soil in the form of organic fertilizers and then accumulate in potatoes, peanuts, vegetables, and other plant foods (Zhao et al., 2019; Geng et al., 2022; Abbas et al., 2023).

The search for new, more effective drugs that do not cause drug resistance and have a pronounced antimicrobial effect, including in relation to antibiotic-resistant strains of microbes, is relevant both in medicine and in veterinary medicine. The best way to solve this problem is to include probiotics in the feed (Niranjan et al., 2023).

Recently, probiotic preparations have been used in animal feeding, which include live microorganism symbionts of the gastrointestinal tract. Probiotic preparations contain strains of live bacteria isolated from the gastrointestinal tract of animals, which stimulate not only the development and vital activity of beneficial symbiont microflora, but also inhibit the growth of pathogenic and opportunistic strains of microorganisms. Probiotic preparations are widely used to improve digestion, increase the efficiency of feed use, improve metabolic processes, as well as prevent and treat gastrointestinal diseases of an infectious and non-infectious nature, resulting from a sharp change in the composition of the diet, violations of feeding regimes, technological stresses, re-establishment, correction of the symbiont microflora of the digestive tract after treatment with antibiotics and antibacterial chemotherapeutic agents, as a substitute for antibiotics, in stimulating non-specific immunity, and in general to increase the productivity of animals (Ma et al., 2018; Chida et al., 2021). In addition, it has been shown that probiotics contribute to an increase in the number of beneficial cellulolytic microorganisms in the rumen of ruminants: representatives of the families Eubacteriaceae, Lachnospiuaceae, Rumnococcaceae. Members of these families have the ability to form cellulase, which allows ruminants to efficiently use the energy of feeds rich in fiber and low in protein (Azad et al., 2017; Xu et al.,2017).

Interest in the symbiotic microbiota of the rumen of reindeer (*Rangifer tarandus*) is due to the adaptation of this species to harsh Arctic conditions, including the ability to effectively use scarce botanical food sources from the tundra, forest-tundra, and northern taiga zones (Mathiesen et al., 2000; Sundset et al., 2009a). In the diet of reindeer, the proportion of lichens containing significant amounts of indigestible polysaccharides - chitin, hemicellulose, etc., reaches 70%; therefore, cellulolytic rumen microorganisms in deer play a decisive role in digestion.

In addition, lichens are active producers of more than 250 secondary compounds; the most well-known metabolite with toxic properties is usnic acid, which is produced by lichens of many genera (Glad et al., 2014). Studies have shown the presence of mycotoxins in various combinations in lichens of different genera and families; besides, it has been found that higher plants in the diet of reindeer are also significantly affected by combinations of mycotoxins (Burkin & Kononenko, 2013; Yildirim et al., 2018).

In recent years, in order to develop highly effective biological products, close attention has been paid to the ability of microorganisms to biodegrade bacterial and fungal toxins into non-toxic compounds. According to several researchers, bacteria associated with the rumen of deer can actively detoxify usnic acid and mycotoxins contained in the components of their diets (Sundset et al., 2009b; Luzina & Salakhutdinov, 2016).

Biotroph LLC screened highly effective isolates from the rumen contents of reindeer to create a collection of bacteria with cellulolytic and antimicrobial properties, as well as the ability to biodegrade mycotoxins (Ilina et al., 2021). Isolated isolates can serve as the basis of highly effective preparations for reindeer husbandry and other branches of animal husbandry. Based on the conducted research, the biological preparation "Rumit" was created, the preparation is an association of bacteria isolated from the rumen of reindeer (*Rangifer tarandus*) (genera Bacillus, Bacteroides, Porphyromonas, Pseudomonas, etc.), applied to sunflower meal. Previously, we have shown the efficacy of this probiotic preparation when added to the diet of young cattle and dairy cows (Smirnova et al., 2023a,b). From

the most promising strain of *Bacillus velezensis* bacteria was identified. Some researchers note that various strains of *B. velezensis* isolated from the microflora of the gastrointestinal tract of animals and humans are capable of synthesizing various enzymes and antimicrobial compounds and therefore can be used as the basis for probiotic preparations (Khalid et al., 2021; Li et al., 2023). Based on the obtained positive characteristics of the *B. velezensis* strain, Biotroph LLC created a new probiotic "Rumit-V". The aim of the research was to determine the effectiveness of the use of the probiotic "Rumit-V" in feeding lactating cows of the Holsteinized Russian Black Pied breed.

Material and methods

Research area
Industrial tests of the microbiological preparation "Rumit-V" were carried out in the conditions of the dairy complex of Zazerkalye LLC in the Gryazovets District, Vologda Region (Russia, Panfilovo 58.978275, 39.873296) from October 2022 to March 2023. This enterprise is sustainable, actively developing, using modern technologies. The livestock of the agricultural enterprise is about 2000 heads of Russian Black Pied cattle of various age categories and levels of productivity. All the manipulations with the experimental animals were performed according to the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Official Journal of the European Union L276/33, 2010). Laboratory tests were carried out at the Center for Agricultural Research and Biotechnology at the Vologda Research Center of the Russian Academy of Sciences.

Information about the test drug
By using a complex of molecular genetic methods (NGS sequencing, T-RFLP analysis) specialists from Biotrof LLC studied the microbiome of the reindeer rumen depending on the seasonal variability of the botanical composition and nutritional value of its diet, toxic load (mycotoxins) and animal health characteristics mycotoxins (Ilina et al., 2021). The contents of deer rumen turned out to be a promising source for searching for bacteria with high biological activity. The data obtained indicate that microorganism isolates from the rumen of reindeer can synthesize cellulases and enzymes that carry out the biodegradation of mycotoxins, which likely allows them to gain a competitive advantage in the rumen of deer consuming diets with an abundance of indigestible fiber and secondary metabolites with toxic properties (Ilina et al., 2020). The basis of the experimental probiotic of the biological product "Rumit-V" is the most promising strain of bacteria *Bacillus velezensis*, isolated from the rumen of reindeer. Microorganisms are applied to sunflower meal in an amount of 2×10^7 CFU/g, then the biomass is dried to obtain a dry concentrate in the form of a powder; the manufacturer of the probiotic is Biotrof LLC (Russia). *B. velezensis* promotes the decomposition of cellulose and other polysaccharides, has antagonistic properties towards several pathogenic microorganisms, and can biodegrade mycotoxins.

Animals, diet and experimental design
To study the effect of the dietary supplement on the level of milk productivity and animal health, two groups of Holsteinized Russian Black Pied breed cows of 30 heads were formed (Table 1).

Table 1. Characteristics of experimental animal groups on the main selection indicators

Group	Bloodline, %	Live weight, kg	Dairy days of current lactation	Last completed lactation	Milk yield for the last completed lactation, kg
Control	91.0 ± 1.61	539 ± 3.5	118 ± 11.5	1.8 ± 0.26	9213 ± 205
Experimental	88.8 ± 1.79	542 ± 3.8	115 ± 9.6	1.9 ± 0.28	9207 ± 198

Note: the absence of significant differences indicates the homogeneity of the formed groups of animals

The mini-herd (miniature herd) method was chosen as the basis for the formation of experimental groups of cows. From the total livestock located on the complex, considering productivity, three groups of animals (mini-stads) were formed using the principle of random selection (draw), and from each of these groups, 15% of the animals in the mini-stad were selected by random sampling. In turn, the required number of animals was selected from each corresponding productivity group, considering the physiological state, age, live weight.

The total duration of the experiment was 134 days, including the period of feeding biological products (90 days). The groups of cows were kept in one room on a leash in accordance with the standards of zoosanitary control. The cows of the control group received a household ration, and the cows of the experimental group received the "Rumit-V" at 50 g per head per day in addition to the main diet during the day.

The enterprise uses its own feed (hay, haylage, silage, grain) to feed cows. To implement standardized feeding, diets have been developed for different groups of cows depending on the physiological state, stage of lactation and milk yield. Cows on the farm received a complex multicomponent feed silage-concentrate mixture twice a day (in the morning and afternoon). All feed used on the farm is of high quality (silage, concentrates and haylage of I – II quality classes), which is required when conducting experiments on animals.

Milk production and sampling
The milk productivity of the studied animals was recorded by control milking once a month for three months. Milk samples were taken after morning and evening milking and stored with the addition of potassium dichromate in

the refrigerator at a temperature of +5°C until analyzed for the content of mass fraction of fat and mass fraction of protein. Milk analysis was carried out using a CombiFoss 7 (Denmark).

Sampling and analysis of ruminal fluid

Sampling of rumen contents was carried out at the beginning and at the end of the experiment from 5 heads in each group according to the N.V. Kurilov method (1972) 2–3 hours after feeding using a yawner (Kurilov & Krotkova, 1972). The collected rumen fluid was filtered through four layers of gauze. The activity of rumen microflora was determined by calculating the time of discoloration of a 0.03% solution of methylene blue in an amount of 1 ml added to 20 ml of rumen contents (methylene blue method according to G. Dirksen) (Meyer et al., 2004).

Sampling and analysis of blood serum

Biochemical and hematological blood tests were carried out before feeding the probiotic and at the end of the experiment (10 cows in each group of cows). Blood samples were collected from the tail vein before morning feeding into sterile tubes with EDTA K2 for hematological studies and a coagulation activator for biochemical studies. Test tubes with blood in a thermal container with refrigerant were delivered to the laboratory within an hour and a half.

For biochemical analysis, blood was centrifuged at 4000 rpm. for 10 minutes to separate the serum, followed by examination. The analysis was carried out using standard test kits from DIAVET (Russia). Blood serum components were determined in triplicate analytical replicates; internal quality control of the test results was carried out using calibration and control materials from DiaSys (Germany): control sera "Norm" (TruLab N), "Pathology" (TruLab P) and a multicalibrator (TruCal U). Automatic analyzers URIT-3020 (China) and iMagic-V7 (China) were used during the work.

Statistical analysis

Statistical processing of research results was carried out using methodological guidelines on biometrics using the computer program Excel and "Statistica for Windows, V 6.0". The values of the obtained results were reflected in the form of the mean and standard error (X±mx). Comparison of data with each other was carried out using Student's t-test at three probability levels ($*p<0{,}05$; $**p<0{,}01$; $***p<0{,}001$).

Results and Discussion

A necessary condition for experiments is selection of healthy animals. When assessing the physiological parameters of health, the body temperature of the animals involved in the experiment ranged from 37.7 to 38.1°C. The average pulse between the groups ranged from 50.0 to 52.2 beats per minute, that corresponded to the recommended values. The respiratory rate of the cows was higher than the physiological norm in all groups from 1.4 to 2.6 movements per minute, which may be due to the fact that the experiment involves highly productive animals with a high level of metabolism, so the respiratory rate in this case can be up to 35 movements per minute. When assessing the frequency of rumen contractions, it was found that in all groups of animals, its value was within the physiological norm from 4.3 to 4.6 times per minute.

Hematological indicators are of no small importance, as they help to timely identify latent pathological processes, more accurately establish their nature, and catch various complications in a sick animal even before the onset of a pronounced clinical manifestation. Table 2 shows the morphological parameters of the blood of experimental animals at the beginning of the experiment.

Table 2. Morphological parameters of animal blood (at the beginning of the experiment)

Indicator	Reference values	Group	
		Control	Experimental
Erythrocytes, 10^{12}/L	5.0 – 10.0	5.93 ± 0.13	5.93 ± 0.16
Hemoglobin, g/L	80.0 – 120.0	98.5 ± 2.06	99.5 ± 2.21
Hematocrit, %	24.0 – 46.0	24.5 ± 0.51	24.3 ± 0.60
Thrombocytes, 10^9/L	100.0 – 800.0	406.6 ± 67.7	388.2 ± 54.3
Leukocytes, 10^9/L	4.0 – 12.0	11.5 ± 0.63	11.7 ± 0.98
Lymphocytes, %	45.0 – 75.0	47.8 ± 2.89	50.0 ± 3.87
Granulocytes, %	27.0 – 46.0	42.5 ± 2.36	43.9 ± 3.35
Average erythrocyte volume, fl	40.0 – 60.0	41.4 ± 0.68	41.1 ± 0.62
Average hemoglobin in erythrocyte, pg	13.20 – 19.80	16.59 ± 0.27	16.76 ± 0.29
Average hemoglobin concentration in erythrocyte, g/L	310.0 – 410.0	402.1 ± 1.13	409.4 ± 2.51
Average thrombocyte volume, fl	4.5 – 6.7	5.88 ± 0.16	5.58 ± 0.07

Note: the absence of significant differences in morphological blood parameters between the groups indicates similar indicators of animal health at the beginning of the experiment

Table 3. Morphological parameters of animal blood (at the end of the experiment)

Indicator	Group	
	Control	Experimental
Erythrocytes, 10^{12}/L	6.56 ± 0.10	6.23 ± 0.15
Hemoglobin, g/L	109.4 ± 2.84	102.1 ± 2.24
Hematocrit, %	26.7 ± 0.50	24.9 ± 0.58
Thrombocytes, 10^9/L	284.5 ± 36.1	218.4 ± 25.6
Leukocytes, 10^9/L	15.7 ± 1.29	11.2 ± 0.48**
Lymphocytes, %	39.7 ± 3.46	43.4 ± 4.10
Granulocytes, %	50.4 ± 3.18	44.8 ± 3.67
Average erythrocyte volume, fl	40.8 ± 0.68	40.0 ± 0.50
Average hemoglobin in erythrocyte, pg	16.64 ± 0.41	16.34 ± 0.23
Average hemoglobin concentration in erythrocyte, g/L	408.6 ± 4.04	409.2 ± 1.98
Average thrombocyte volume, fl	7.18 ± 0.19	6.47 ± 0.12

Note: the differences are significant when comparing these groups **$p \leq 0.01$

Table 4. Rumen microflora activity of animals

Group	Indicator discoloration time, minutes	
	At the beginning of the experiment	At the end of the experiment
Control	6.10 ± 0.34	5.85 ± 0.24
Experimental	6.24 ± 0.41	5.26 ± 0.19*

Note: The differences are significant when comparing these groups *$p \leq 0.05$

The data presented in the table show that the hematological parameters of the studied groups of animals were within the recommended values. Thus, the content of leukocytes in the blood of the control and experimental groups of cows was 11.5 and 11.7×10^9/L, respectively, which may indicate the absence of latent infectious and inflammatory processes in the animal's body. The concentration of erythrocytes in the blood in the control and experimental groups was 5.93×10^{12}/L, with a hemoglobin level of 98.5 and 99.5 g/L, respectively.

Thus, the physiological and hematological parameters of the studied animals were within the limits of norms and did not differ significantly between the cows of the control and experimental groups, so clinically healthy animals participated in the experiment. After finishing feeding the biological preparation in cows, a repeated study of morphological blood parameters was carried out (Table 3).

Hematological analysis of the blood of the studied animals showed that, in general, all indicators were within the permissible values, only in the control group of cows the level of inflammatory processes increased, which is evidenced by a higher content of leukocytes in the blood (by 40.2%, $p \leq 0.01$) compared to the experimental group, while in the experimental group of cows the content of the studied indicator slightly decreased compared to the initial one. This may indirectly indicate the positive effect of the probiotic on reducing infectious and pathological processes in the body of animals. It should be noted that in the control group of cows, there is a decrease in the content of lymphocytes by 11.8% and an increase in granulocytes by 8.7%, which is associated with a large load on the metabolic processes of cows and the tension of the immune system, as well as an increase in antimicrobial reactivity (granulocytes).

It is known that feeding, and, as a result, rumen digestion is one of the leading factors in ensuring high productivity of dairy herds (Hua et al., 2022; Gross, 2022). This is due to the fact that during lactation, the animal's body is in a state of increased functional activity, complex processes of fermentation of feed by means of a huge number of bacteria, fungi, protozoa, as well as the absorption of nutrients and the synthesis of new ones take place in the forestomach. All this provides the animal with the necessary energy and nutrients, affects the physiological processes occurring in the body, which, in turn, contributes to the strengthening of metabolic processes, productive and reproductive phenomena. An important point in these processes is the ability to control rumen digestion by adjusting the diet. In this regard, we studied the effect of feeding the probiotic "Rumit-V" on some indicators of the rumen content of animals (Table 4).

At the beginning and at the end of the experiment, all the samples were good in terms of organoleptic parameters: the color was from gray-green to brown-green, the smell was specific, fragrant (without mustiness and sourness), the consistency was slightly viscous. As follows from the data of Table 4, at the beginning of the experiment, the activity of the rumen microflora of the animals in the control and experimental groups was approximately at the same level. After feeding the probiotic, there was a decrease in time by almost 1 minute. Thus, the activity of scar microflora for 90 days of the experiment in the experimental group significantly increased (by 18.6%.). Our results, which indirectly indicate the positive effect of the probiotic on the functioning of the animal rumen microbiome, correspond with the data of several studies. It was shown that the addition of a probiotic based on *Lactobacillus rhamnosus* and *Enterococcus faecalis* to the diet of goats contributed to the dominance of beneficial fibrolytic or cellulolytic bacteria in the rumen (Maake et al., 2021). Similar data were obtained when using a probiotic on cattle (Pinloche et al., 2013). The use of a probiotic based on *Bacillus subtilis*, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* in calves significantly increased the average daily weight gain in the first eight weeks after birth, while improving immune

function and reducing the incidence of diarrhea. This effect was associated with an increase in the relative abundance of fiber-degrading Ruminococcaceae and bifidobacteria, which play an important role in immune support (Wu et al., 2021).

In the conditions of intensive milk production technology, metabolic load of the animal's body increases. To maintain active metabolic processes, it is necessary to provide the optimal amount of all regulated substances and elements in rations. In order to deepen control over the completeness of cow feeding and ensure prompt response to nutritional imbalances and adjustment of rations, it is necessary to determine the biochemical parameters of the blood. Of particular importance is the correct choice of indicators that best reflect all aspects of metabolism (protein, carbohydrate, fat, mineral) (Overton et al., 2017). Having studied the level of metabolic processes in the animal's body at the beginning of the experiment, it was found that there were no deviations from the recommended values for the main parameters of blood serum reflecting protein, carbohydrate, energy, and mineral metabolism. However, there was a change in the concentration of aspartataminotrasferase and alanine aminotransferase in almost all experimental animals, and since aminotransferases are liver enzymes and are in the cytoplasm of cells (hepacytes, myocytes), such changes are primarily related to the intensive metabolism of highly productive animals and therefore an increase in the functional load on the liver.

Changes in the metabolic processes of the cows' body during feeding of the microbiological preparation "Rumit-V" were also assessed after feeding (Fig. 1, 2). At the end of the experiment, there was a positive trend in some biochemical parameters, which may indicate the normalization of metabolic processes in the experimental group of animals.

In cows, carbohydrate metabolism plays a huge role in determining the intensity of other metabolic processes. The main indicator of carbohydrate metabolism is the concentration of glucose, which is a source of energy for almost all vital physiological processes. The level of glucose in the blood serum of the studied animals in the experimental group at the beginning of the experiment was slightly higher than in the control one, after the end of feeding the probiotic, the opposite trend was observed. The results obtained are consistent with the data of several studies showing that under the influence of feeding probiotics in the blood serum of cattle, fatty acids are mobilized, and the glucose content increases (Overton et al., 2017).

When analyzing the protein metabolism data, it should be noted that in all experimental animals, the concentration of total protein in the blood serum was within the recommended values, but at the end of the experiment in the experimental group, its content was higher than in the control group by 4.3%.

Urea is the main final nitrogen-containing product of protein breakdown in animals and is related to the protein supply of animals. When analyzing the content of urea in the blood of cows, it was found that under the influence of feeding the probiotic "Rumit-V" there was a decrease in its concentration, as compared to previous blood tests, by 7.8% ($p \leq 0.05$), and its content was also lower by 11.3% than in the animals of the control group ($p \leq 0.01$). The positive dynamics of protein metabolism in the body of animals indicates an increase in the efficiency of feed nitrogen use, including for the synthesis of microbial protein, and can also be associated with the activation of protein synthesis and renewal processes, and more intensive use of amino groups, but not for the formation of urea.

The activity of amination enzymes was analyzed, as they are the most significant diagnostic parameters since they are extremely sensitive as indicators of cytolytic syndrome (Fig. 2). Studies show that the use of a probiotic supplement in feeding reduces the functional load on the liver. At the end of the experiment, the content of lactate dehydrogenase in the experimental group, compared to the control, was lower by 12.1%, aspartatate aminotrasferase by 8.3%, alanine aminotransferase – by 11.7%. The data obtained probably indicate a positive effect of the probiotic "Rumit-V" on cows, since it is known that an increase in the level of a number of enzymes in animals is observed with liver diseases, ketosis, endometritis, etc. (Waage, 1984; Du et al., 2017).

Analysis of animal blood serum for the content of macro- and microelements did not reveal deviations from the recommended values. The results of monthly control milkings allowed determining the positive impact of the enzymatic-probiotic effect in the diets of lactating cows on their productive qualities and subsequent efficiency of milk production (Table 5).

From the data of the table, it follows that the average daily milk yield of basic fat content in the cows of the control and experimental groups was 31.5 and 34.2 kg per head per day, respectively, thus the productivity of the animals receiving the supplement was higher than in the control group by 8.6%. The gross milk yield per animal for 90 days of the experiment in the experimental group was higher, compared to the control group, by 211 kg. Taking into account the cost of the consumed ration, the profit from milk sales per animal per day in the control group was 8.96 US dollars, while in the experimental group it was 9.76 US dollars. Thus, the use of the probiotic resulted in an additional profit of 0.8 US dollars per animal per day.
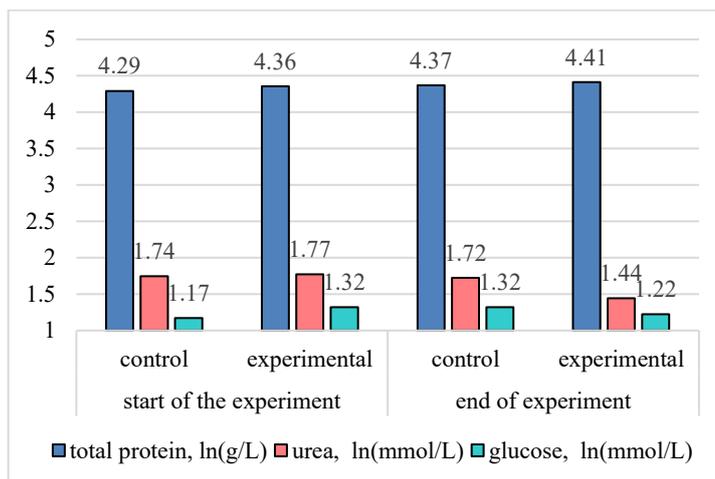
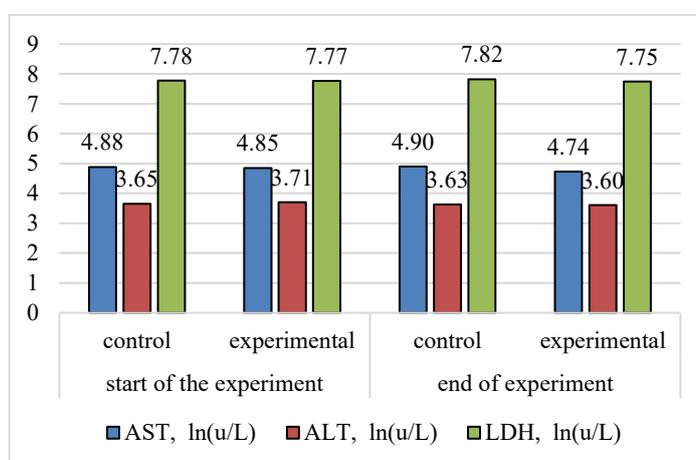**Fig. 1**. Biochemical parameters of animal blood. Logarithm of data has been performed to bring it to a uniform scale.

Fig. 2. Enzyme activity indicators. Logarithm of data has been performed to bring it to a uniform scale

Table 5. Economic efficiency of milk production (per one head of cattle).

Indicators	Group	
	Control	Experimental
Average daily milk yield of basic fat content, kg*	31.5 ± 0.7	34.2 ± 0.5**
Mass fraction of fat, %	3.55 ± 0.05	3.61 ± 0.06
Gross milk yield of basic fat content for the period of experience, kg	3008 ± 72	3219 ± 65**
Selling price of 1 kg of milk, US dollar	0.40	0.40
Income from the sale of produced milk, US dollar	1203.2	1287.6
Cost of consumed supplement per animal per day, US dollar	–	0.138
Cost of the consumed additive for the period of experience, US dollar	–	12.4
Diet cost for the period of experience, US dollar	396.7	409.1
Profit from the sale of milk for the period of experience, US dollar	806.5	878.5
Additional profit from the sale of milk for the period of experience, US dollar	–	72.0
Additional income per animal, US dollar per day	–	0.8

Note: * in the Russian Federation, the base fat content of milk is 3.4%.
** the differences are significant when comparing these groups p ≤ 0.05

Conclusion

According to the results of the studies, it was found that the inclusion of the enzymatic-probiotic preparation "Rumit-V" in the main diet of cows of the Holsteinized Russian Black Pied breed contributed to an increase in the activity of rumen microflora by 18.6% and an improvement in certain biochemical parameters of the blood. More intensive processes in the body of animals led to an increase in milk productivity by 7.0%, and, as a result, to a reduction in production costs and an additional profit from the sale of milk in the amount of 0.8 US dollars per animal per day.

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