The effect of the developed complex symbiotic composition on the immune and cytokine profile in young cattle in neonatal ontogenesis

Anastasia Zhivoderova[1*], *Viktor* Samoylenko[1], *Nadezhda* Ozheredova[2], *Bogdan* Pyanov[1], and *Anastasia* Lapina[1]

[1]North Caucasus Federal University, 1, Pushkin street, 355009, Stavropol, Russia
[2]Stavropol State Agrarian University, 12, Zootekhnicheskiy lane, 355017, Stavropol, Russia

Abstract. The humoral link of immunity plays an important role in protecting the body of newborn young animals from diseases of bacterial etiology. Diseases of the gastrointestinal tract in young cattle constitute a significant part. In recent years, more and more attention has been paid to research on probiotherapeutic agents and their effect on animal immunity. In this regard, it is extremely important to develop drugs based on target components, namely certified strains B-2585 Lactobacillus acidophilus 13 and B-2579 Enterococcus faecium K-50 with the inclusion of auxiliary components – inulin and FOS. It has been experimentally proven that the complex synbiotic composition has an effect on increasing the bactericidal and lysozyme activity of blood serum, the absorption and metabolic activity of neutrophils, reducing the content of pro-inflammatory cytokines and their optimal ratio with anti-inflammatory mediators. In addition, probiotic microorganisms included in the product increase the colonization potential of the microflora of the gastrointestinal tract, act as primary antigens for native leukocytes, stimulating the development of an immune response in contact with foreign antigens. These data can be used for further development of drugs based on probiotic microorganisms, in order to increase the nonspecific immunity of the animal.

1 Introduction

The formation of nonspecific immunity in calves during the newborn period is of great importance for their health and survival. Nonspecific immunity is the initial defense mechanism that helps the calf cope with various infectious agents before its adaptive immunity is fully formed [1-17]. In newborn calves, which are more vulnerable during this period, nonspecific immunity begins to form even before birth. In the last weeks of pregnancy in cattle, immunoglobulins are transmitted from mothers through the placenta to the fetus. This passive transfer of antibodies provides the newborn calf with initial protection in the first days of its life [8, 9, 10].

However, despite the fact that immunoglobulins are transmitted to the calf while it is still in the womb, the concentration of antibodies in the blood serum of calves at birth is very low,

* Corresponding author: nastya.zhivoderova007@mail.ru

as a result of which the calf's body is without proper protection from many bacterial infections and diseases that can pose a threat to its health [11, 15].

The main source of immunoglobulins for calves in the critical phase after birth is colostrum, which contains a high concentration of antibodies. Therefore, the first mother's milk is of great importance for the successful formation of nonspecific immunity [7].

In the first hours of calves' life, the intestines are permeable to antibodies from the intestinal contents, and this process is called passive transfer. The more active the transfer, the more antibodies are absorbed and the better non-specific immunity develops in calves [9].

However, over time, the intestine becomes less permeable to antibodies, and therefore the first day of a newborn calf's life, during which he consumes the first portion of colostrum with a high concentration of maternal immunoglobulins, is extremely important for the successful development of the immune process [3].

During the first week of life, the concentration of antibodies in the blood serum of calves increases significantly. At this time, the intestine is already fully formed, and passive antibody transfer does not happen. Therefore, after the first day of life, colostrum is no longer able to provide calves with the necessary amount of antibodies to support the development of nonspecific immunity [5].

The gastrointestinal tract of animals is a complex system in which an effective mechanism of immunity develops. The source of its development is the numerous bacteria, fungi and viruses that exist there. One of the key indicators characterizing the dynamism of the intestine is the diversity of microflora, which is involved in maintaining a stable immune system. Thus, beneficial lactobacilli effectively compete with pathogenic microorganisms, contributing to the strengthening of the immune system [12].

Moreover, studies show that immune cells are present in the intestine, namely T- and B – lymphocytes, which are actively involved in protecting the body from pathogens. They form antibodies and cytokines that help control and prevent the development of inflammatory process. The intestine acts as an important organ in which the synthesis of immune cells takes place, which in turn contributes to the maintenance of stable immunity [13].

An important aspect in the dynamics of intestinal functioning is the ratio between its microflora and food substances entering the body, they are the indispensable substrate for the reproduction of beneficial bacteria [14].

In recent years, more and more attention has been paid to the study of the interaction between the intestinal microflora and the body's immune system. Of particular interest are probiotherapeutic agents, which include strains of probiotic bacteria. The prospect of their use is their tendency to increase the concentration of antibodies in the blood serum of newborn calves, which is a key point in maintaining and strengthening the immune system [15].

The humoral link of the immune system plays an important role in protecting the body from various bacterial pathogens. It is a component of the immune system in which antibodies, also known as immunoglobulins (IgM, IgG, IgA), play a key role. These proteins perform many functions, including the capture and destruction of pathogenic microorganisms, stimulation of cells of the immune system and regulation of inflammatory processes [16].

It is known that the composition and balance of the intestinal microflora can affect the immune system. Probiotic strains of Lactobacillus microorganisms are able to colonize the intestine and create favorable conditions for the development of beneficial microbiota [1,3].

In addition, they are able to affect cells of the immune system, including immune enhancement cells (T- and B – lymphocytes) and cells responsible for the production of antibodies [17].

Recent studies demostrate that calves have an increased immune response when they are

given probiotic medications based on strains of probiotic bacteria. In particular, there is an increase in the level of immunoglobulins in the blood and cytokine profile. This indicates that probiotic microorganisms can stimulate the production of antibodies and enhance the protection functions of the body [1].

However, for a more complete assessment of probiotherapeutic agents on the humoral link of immunity, additional studies are needed. It is important to take into account various factors, such as health status and dosage of medications [12, 15].

Probiotherapeutic agents based on strains of probiotic bacteria are a potentially important tool for strengthening all parts of the immune system in newborn calves. Their ability to stimulate the production of antibodies and enhance the protection functions of the body can be used to more effectively combat diseases of bacterial etiology and reduce the risk of their development [7.10].

2 Materials and methods of the studies

On the basis of the educational vivarium of the Institute of Veterinary Medicine of the Federal State Budgetary Educational Institution of Higher Education "Stavropol State Agrarian University", in the period from November 2023 to December 2024. The main studies were conducted to examine the effect of a complex synbiotic composition developed with the inclusion of deposited certified strains B-2585 Lactobacillus acidophilus 13, B-2579 Enterococcus faecium K-50 with a concentration of microorganisms 10^8-10^{12} CFU/g and excipients — Organophosphorus chemistry, inulin in dry form and a protective sucrose-gelatin medium, on the immune and cytokine profile in young cattle in neonatal ontogenesis.

The object of the study was 6 clinically healthy one-day-old dairy calves of the Holstein-Frisian breed, divided into two groups of 3 heads (control and experimental groups). In the experimental group, calves with the second portion of colostrum were given a complex synbiotic composition for 15 days based. In the control group, according to the method used, individuals were given a saline solution at the rate of 2 ml per 1 kg of live body weight. The microflora of the gastrointestinal tract of calves of the control and experimental groups was evaluated according to the guidelines for the bacteriological diagnosis of colibacteriosis (escherichiosis) of animals approved by the Ministry of Agriculture of the Russian Federation No. 13-7-2/2117 dated 07/27/2000.

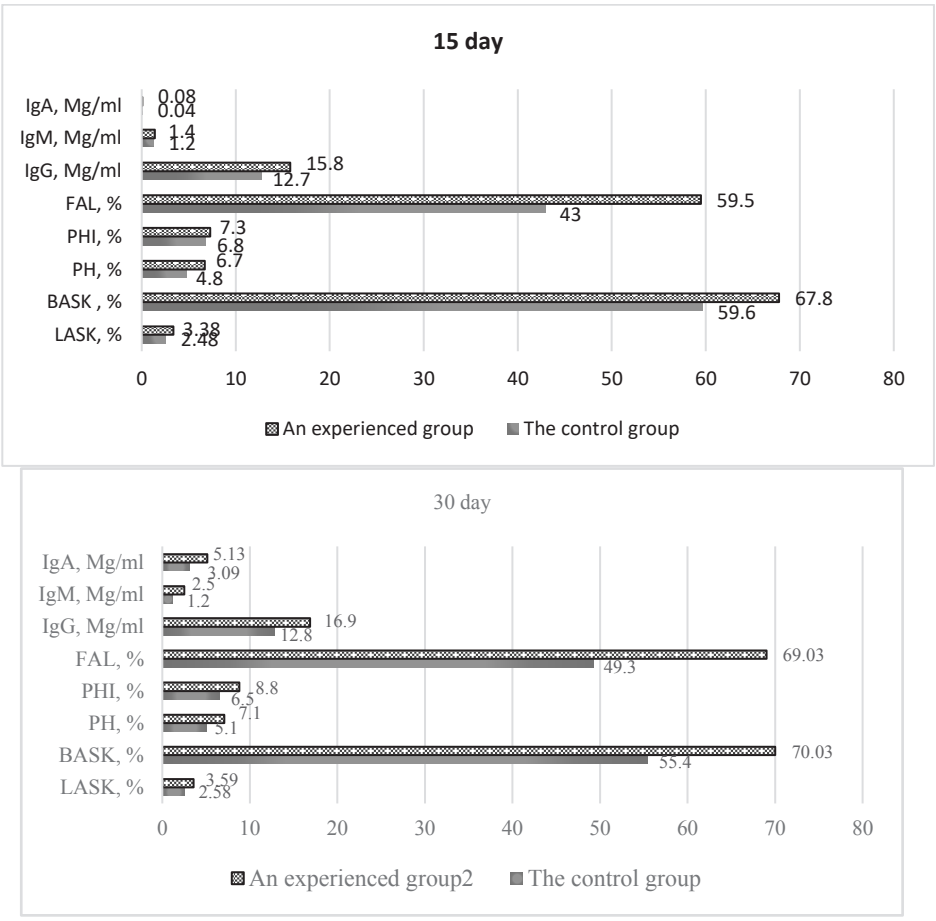
The delayed beneficial effect was evaluated on the 15th day after blood sample collection 2-3 hours before morning feeding from all individuals from each group. The study of the immune status in young cattle in neonatal ontogenesis was carried out on the 15th and 30th days of life, and the cytokine profile on the 1st and 15th days of life. The immune status of calves was determined in the laboratory of veterinary medicine of the All-Russian Scientific Research Institute of Sheep and Goat Breeding, a branch of the North Caucasian Federal Scientific Agrarian Center. The cytokine profile was evaluated on the basis of the interdepartmental scientific and educational laboratory of Experimental Immunomorphology, Immunopathology and Immunobiotechnology of the Federal State Autonomous Educational Institution of Higher Education "North Caucasus Federal University".

Thus, the determination of the phagocytic activity of neutrophils, phagocytic index, phagocytic number was carried out according to the method of A.M. Gorchakov and co-authors (2003), in relation to the test object Staphylococcus aureus (strain ATCC 6538P), the content of the main classes of immunoglobulins IgG, IgM, IgA (according to the method of Mancini et al., 1965). Bactericidal activity (BASK) was evaluated in blood serum according to the method of D.A. Petrachev (1981) in relation to the test object Escherichia coli (strain K-12 J53) and lysozyme activity (LASK) in relation to the test object Micrococcus lysodeik ticus (strain 2665), according to the method of V.Ya. Sarukhanova and co-authors (2012).

The expression of the main cytokine profile parameters was assessed by adding 1:1 to 0.5ml of heparin-stabilized blood (5000Ed/ml), inactivated Staphylococcus aureus test antigen (strain ATCC 6538P) and co-incubation at 37^0C for 24 hours. Quantitative determination of interleukin-2 (IL-2), interleukin-10 (IL-10) and interleukin–4 (IL-4) was determined by enzyme immunoassay (ELISA), followed by taking into account the results on the Uniplan-TM spectrophotometer in accordance with the approved guidelines for diagnostic kits.

3 Results

As a result of the studies conducted to study the effect of the developed complex synbiotic composition on the immune and cytokine profile in young cattle, it was found that the level of immune status in animals of the experimental group was significantly higher and was at the most stable level, thereby reducing the severity and duration of diseases with diarrheal syndrome in the neonatal period of life (Figure 1).



Note: *P ≤0.05 – the differences are significant (relative to the control)
** P ≤0.001 – the differences are highly significant (relative to the control)

Fig. 1. Indicators of immune effects of calves

According to the data presented in Table 1, the inclusion in the feeding diet of the developed complex synbiotic composition including probiotherapeutic strains of microorganisms contributes to maintaining a stable level of humoral immunity and natural resistance during the physiological adaptation of the newborn organism to new conditions and the formation of an immunological system in animals with preventive measures provided for by production.

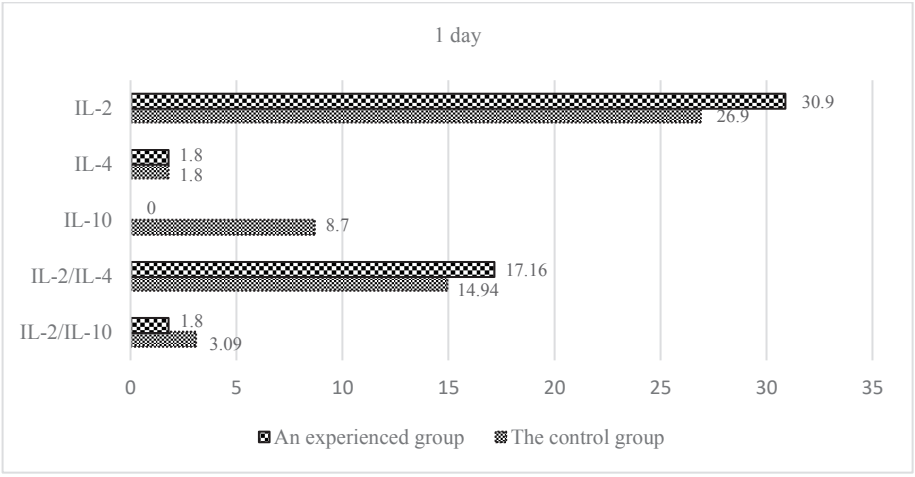
Thus, in animals of the experimental group, the level of BASK and LASK on day 15 of life was significantly higher than in the control group by 13.8% and 36.3%. The metabolic activity of neutrophils and intracellular intensity of phagocytes were significantly higher than in the control group, namely FAL by 38.4%, PHI by 7.6%, PH 39.6%.

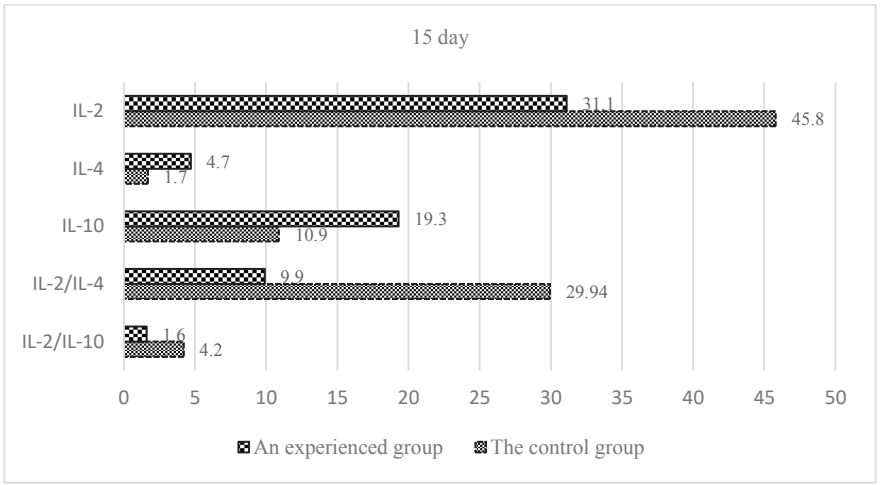
On the 30th day, the trend continued. In calves from the experimental group, the immunological status was the most stable than in the control group, namely BASK by 26.4%, LASK 39.1%, PHAL by 40.0%, PHI 35.4%, PHN 39.2%. Established changes in the immunological status of animals from the experimental group on day 30 indicated an increase in cytotoxicity with a clear immunocorrective effect of the developed remedy, and characterized a stable condition of nonspecific resistance of the body within the physiological norm.

In the process of research, 100% of the animals in the control group had signs of gastrointestinal diseases with diarrhea syndrome. Thus, on the first day, according to the data obtained during the assessment of the microflora of the gastrointestinal tract in 2 calves from the control group (66.7%), a violation of the colonization potential of the gastrointestinal tract was detected (with an average duration of 6 to 4 days). The disease was severe.

In the experimental group, in calves treated with a complex synbiotic composition, gastrointestinal diseases were recorded in 33.3% of cases with an average duration of 3 days. The disease was mild.

On the 15th day of life, calves from the control group had a high content of proinflammatory IL-2 and anti-inflammatory IL-10 in the cytokine profile, and the amount of IL-4 stimulating the humoral link of the immune response was lower than in animals from the experimental group. The ratio of pro- and anti-inflammatory cytokines IL-2/IL-4, IL-2/IL10 were high, which characterizes the course of an acute inflammatory process in animals (Figure 2).





Note: *P ≤0.05 – the differences are significant (relative to the control)
** P ≤0.001 – the differences are highly significant (relative to the control)

Fig. 2. Cytokine profile in patients with daily stimulation of Staphylococcus aureus leukocytes (pg/ml).

According to a comparative analysis of the data presented in Table 2, it was found that cytokine profile parameters under stimulation of cells with the Staph. aureus antigen, significant differences were revealed in calves in the experimental and control groups. Thus, the ratio of IL-2/ IL-4 in calves from the experimental group was significantly lower and more stable than in animals from the control group by 66.9%, which indicates a rapid transition from an inflammatory reaction to a cellular immune response to stimulation of the Staph. aureus test antigen. An increase in the concentration of IL-10 in calves from the experimental group correlates with the activation of the release of IL-4, responsible for humoral immunity and immunoglobulin synthesis, which is explained by the presence of specific B cells and memory cells in the blood.

An increase in the concentration of IL-10 in calves of the control and experimental groups when exposed to the Staph. aureus test antigen is characterized by the need to reduce the inflammatory response of the animal body to the presence of pathogens. In calves whose feeding ration included the developed synbiotic composition, stimulation of Staph. aureus did not cause a similar reaction, which, with stable synthesis of interleukin-2, indicates a more pronounced cellular reaction.

Thus, the ratio of cytokines synthesized by Th-1 and Th-2 cells in the calves of the experimental group decreased by an average of 12%. These changes reflect the activation of the humoral immune response as a result of the use of a complex synbiotic composition developed with the inclusion of deposited certified strains B-2585 Lactobacillus acidophilus 13, B-2579 Enterococcus faecium K-50 with a concentration of microorganisms 10^8-10^{12} CFU/g and excipients — FOS, inulin in dry form and a protective sucrose-gelatin medium.

4 Conclusions

In the course of research conducted to study the effect of a complex synbiotic composition developed with the inclusion of deposited certified strains B-2585 Lactobacillus acidophilus 13 and B-2579 Enterococcus faecium K-50 on the immune and cytokine profile in young cattle in neonatal ontogenesis, it was determined that the target components included in the product contribute to an increase in bactericidal and lysozyme activity blood serum,

absorption and metabolic activity of neutrophils,, reducing the content of pro-inflammatory cytokines and their optimal ratio with anti-inflammatory mediators.

In addition, probiotic microorganisms included in the product, which increase the colonization potential of the microflora of the gastrointestinal tract, act as primary antigens for native leukocytes, stimulating the development of an immune response in contact with foreign antigens, as well as synthesizing vitamins of group B and K increase the antioxidant status of the animal's body.

References

1. Raheem A. Modulatory Effects of Probiotics During Pathogenic Infections With Emphasis on Immune Regulation / A. Raheem, L. Liang, G. Zhang, S. Cui // Front Immunol. – 2021. – P. 12-16. doi: 10.3389/fimmu.2021.616713.
2. Mazziotta C. Probiotics Mechanism of Action on Immune Cells and Beneficial Effects on Human Health. / C. Mazziotta, M. Tognon, F. Martini, E. Torreggiani, J.C. Rotondo // Cells. – 2023. – **V. 12**(1) – P. 184-189. doi: 10.3390/cells12010184.
3. Cortes-Perez N.G. Probiotics and Trained Immunity / N.G. Cortes-Perez, A. de Moreno de LeBlanc, J.G. Gomez-Gutierrez, J.G. LeBlanc, L.G. Bermúdez-Humarán // Biomolecules. – 2021. –**V.11**(10). – P. 140-142. doi: 10.3390/biom11101402.
4. Wang X, Zhang P, Zhang X. Probiotics Regulate Gut Microbiota: An Effective Method to Improve Immunity. / X. Wang, P. Zhang, X. Zhang // Molecules. – 2021 – **V. 26**(19). – P. 606-615. doi: 10.3390/molecules26196076.
5. Rousseaux A. Immunomodulation of B Lymphocytes by Prebiotics, Probiotics and Synbiotics: Application in Pathologies. / A. Rousseaux, C. Brosseau, M. Bodinier // Nutrients. – 2023. – **V. 15**(2). – P. 269-275. doi: 10.3390/nu15020269.
6. Huang C, Hao W, Wang X, Zhou R, Lin Q. Probiotics for the treatment of ulcerative colitis: a review of experimental research from 2018 to 2022. Front Microbiol. –2023 Jul 6;**14**:1211271. doi: 10.3389/fmicb.2023.1211271.
7. Bloemendaal M. Probiotics-induced changes in gut microbial composition and its effects on cognitive performance after stress: exploratory analyses / M. Bloemendaal, J. Szopinska-Tokov, C. Belzer, D. Boverhoff, S. Papalini, F. Michels, S. van Hemert, A. Arias Vasquez, E. Aarts // Transl Psychiatry. –2021. – **V.11**(1). – P. 300-305.doi: 10.1038/s41398-021-01404-9.
8. Du.W. Modulating gastrointestinal microbiota to alleviate diarrhea in calves / W. Du, X. Wang, M. Hu, J. Hou, Y. Du, W. Si, L. Yang, L. Xu, Q. Xu // Front Microbiol. – 2023. – **V. 14.** – P. 118-122. doi: 10.3389/fmicb.2023.1181545.
9. Chuang, S.T. Development of Next-Generation Probiotics by Investigating the Interrelationships between Gastrointestinal Microbiota and Diarrhea in Preruminant Holstein Calves / S.T. Chuang, C.T. Chen, J.C. Hsieh, K.Y. Li, S.T. Ho, M.J. Chen // Animals (Basel). – 2022. – **V. 12**(6). – P. 695-703. doi: 10.3390/ani12060695.
10. Khaziakhmetov, F. The influence of "Stimix Zoostim" and "Normosil" probiotics on fecal microflora, hematologic indicators, nutrient digestibility, and growth of mother-bonded calves / F. Khaziakhmetov, A. Khabirov, K. Tagirov, R. Avzalov, G. Tsapalova, A. Basharov // Vet World. – 2020. –**V. 13**(6) – P. 1091-1097. doi: 10.14202/vetworld.2020.1091-1097. Epub 2020 Jun 15.
11. Li,Y. Effects of two strains of Lactobacillus isolated from the feces of calves after fecal microbiota transplantation on growth performance, immune capacity, and intestinal barrier function of weaned calves / Y. Li, X. Li, C. Nie, Y. Wu, R. Luo, C. Chen, J.

Niu, W. Zhang // Front Microbiol. – 2023. – **V. 31.** – P. 249-254. doi: 10.3389/fmicb.2023.1249628.

12. Wu, Y. Effects of Multispecies Probiotic on Intestinal Microbiota and Mucosal Barrier Function of Neonatal Calves Infected With E. coli K99 / Y. Wu, C. Nie, R. Luo, F. Qi, X. Bai, H. Chen, J. Niu, C. Chen, W. Zhang // Front Microbiol. – 2022. – **V. 26.** – P. 813-818. doi: 10.3389/fmicb.2021.813245.
13. Dar, A.H. The effects of probiotic Lactobacillus acidophilus and/or prebiotic mannan oligosaccharides on growth performance, nutrient utilization, blood metabolites, faecal bacteria, and economics of crossbred calves. / A.H. Dar, S.K. Singh, J.U. Rahman, S.F. Ahmad // Iran J Vet Res. – 2022. – **V. 23**(4). – P. 322-330. doi: 10.22099/IJVR.2022.42992.6259.
14. Kober AKMH, Riaz Rajoka MS, Mehwish HM, Villena J, Kitazawa H. Immunomodulation Potential of Probiotics: A Novel Strategy for Improving Livestock Health, Immunity, and Productivity / AK.H. Kober, M.S. Riaz Rajoka, H.M. Mehwish, J. Villena, H. Kitazawa // Microorganisms. – 2022. – **V. 10(**2). – P. 388-391. doi: 10.3390/microorganisms10020388.
15. Wang, Y. Effects of Milk Replacer-Based Lactobacillus on Growth and Gut Development of Yaks' Calves: a Gut Microbiome and Metabolic Study / Y. Wang, M. An, Z. Zhang, W. Zhang, M.F. Kulyar, M. Iqbal, Y. He, F. Li, T. An, H. Li, X. Luo, S. Yang, J. Li // Microbiol Spectr. –2022. – **V. 10**(4). – P. 115-122. doi: 10.1128/spectrum.01155-22.
16. Sharma, A.N. Effect of synbiotics on growth performance, gut health, and immunity status in pre-ruminant buffalo calves. / A.N. Sharma, P. Chaudhary, S. Kumar, C.R. Grover, G. Mondal // Sci Rep. –2023. – **V. 13**(1). – P. 101-105. doi: 10.1038/s41598-023-37002-6.
17. Mounir, M. Synbiotics and Their Antioxidant Properties, Mechanisms, and Benefits on Human and Animal Health: A Narrative Review. / M. Mounir, A. Ibijbijen, K. Farih, H.N. Rabetafika, H.L. Razafindralambo // Biomolecules. – 2022. – **V. 12**(10). – P. 1443-1449. doi: 10.3390/biom12101443.