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Research Article

Increasing Immunity in Young Cattle Through the Use of the Probiotic Preparation Lactobact-ARZ

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Abstract: According to the Kazakhstan national animal health monitoring system, the mortality rate of young farm animals ranges from 4.2% to 7.8%, with gastrointestinal diseases being the main registered condition. This study aimed to create a locally produced probiotic, formulated using a consortium of lactic acid bacteria, to enhance the immune function of young farm animals. The study utilized a newly developed probiotic designed to prevent and treat gastrointestinal diseases in young cattle. Modern molecular biological, zootechnical, biochemical, hematological, and immunological methods and techniques were used in the research. The potential advantages of the developed probiotic preparation Lactobact-ARZ are demonstrated, with optimization of its dosage in the diet to improve growth and development, increase live weight, and enhance immunity in young cattle. Calves that received probiotic-enriched milk demonstrated improved growth, development, feed utilization efficiency, survival rate, and hematological profile.

Keywords: Immunity, Microorganisms, Productivity, Preparation, Calves, Strain

Introduction

In Kazakhstan, infectious diseases affecting the gastrointestinal tract of young livestock and poultry result in significant economic losses for the agricultural sector. Thus, in the Kazakhstan national animal health monitoring system, the mortality rate of young farm animals ranges from 4.2 to 7.8%, while gastrointestinal diseases are the main reported disease. The highest mortality rates (up to 11.25 cases per 1,000 head) were recorded in the North Kazakhstan and East Kazakhstan regions, where the combination of extreme climate conditions and the predominance of small-scale farming limits the implementation of preventive measures. These figures exceed those reported for the European Union (5–6%) (Ismail & Muhaffel, 2022), but remain below the average values observed in developing countries (20–30%) (Otte et al., 2024). Therefore, bacterial infections of young animals and birds remain the main urgent problem today. In this regard, the study of the biological properties of epizootic strains of microorganisms isolated from animals and currently circulating in epizootic foci of infections is extremely important (Shokhan et al., 2024).

Among gastrointestinal infections affecting young livestock and poultry, enterotoxigenic E. coli is a leading cause of dysbiosis, particularly in the early weeks of life. Bacterial enterotoxins increase water secretion into the intestines, resulting in diarrhea (Izhar et al., 2024). The disease progresses quickly, causing poor absorption of nutrients, weight loss, weakness, and severe dehydration, which can be fatal within a day. Death ensues within 24 hours. The use of antibiotics to treat these diseases dates back quite some time. Although antibiotics have long been used to treat these conditions, their application in animal husbandry remains controversial due to the risk of resistance and residues in animal products (Chavdarov et al., 2024). Recent studies indicate that antibiotics have limited impact on disease outcomes, with any benefits typically appearing only after several days. This highlights the need to develop effective alternatives to antibiotics.

In recent years, biologically active substances such as probiotics have been increasingly utilized to prevent and treat diseases in farm animals (Park et al., 2024). Research indicates that administering probiotics orally can effectively combat a range of intestinal pathogens in livestock. Probiotics contribute to maintaining a healthy balance of gut microflora, safeguarding animals from digestive disorders, enhancing feed utilization and weight gain, and strengthening the immune response (El-Desokey et al., 2019). Additionally, supplementing milk

with specific probiotic strains has been shown to promote better growth and overall health in calves. The analysis shows that probiotics with a single strain of lactic acid bacteria are mainly used. There is practically no information on the combined effects of several strains on the oxidative and immune stress of a calf before weaning from a cow (Grigore et al., 2020).

Research in this area, using modern equipment, will enable the development of a scientifically sound veterinary intervention scheme. The dysbiosis of newborn calves is registered throughout Kazakhstan, and gastrointestinal infections are widespread in all regions of the country, causing enormous economic damage to farms and large industrial enterprises, as well as the private sector. It should be especially noted that large losses from gastrointestinal infections of young animals are also suffered by breeding farms, on the effective operation of which the dynamic development of the industry as a whole depends (Krasochko et al., 2018).

Various environmental stressors can negatively impact the health and physiological state of animals, especially newborn and recently weaned calves. Factors such as inadequate nutrition and poor living conditions contribute to physiological stress, increasing the risk of pathogenic bacterial infections and disrupting the balance of gut microbiota. Such intestinal dysbiosis is associated with a higher incidence of diseases, inflammatory responses, and impaired growth in young animals (Arbuzova, 2010).

Treatment of animals with antibiotics has been used for a long time. Extensive antibiotic use in both medicine and animal agriculture has led to a growing body of research on their associated side effects. Secondary products of antibiotic metabolism harm human health through the consumption of milk and meat. This effect manifests itself in the form of various hormonal disorders and allergies, due to the suppression of beneficial bacteria in the small intestine and an increase in the number of pathogenic microflora. Another significant concern is that administering antibiotics in subtherapeutic doses through animal feed promotes the emergence of antibiotic-resistant strains of harmful bacteria, including *E. coli*, *Salmonella spp.*, and *Campylobacter spp.* (Shkuratova et al., 2015).

For this reason, incorporating modern biologically active substances into the diets of young farm animals is essential, as they offer beneficial effects without causing harm.

Growing concerns about the side effects of antibiotics and the move to eliminate their use as growth promoters in young animals have prompted both producers and consumers to seek alternative solutions.

There is evidence in the literature that probiotics have begun to fill this gap, and many livestock farms in the country have begun to give them preference, using them for the prevention and treatment of young animals, in particular calves. For example, the authors of this project have been testing such domestically produced probiotics as Lactobacterin TK2 (Boranbayeva et al., 2020), Acidophilin B-143 (Tulemisova et al., 2011) and Torulakt (Alpeisov, 2022) in livestock and poultry farms in the Almaty region for several years. Research has demonstrated that the probiotics mentioned above enhance both the survival rates of young livestock and the productivity of farm animals and poultry. (Konishheva et al., 2022; Luk'janova et al., 2012; Maksimovich et al., 2019; Pirozhkov et al., 2011).

Gastrointestinal tract diseases in calves are complex and multifactorial, involving both systemic and local factors that influence the health status and disease outcome (Myktybayeva et al., 2025). Therefore, incorporating probiotics into comprehensive treatment strategies is highly recommended. Understanding the mechanism of their action and predicting the expected preventive effect makes it possible to use them in various variants with other antimicrobial preparations, special feed mixtures, or compounds that suppress the symptoms of diarrhea (Belkin et al., 2019; Hamal et al., 2006; Kim et al., 2000; Rahman et al., 2013; Xu et al., 2011).

Of great interest is the use of combined probiotics enriched with several highly productive strains of lactic acid bacteria obtained by sequencing deoxyribonucleic acid (DNA), which will make it possible to more effectively combat gastrointestinal diseases of young and adult animals (Zábranský et al., 2013). Additionally, data collected on the morphological, staining, cultural, and biochemical characteristics of lactic acid bacteria can aid in distinguishing and identifying new strains (Renaud et al., 2019). Some strains characterized by intensive accumulation of biologically active substances in the environment may be recommended for the production of harmless probiotic biologics (Stefańska et al., 2020).

Thus, the scientific justification of the use of biologically active supplements of a new generation, the study of physiological and biochemical metabolic processes, immune status, growth, development, and productivity of farm animals arouse great scientific interest among scientists in the field of veterinary medicine in many countries and are promising.

The objective of this research was to create a locally produced, eco-friendly probiotic derived from a consortium of lactic acid bacteria, designed to enhance immunity and support the wellbeing of young livestock.

Materials and Methods

Materials

The material for this study was the developed probiotic preparation Lactobact-ARZ based on an association of nine novel strains of lactic acid bacteria: *Lacticaseibacillus casei* strain 7K-2L (collection number

B-RKM 1123), *Lactobacillus casei* strain 7K-6L1 (B-RKM 1124), *Lactobacillus casei* strain 5K-9L1 (B-RKM 1125), *Lactobacillus casei* strain 7K-16L (B-RKM 1126), *Lactobacillus paracasei* strain 17K-16L12 (B-RKM 1127), *Lactobacillus paracasei* strain RN-01 (B-RKM 1198), *Lactobacillus helveticus* strain TRk-03 (B-RKM 1199), *Lactobacillus acidophilus* strain TRk-09 (B-RKM 1200), and *Lactobacillus casei* strain Sh015 (B-RKM 1008). This preparation is intended to enhance the immune system in young farm animals. Five of these strains have been included in the GenBank genetic sequence database of the National Institutes of Health (USA).

1. Lacticaseibacillus paracasei strain 17K-6L12 16S ribosomal RNA gene, partial sequence GenBank: OR722737.1 (Myktybayeva et al., 2023e)
2. Lacticaseibacillus casei strain 7K-16L1 16S ribosomal RNA gene, partial sequence GenBank: OR711028.1 (Myktybayeva et al., 2023d)
3. Lacticaseibacillus casei strain 5K-9L1 16S ribosomal RNA gene, partial sequence GenBank: OR711014.1 (Myktybayeva et al., 2023a)
4. Lacticaseibacillus casei strain 7K-6L1 16S ribosomal RNA gene, partial sequence GenBank: OR711013.1 (Myktybayeva et al., 2023c)
5. Lacticaseibacillus casei strain 7K-2L 16S ribosomal RNA gene, partial sequence GenBank: OR673093.1 (Myktybayeva et al., 2023b)

Experimental and control groups of calves were created by random sampling. Randomization was performed using a random number generator using Microsoft Excel. Each calf had an identification number, which were sorted randomly.

Research Location

The research utilized a newly formulated probiotic designed to prevent and treat gastrointestinal diseases in young cattle. In total, 80 heads of experimental calves of the Holstein breed were selected, and 8 experimental and control groups of 10 heads each were formed. The research was carried out in 3 regions of Kazakhstan, Almaty, Zhambyl, and Qyzylorda, as shown in Figure 1.

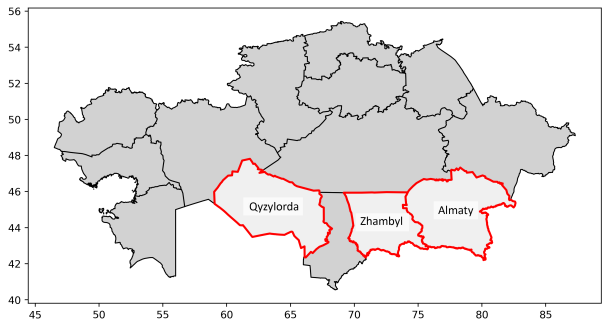

Fig. 1: Areas of research conducted

Statistical Analysis and Equipment

Modern molecular biological, zootechnical, biochemical, hematological and immunological methods and techniques were used in the research.

The analytical part of the research included the search for information and statistical materials, and literary scientific data published in domestic and foreign publications.

T Statistical analysis of the results was performed using ANOVA in the Minitab17 software. Biometric differences between the groups were revealed by the Tukey test $P<0.05$. The studied samples, with an average value and a probability of deviation from the standard, were conducted in parallel using Microsoft Excel.

Analytical studies were conducted in the T-Helper Laboratory of Omicron 3D LLP and the Antiviral Protection Laboratory of the Microbiology and Virology Research and Production Centre.

Test cultures were used to study the antagonistic activity of selected lactic acid bacteria such as *Proteus vulgaris, Bacillus subtilis, Escherichia coli, and Micrococ. luteus, Staph.Albus, Staph.aureus, Salmonella typhimirium Sal.dublin, Sal.cholerae-suis*.

Traditional Kazakh fermented milk samples of koumiss from different regions of Kazakhstan were used to isolate lactic acid bacteria. The morphological and physiological properties of new strains of lactic acid bacteria have been studied.

A mathematical assay was employed to assess cell surface hydrophobicity by measuring adhesion to hydrocarbons (Otero et al., 2004).

The 16 sRNA gene fragment was sequenced on the Applied Biosystems 3500 Genetic Analyser using the Sanger method using the BigDye Terminator v3.1 kit according to the manufacturer's recommendations.

When studying the zootechnical parameters of the calves, weekly live weight weighing and body measurements were carried out before weaning them from the cow.

To identify and confirm the effectiveness of the immune system and oxidative stress, blood samples were taken from calves. An immunological study determined the effect of a probiotic preparation on cellular and humoral immunity factors. The leukocyte formula and the main indicator of the natural resistance, that is, the phagocytic activity of leukocytes, have been determined from cellular immunity factors. The studies were performed on the FT-2 immunoanalyzer. TAS, TOS, PON-1, TTL, NTL, TDH, CAT, SOD, and MDA analyses were performed using the Mindray BS300 automatic biochemical blood analyser with the Relassy kit. IgE, IgA, and IgG assays were determined using an electrospectrometer with a wavelength of 600 nm.

Analytical studies were conducted according to methodological recommendations (Fan et al., 2021; Frizzo et al., 2011).

Potential Limitations of Translating Mouse-Based Findings to Calves

The results of murine models regarding safety and efficacy require cautious interpretation when extrapolated to cattle; field trials in calves (such as those conducted in this study) are necessary for confirmation and evaluation under real-world conditions. This combination of experiments helps to bridge the gap between preclinical and clinical data, providing a comprehensive assessment of the probiotic.

Results and Discussion

The results of assessing the antagonistic activity at various concentrations-10^7, 10^8, and 10^9 CFU/cm³-of the associated probiotic preparation Lactobact-ARZ under in vivo conditions in laboratory animals (white mice), administered orally after infection with a virulent culture of Salmonella typhimurium strain 371 at a dose of 10^9 CFU/cm³, are presented in Tables 1, 2 and 3.

For the experiment, three trials were conducted, each divided into four groups: three experimental groups (Groups 1, 2, and 3) and one control group (Group 4). Each group consisted of 15 mice. In total, 180 white mice weighing 14-16 g were used in the experiment.

In all experiments, the infected white mice were administered the probiotic preparation Lactobact-ARZ orally at three different concentrations, with increasing dosage: Experimental Group 1 received ad libitum feed plus the probiotic at a concentration of 10^7 CFU/cm³; Experimental Group 2 received ad libitum feed plus the probiotic at 10^8 CFU/cm³; Experimental Group 3 received ad libitum feed plus the probiotic at 10^9 CFU/cm³. The fourth, control group received ad libitum feed and was administered only physiological saline without the probiotic.

In the first experiment, the infected white mice were administered the probiotic preparation orally from the first day for ten consecutive days (Table 1).

Table 1: Antagonistic Activity of the Probiotic Preparation Lactobact-ARZ from the Day One of Oral Administration to Infected White Mice

Groups	Administration Method and Dose (CFU/cm³)	Results of Infection with Virulent S. typhimurium 371			
		Diseased	Left alive	Fallen, heads	Survived (%)
Group 1 – Experimental	10^7 orally	-	15	-	100
Group 2 – Experimental	10^8 orally	-	15	-	100
Group 3 – Experimental	10^9 orally	-	15	-	100
Group 4 – Control	Physiological saline, orally	15	-	15	-

Table 2: Antagonistic Activity of the Probiotic Preparation Lactobact-ARZ from the Day Two of Oral Administration to Infected White Mice

Groups	Administration Method and Dose (CFU/cm³)	Results of Infection with Virulent S. typhimurium 371			
		Diseased	Left alive	Fallen, heads	Survived (%)
Group 1 – Experimental	10^7 orally	2	14	1	93,3
Group 2 – Experimental	10^8 orally	1	15	-	100
Group 3 – Experimental	10^9 orally	-	15	-	100
Group 4 – Control	Physiological saline, orally	15	-	15	-

Table 3: Antagonistic Activity of the Probiotic Preparation Lactobact-ARZ from the Day Three of Oral Administration to Infected White Mice

Groups	Administration Method and Dose (CFU/cm³)	Results of Infection with Virulent S. typhimurium 371			
		Diseased	Left alive	Fallen, heads	Survived (%)
Group 1 – Experimental	10^7 orally	2	13	2	86.6
Group 2 – Experimental	10^8 orally	1	14	1	93.3
Group 3 – Experimental	10^9 orally	-	15	-	100
Group 4 – Control	Physiological saline, orally	15	-	15	-

According to the data presented in Table 1, in the three experimental groups (10^7, 10^8, 10^9 CFU/cm³), where associations of probiotic strains Lactobact-ARZ were administered orally from the first days of the experiment, no cases of disease were observed among the 45 mice. The animals remained active, their fur was shiny and smooth, and they responded quickly to external stimuli. In contrast, all white mice in the control group developed disease and subsequently died following infection.

In the second experiment, the infected white mice were administered the probiotic preparation orally for nine consecutive days, starting from the second day of the experiment (Table 2).

According to the data presented in Table 2, three mice developed illness. However, the disease manifested in a mild form, and within the following two days. Two mice recovered while one died. In the first experimental group, which received a concentration of 10^7 CFU/cm³, one of the two diseased mice died. In the second

experimental group, which received the probiotic preparation at a concentration of 10^8 CFU/cm³ administered orally, the one diseased mouse recovered. In the third experimental group, which received the probiotic preparation at a concentration of 10^9 CFU/cm³, a 100% survival rate was observed, with no cases of illness or mortality. In the fourth (control) group, all animals developed illness and died.

In the third experiment, the infected white mice were administered the probiotic preparation orally for eight consecutive days, starting from the third day of the experiment (Table 3).

According to the data presented in Table 3, three mice developed illness, and in all cases, the disease progressed severely, resulting in the death of all three animals within the following two days. In the first experimental group, which received a concentration of 10^7 CFU/cm³, recovery was not observed among the two diseased mice, as all affected animals died. In the second experimental group, which received the probiotic preparation at a concentration of 10^8 CFU/cm^3 administered orally, the single diseased mouse also died. In the third experimental group, which received the probiotic preparation at a concentration of 10^9 CFU/cm^3, a 100% survival rate was observed, with no cases of illness or mortality. In the fourth (control) group, all animals developed illness and died. In all control groups, all white mice developed illness and died. The affected animals exhibited signs of depression and toxicosis (profuse salivation, unsteady gait), and upon necropsy, necrotic foci were detected in the liver.

Thus, it was established that under in vivo conditions, the antagonistic activity of the developed probiotic preparation Lactobact-ARZ comprising an association of strains, was high, as infected mice achieved a 100% therapeutic outcome (first experiment). On the second and third days after infection, when clinical signs of disease appeared, the probiotic also demonstrated a high therapeutic effect, indicating its antagonistic properties against Salmonella. According to Table 3, in the third experimental group, all 15 diseased mice had recovered by the end of the experiment, and at a probiotic concentration of 10^9 CFU/cm³, the therapeutic outcome was 100%. With oral administration of the probiotic at a concentration of 10^8 CFU/cm³, the therapeutic effect was 93.3% survival, and at a concentration of 10^7 CFU/cm³, the therapeutic effect was 86.6% survival (third experiment).

Based on the results of laboratory studies in white mice, we draw a preliminary conclusion that a newly developed, therapeutically highly effective associated probiotic preparation has been created, which includes lactic acid bacteria strains *Lacticaseibacillus casei* strain 7K-2L (B-RKM 1123), *Lactobacillus casei* strain 7K-6L1 (B-RKM 1124), *Lactobacillus casei* strain 5K-9L1 (B-RKM 1125), *Lactobacillus casei* strain 7K-16L (B-RKM 1126), *Lactobacillus paracasei* strain 17K-16L12 (B-RKM 1127), *Lactobacillus paracasei* strain RN-01 (B-RKM 1198), *Lactobacillus helveticus* strain TRk-03 (B-RKM 1199), *Lactobacillus acidophilus* strain TRk-09 (B-RKM 1200), and *Lactobacillus casei* strain Sh015 (B-RKM 1008).

The results obtained are consistent with the research of a number of scientists who believe that probiotics in animals and humans produce broad-spectrum antibiotic substances, as well as proteolytic enzymes (Rabetafika et al., 2023).

In the second phase of the study, four farms were chosen to evaluate calf growth and development before and after probiotic administration. These included the Dostyk Farm in Merken District of the Zhambyl Region, Zaman Ata Farm and Zher-Kazyna LLP in the Zhanakorgan District of the Kyzylorda Region, and Baiserke-Agro LLP in Talgar District of the Almaty Region.

In the experimental groups of each farm, calves received probiotics for the first 12 days for preventive purposes at a dose of 3 g per head 2 times a day (morning and evening). Milk with probiotics was given from a diameter of the special dish with a nipple hole of no more than 2 mm. The control group was fed a standard diet consisting of milk without any probiotic supplementation. In the evening before weighing, the feed and water supply was stopped and the calves were weighed on an empty stomach.

Using the example of the Dostyk Farm in Zhambyl Region, the following can be noted. On this farm, healthy newborn calves of the Holstein breed were selected by random sampling, which were divided into 2 groups of 10 heads each.

The results obtained on live weight and live weight gain in the experimental and control groups of calves in the Dostyk Farm in the Zhambyl Region are shown in Table 4.

At the conclusion of the experiment, calves in the experimental group showed significantly higher live weight values compared to the control group ($P < 0.95$). The experimental group also demonstrated improved calf survival, with two more animals remaining healthy compared to the control group. Administering the probiotic from the first to the twelfth day of life resulted in a greater increase in live weight among treated calves. While the control group had an average daily weight gain of 11.92 kg, the experimental group achieved a gain of 13.34 kg, representing an 11.9% improvement. These data indicate that there is a direct correlation between the daily intake of probiotic calves and the average daily weight gain.

The same relationship was noted between the dose of probiotics and the consumption of calf feed.

Table 4: Comparative effects of probiotic Lactobact-ARZ on weight gain in experimental and control calf groups

Inventory Number	Live Weight (kg)		Live Weight Gain (kg)	
	Start Weight	End Weight	During Experimental Period	Daily Average
Experimental Group				
46540708	39.20	57.80	18.60	1.55
46540709	38.10	56.55	18.45	1.54
46540710	37.90	56.20	18.30	0.69
46540712	38.20	57.10	18.90	1.58
46540708	38.90	58.25	19.35	1.61
46540711	38.40	56.10	17.70	1.48
46540713	39.60	57.45	17.85	1.49
46540479	38.80	55.90	17.10	1.43
46540471	38.50	56.95	18.45	1.54
46540472	39.40	56.50	17.10	1.43
Total	387	568.8	181.7	13.34
Control Group				
46540470	37.15	51.25	14.10	1.18
46540473	38.21	53.36	15.15	1.26
46540478	37.70	52.10	14.40	1.20
46540480	38.40	53.40	15.00	1.25
46540474	37.93	52.63	14.70	1.23
46540477	38.00	51.20	13.20	1.10
46540475	37.11	51.21	14.10	1.18
46540476	37.64	51.44	13.80	1.15
46540481	37.11	52.41	15.30	1.28
46540482	38.55	51.60	13.05	1.09
Total	337.8	520.6	142.8	11.92

According to the results of the experiment, the experimental group had a higher feed intake, which was in the range of 479 g, and in the control group, this indicator was in the range of 460 g. This suggests that the probiotic dose used improved the calves' feed intake, which led to a higher body weight and, consequently, an increase in body weight.

Final measurements revealed that the combined weight of calves in the experimental group reached 568.8 kg, exceeding the control group by 48.2 kg.

Body measurements were taken to identify the dynamics of growth and development in calves. In summary, calf body length varied between groups, measuring 10.5 cm in the first control group and reaching 15.5 cm in the second experimental group.

Calves in the second experimental group had a body height of 12 cm, compared to 6.5 cm in the control group, indicating that probiotic supplementation positively influenced body depth. A similar trend was observed for withers height, which measured 12 cm in the experimental group versus 7.5 cm in the control group.

Chest circumference measured 10.5 cm in the first control group, while the second experimental group showed a larger measurement of 16.5 cm.

Summarizing the above, it can be stated that the probiotic use of the developed for calves contributed to faster weight gain compared to the control group, where standard feeding was used. It should also be noted that the developed probiotic on the growth and development of calves, as evidenced by body measurements taken. It is also important to note that providing the optimal probiotic dose improves feed conversion efficiency.

Blood samples were collected from the jugular veins of all experimental calves using gel vacuum tubes to assess immune status and other physiological parameters at 4 days old, 28 days old, and at weaning. (Wu et al., 2021). A hematological blood test was performed in the T-Helper laboratory of Omicron 3D Limited Liability Company.

Table 5 shows the average laboratory results for blood hematology obtained from experimental calves raised in the Dostyk Farm in the Zhambyl region.

Table 5 shows that there were no significant differences between the experimental and control groups in clinical indicators or in measures of the humoral immune response.

Overall, immunoglobulin levels showed only minor fluctuations, likely due to the young age of the animals and the brief observation period. However, an upward trend in hemoglobin levels was observed, especially in the experimental groups, suggesting an improvement in the blood's total oxidative capacity. The probiotic strains *Lactobacillus casei* and *Lactobacillus acidophilus*, present in the Lactobact-ARZ formulation, may also promote the production of short-chain fatty acids

(SCFAs) such as butyrate. These metabolites decrease the pH of the intestinal environment, improving the solubility and absorption of iron (Alkhalf et al., 2010; Mazziotta et al., 2023). At the same time, probiotics compete with pathogenic microflora for resources, thereby reducing the colonization of iron-consuming bacteria (for example, E. coli) (Wieërs et al., 2020). In the experimental group, leukocyte levels tended to normalize, indicating a lower susceptibility to neonatal intestinal infections and a faster adaptation to farm conditions compared to the control group. Additionally, the experimental group showed a modest rise in total protein levels, which contributed positively to the calves' overall health. The moderate rise in serum total protein observed in the experimental group indicates improvements in protein metabolism. According to international studies, probiotics stimulate amino acid absorption by enhancing the integrity of the intestinal barrier and modulating TLR receptors (Bajagai et al., 2016; Wang et al., 2023). The rise in protein levels may be associated with the synthesis of immunoglobulins (IgA, IgG), as supported by studies in which probiotics increased humoral immunity (Özkaya et al., 2023; Uyeno et al., 2015). Moreover, a reduction in markers of oxidative stress (MDA) creates conditions conducive to stable albumin synthesis, which serves an antioxidant function (Wang et al., 2023). Although the observed change did not reach statistical significance, its biological relevance is supported by similar findings in studies by Smirnova et al. (2023) (Smirnova et al., 2023). There is a noticeable decrease in the indicators of alanine aminotransferase and aspartate aminotransferase, which may characterize improvements in the general condition of the calves in the experimental group compared to the control group during the experiment. The level of alkaline phosphatase remained relatively stable throughout the observation period in both the experimental and control groups, which is consistent with the physiological role of this enzyme in bone tissue formation during the growth of calves. During the study, the level of alkaline phosphatase (ALP) in calves remained unchanged across all groups, indicating stability in bone formation and mineralization processes (Cheng & Zhao, 2023; Golub & Boesze-Battaglia, 2007; Vimalraj, 2020). Alkaline phosphatase is a key enzyme responsible for the hydrolysis of phosphate esters and the maintenance of the required concentration of inorganic phosphate for bone tissue formation. The absence of changes in ALP levels suggests that the probiotic did not have a direct effect on bone metabolism and that the liver and bone functions in the animals remained within physiological norms throughout the experiment. This also confirms the absence of negative effects of probiotic administration on calf health. For a more detailed investigation of the influence of probiotics on mineral metabolism and bone growth, it is recommended to conduct long-term studies with additional biochemical and morphometric indicators.

Table 5: Results of hematology of blood of calves raised in the Dostyk Farm of Zhambyl Region

Name	Experimental Group			Control Group		
	1st Sampling (17.04.2024)	2nd Sampling (23.04.2024)	3rd Sampling (27.04.2024)	1st Sampling (17.04.2024)	2nd Sampling (23.04.2024)	3rd Sampling (27.04.2024)
Hemoglobin	97±12.79	101,11±16.23	106±13.09	101±10.52	96,87±13.54	101±10.54
Red Blood Cells	8,16±1.84	9,02±1.56	9,44±1.35	9,05±1.65	9,12±1.98	8,66±1.98
White Blood Cells	8,80±2.65	7,46±2.65	9,29±4.58	8,97±5.21	9,25±3.25	10,23±3.54
Hematocrit	26,89±4.61	28,58±3.87	29,39	27,81±3.95	28,62±4.25	28,21±4.85
Platelets	374,01±250.05	319±132	316,22±87.9	384,12±230.12	349±145.25	345,23±122.54
Alanine Aminotransferase	38,67±5.36	41,86±6.25	43,25±7.20	36,87±6.35	44,56±4.52	44,52±4.32
Aspartate Aminotransferase	32,11±4.89	39,88±8.25	39,62±6.58	34,21±6.02	40,21±4.32	38,21±4.01
Alkaline phosphatase	197.85±23.85	201,54±23.01	203,6±22.85	201,56±20.31	210,56±23.1	204,51±20.36
GRA%	36,11±16.12	38,77±16.54	39,78±12.54	37,25±15.62	39,22±12.25	40,23±13.54
EO%	0	0	0	0	0	0
MID%	7,22±2.65	7±2.35	7,11±1.95	7,96±2.54	7,86±1.36	8,02±2.01
LIM%	56,6±19.54.	54,22±15.87	53,11±16.25	54,79±10.54	52,92±11.02	48,25±10.54
ESR	1	1	1	1	1	1
Glu	4,25±0.87	4,62±0.82	4,25±0.92	4,36±0.087	4,32±0.95	4,15±0.87
UREA	3.74±1.56	4,21±1.55	4,15±1.36	3.98±0.99	4,62±1.54	4,25±1.54
CREAT	68.24±10.54	69,85±8.24	70,21±8.21	69.24±7.85	70,23±6.89	69,68±7.25
Total Pro	63.51±3.48	68,52±3.65	65,21±4.58	64.51±3.25	64,35±3.25	63,58±3.48
Bil	7,22±1.82	7,85±1.66	7,05±1.25	7,36±1.52	7,36±1.32	8,01±2.15
IgG	10,04±1.58	10,55±1.36	10,74±1.28	10,12±1.34	10,20±1.24	10,74±1.81
IgA	1,36±0,21	1,46±0.27	1,33±0.412	1,39±0,54	1,44±0.39	1,45±0,44
IgM	0,93±0.015	0,91±0.070	0,95±0.062	0,91±1.01	0,86±0.017	0,95±0.054
IgE	48,73±10.54	46,27±9.54	51,41±12.54	36,98±13.25	43,26±16.25	51,41±10.54

The results obtained indicate that, in general, the hematological blood parameters of the calves in the second experimental group were higher, and therefore their immune function and blood oxygenation were advantageous compared with the control group. In general, the experimental group showed better growth rates, more efficient feed use, and better blood hematology.

In contrast to the work by Boranbayeva et al. (2020), which examined the effects of individual probiotic preparations, the present study is the first to develop and test a domestically produced probiotic based on an association of nine novel lactic acid bacteria strains isolated from traditional Kazakh fermented dairy products (Boranbayeva et al., 2020). Additionally, the study was carried out across three distinct regions of Kazakhstan, which enabled the assessment of local microbiota features and the prevailing epizootic conditions. In contrast to Renaud et al. (2019), who used a multi-strain probiotic as an adjunct treatment for diarrhea, our research focused on prevention and included an in-depth evaluation of immune function and oxidative stress in calves (Renaud et al., 2019).

Overall, the experimental group exhibited more significant improvements in clinical, biochemical, and immunological parameters compared to the control group. Similar positive trends were also observed in calves from other farms in the Almaty and Kyzylorda regions where the research was conducted.

Conclusion

Summarising the results of the conducted research, it should be noted that, in general, our research is consistent with the work of several scientists who note that probiotics in the body of farm animals produce antibiotic substances with a wide spectrum of action, as well as proteolytic enzymes.

Thus, after conducting comparative studies on the antagonistic activity against salmonella of the virulent culture of Salmonella typhimurium (strain 371) in vivo on white laboratory mice, we made sure that the developed probiotic preparation in the form of an association of *Lactobacillus* strains has a high therapeutic efficacy.

Based on the results of the studies, a new therapeutically highly effective associated probiotic preparation, Lactobact-ARZ was developed. This preparation includes lactic acid bacteria of the following strains: *Lacticaseibacillus casei* strain 7K-2L (V-RKM 1123), *Lactobacillus casei* strain 7K-6L1 (V-RKM 1124), *Lactobacillus casei* strain 5K-9L1 (V-RKM 1125), *Lactobacillus casei* strain 7K-16L (V-RKM 1126), *Lactobacillus paracasei* strain 17K-16L12 (V-RKM 1127), *Lactobacillus paracasei* strain RN-01 (B-RKM 1198), *Lactobacillus helveticus* strain TRk-03 (B-RKM 1199), *Lactobacillus acidophilus* strain TRk-09 (B-RKM 1200), and *Lactobacillus casei* strain Sh015 (B-RKM 1008).

Additional trials with the probiotic in calves across the Zhambyl, Kyzylorda, and Almaty regions of Kazakhstan confirmed its strong therapeutic and preventive effects. The results indicate that calves receiving the probiotic showed improved hematological parameters, enhanced immune function, and better blood oxygenation compared to the control group. These animals also demonstrated superior growth, more efficient feed utilization, higher survival rates, and a more robust hematological profile. Overall, the findings highlight the advantages of incorporating the optimized dosage of our Lactobact-ARZ probiotic into young cattle diets to support weight gain, development, and immune health.

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Author's Contributions

Myktybayeva Raya: Conceptualized and designed the overall study; led manuscript drafting, editing, and final approval.

Alpeisov Shokhan: Designed experimental protocols, analyzed data, and contributed to results interpretation and methodology.

Kozhakhmetova Zubaira: Planned and performed statistical analyses, interpreted quantitative data, contributed to methodological robustness.

Kyrgyzbay Nazym: Literature review, reviewed and revised the manuscript to improve structure, clarity, and scientific accuracy.

Conflict of Interest

The authors have no conflict of interests related to this manuscript.

Ethics

This study was conducted in accordance with the principles of bioethical expertise and scientific integrity. All experiments involving animals adhered to established ethical standards and were performed with minimal stress. The research methodology was approved by the Bioethics Commission of the Kazakh National Agrarian Research University on November 11, 2022, prior to the commencement of the research project. All authors confirm that the work does not contain plagiarism, duplicate publications, and scientific fraud. Each procedure was conducted in compliance with the ARRIVE guidelines for in vivo studies.

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