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Research Article

Determination of the Probiotic Potential and Metabolic Function of Lactobacillus Strains for Healthy Calf Rearing

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Abstract: This study examines the effects of probiotic supplementation on the growth, health, and physiological parameters of Holstein calves. Thirty calves were randomly assigned to three groups: a control group and two groups receiving probiotics at doses of 1 g/10 kg and 1.5gt/10 kg live weight. Key parameters such as Live Weight (LW), Body Measurements (BMs), Feed Intake (FI), Feed Conversion Ratio (FCR), haematological and biochemical blood markers, and oxidative stress indicators were evaluated. The results revealed that probiotic supplementation significantly improved growth performance, reduced the number of illness days and diarrhoea cases, and promoted rumen development. However, the observed improvements in FCR and BMs were not statistically significant. Hematological and biochemical parameters remained within normal physiological ranges across all groups, confirming the safety of the supplementation. Overall, this study highlights the potential of probiotics as a valuable dietary strategy to enhance the health and growth of Holstein calves without adverse effects.

Keywords: Probiotic Supplementation, Growth, Health, Blood Parameters, Oxidative Stress, Immune Response, Calves

Introduction

Probiotic microorganisms, especially those from to the *Lactobacillus* genus, have been widely recognized for their essential contributions to food fermentation, health maintenance, and therapeutic innovations. Species such as *L. casei*, *L. acidophilus*, *L. helveticus*, *L.paracasei*, and *L. lactis* have been extensively explored due to their diverse metabolic activities and physiological functions. Beyond their tradional roles in improving the quality of fermented foods, these bacteria play a vital role in modulating the gut microbiota, strengthening the immune system, and generating health-promoting bioactive substances (Cai *et al.*, 2009; Vissers *et al.*, 2010).

The *Lactobacillus* group encompasses Gram-negative, facultative anaerobic bacteria that are naturally present in various environments, including fermented foods and the gastrointestinal tract Notably, strains such as *L. casei*, *L. paracasei*, and *L. helveticus* have demonstrated the ability to survive in challenging conditions like pH and high bile concentrations, making them highly effective probiotics (Huang *et al.*, 2018). Their primary metabolic output, lactic acid, significant contributes to gut health by suppressing the proliferation of pathogenic microbes and fostering a balanced intestinal environment (Hill *et al.*, 2018).

Within the *L. casei* cluster, strains such as *L. casei, L. paracasei,* and *L. rhamnosus*, have garnered considerable scientific interest. These probiotics are extensively employed in functional food industries and supplementation due to their capacity to produce antimicrobial peptides, aid lactose metabolism, and modulate host immune responses (Cai *et al.*, 2009; Huang *et al.*, 2018). Their metabolic flexibility also allows to synthesize key nutrients, including vitamins, organic acids, and bioactive peptides, thereby enhancing host well-being (Huang *et al.*, 2018).

Among the studied strains, *L. acidophilus* TRk 09 and *L. helveticus* TRk 03 have shown superior probiotic capabilities, notably by reinforcing gut barrier functions and regulating immune activities. Evidence suggests these strains promote intestinal integrity and secrete molecules with anti-inflammatory properties (Hill *et al.*, 2018; Huang *et al.*, 2018). Similarly, *L. paracasei* and *L. lactis* contribute significantly to food quality by enhancing fermentation efficiency and producing flavor-enhancing compounds (Cai *et al.*, 2009).

This study aims to assess the functional properties of *Lactobacillus casei* 7K-2L, *Lactobacillus acidophilus* TRk 09, *Lactobacillus helveticus* TRK 03, *Lactobacillus paracasei,* and *Lactobacillus lactis* strains in calf rearing practices. It specifically focus on their effects on growth performance, oxidative stress regulation, and immune system responses. By evaluating their metabolic characteristics and probiotic potential, this research intends to clarify their contributions to improving nutrients obsorption, gut health, and immunğty in calves, thereby supporting more efficient and sustainable calf rearing strategies.

Materials and Methods

Animals and Desing

The study was conducted at two locations: the Research and Application Farm of the Faculty of Agriculture, Isparta University of Applied Sciences, Türkiye (n = 15 calves), and the Yaşar Dairy Cattle Farm located in Sazak village, Yeşilova district, Burdur, Türkiye (n = 15). Both locations share a similar continental-Mediterranean transition climate. Thirty healthy newborn Holstein calves were randomly assigned to three groups based on LW. Group 1 (Control, G1): fed calf starter + whole milk (mean LW = 41.00±2.05 kg); Group 2 (G2): Calf starter + whole milk supplemented with 1g/10kg LW probiotic (mean LW=40.45±2.05 kg); Group 3 (G3): Calf starter+ whole milk supplemented with 1.5g/10kg LW probiotic (mean LW = 39.10±2.05 kg). The probiotic dosages were based on recommendations from Karslı and Aydoğdu (2020).

Feeding Regimen

All calves received colostrum during the first three days of life. Calf starter feed, in pelleted from (1.5 cm particle size) containing 18% crude protein and 2800 kcal/kg ME, was offered ad libitum. Milk feeding (4 liters/day) was administered twice daily (2L in the morning, 2L in the evening) until weaning. The probiotic blend contained approximately $5.5x10^9$ CFU/g, including strains *L. casei, L. acidophilus, L. helveticus, L. paracasei,* and *L. lactis* strains.

Housing Conditions

Calves were individually housed in natural ventilated measuring 110x218 cm. Hygiene and environmental parameters were consistently monitored throughout the trial.

Data Collection

The chemical composition of the calf starter and whole milk was analyzed (Table 1).

Table 1: Composition of whole milk and calf starter

	Milk	Calf starter
Dry matter, %	13.08	90.26
Crude protein, %	3.56	17.55
Ether extract (fat for milk), %	4.18	3.45
Ash, %	-	6.68
Lactose, %	5.34	-
Crude fibre, %	-	7.13
Metabolic energy, kcal/kg	-	2848

Kjeldahl method for crude protein (AOAC, 2005a), Soxhlet method for ether extract (AOAC, 2005b) were used. ANKOM 220 Fiber Analyzer (ANKOM Tech., Macedon, NY, USA) was used for crude fibre. Milk composition is determined using a Milk Test Analyzer (Has Vet, Türkiye).

Growth Measurements

Weekly LW and BMs (body length-BL (8), chest girth-CG (7), withers high-WH (1), hip high-HH (2), and body depth-BD (5)) were recorded (Figure 1). Calves were weighed using an electronic scale (JADVER, Jadver Electronic Scala System, Eyüp Sultan, İstanbul, Türkiye) after fasting overnight. BMs were measured using a stick and tape (Hauptner & Herberholz GmbH & Co. KG, Solingen, Germany) following Özkaya and Bozkurt (2008).

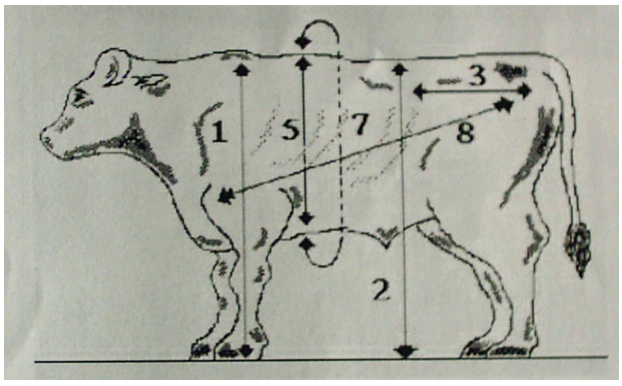

Fig. 1: Taking BMs of animals

Feed Intake and Feed Conversion

Starter FI was recoreded daily using a 1-gram precision scale (TESS, Coymak tartı Ltd. Türkiye). The FCR was calculated by dividing totasl FI by total LW gain by the following formula:

FCR = Total FI (kg)/Total LW gain (kg)

Fecal Consistency Scoring

Feces were evaluated daily using a 4-point scale: 1 = normal, 2 = soft, 3 = runny, 4 = watery (Larson *et al.*, 1977).

Blood Sampling and Analysis

Blood samples were collected via the *Jugular vein* at the beginning of the study, on 28-day-old and at the end of the trial. Blood samples collected in 5 ml gel separator tubes were kept at room temperature and centrifuged at 3000 rpm for 10 min. Serum samples were analyzed for biochemical markers (Total Cholesterol (TC), Glucose (GLU), Blood Urea Nitrogen (BUN), Total Protein (TP), Albumin (ALB), Alanin Transaminase (ALT), Gamma-Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Creatine (CREA), Calcium (Ca), Inorganic Phosphorus (IP), Lactate Dehydrogenase (LDH), Triglycerides (TG), and Total Bilirubin (TBil)) using a Mindray BS-120 biochemical blood analyzer (Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, China). Hematological parameters were assessed with Mindray BC-30 Vet automatic hematological blood analyzer (Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, China).

Oxidative stress indicators (Total Oxidant Status (TOS), Malondialdehyde (MDA)) and antioxidative defense mechanism (Total Antioxidant Status (TAS), Paraoxonase-1 (PON-1), Thiol/Disulfide Homeostasis (TDH), Catalase (CAT), Total Thiol (TT), Native Thiol (NT), Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPx)) were measured using commercial kits (RelAssay, Mega Tıp San. Tic. Ltd. Sti., Gaziantep, Türkiye). The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS (Yumru *et al.*, 2009):

OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L)/TAS (μmol Trolox equivalent/L)

Levels of immunoglobulins (IgA, IgG, IgM and IgE) were performed by Otto Scientic (Ankara, Türkiye) determined using colorimetric methods with specific reagent (RelAssay, Mega Tıp San. Tic. Ltd. Sti., Gaziantep, Türkiye). All the tests were run according to the manufacturer's protocols of reagents.

Health Monitoring

Daily health monitoring included recording incidences of diarrhea, respiratory problems, and general clinical signs. Calves scoring ≥3 in fecal consistency were classified as having diarrhea. Clinical care was provided immediately when needed.

General appearance was scored (Heinrichs *et al.*, 2003): 1 - normal, awake 2 - ears drooping, 3 - dull eyes, 4 - severe lethargy, 5 - critically unresponsive.

Statistical Analyses

Repeated measurements ANOVA was used to evaluate LW, BMs and blood parameters. One-way ANOVA was used for FI, FCR, respiratory and pulse rate, rectal temperature, fecal scores and general appearance. Statistical significance was accepted at $P<0.05$, with trends noted when $0.05<P<0.10$. Tukey's test was applied for multiple comparisons.

Results and Discussion

Live Weight and Body Measurement

The LW gain and BMs of the groups are presented in Table 2.

Table 2: Live weight and body measurements of calves

	G1 (Mean±S.E.)	G2 (Mean±S.E.)	G3 (Mean±S.E.)	P
LW, kg				
Initial	41.00±2.05	40.45±2.05	39.10±2.05	0.99
4wk	48.90±2.40	48.45±2.40	47.80±2.40	
8wk	64.30±3.56	66.85±3.56	68.65±3.56	
BL, cm				
Initial	62.40±2.22	63.75±2.22	64.45±2.22	0.83
4wk	70.50±1.33	71.35±1.33	71.40±1.33	
8wk	77.00±1.33	78.75±1.33	79.25±1.33	
BD, cm				
Initial	28.05±1.13	28.75±1.13	28.05±1.13	0.92
4wk	32.10±1.08	32.65±1.08	32.35±1.08	
8wk	36.95±1.15	37.85±1.15	37.75±1.15	
WH, cm				
Initial	75.85±1.06	74.45±1.06	75.25±1.06	0.65
4wk	81.00±0.91	80.80±0.91	79.70±0.91	
8wk	85.80±0.91	86.85±0.91	85.60±0.91	
HH, cm				
Initial	78.90±0.92	79.95±0.92	78.65±0.92	0.59
4wk	84.95±1.07	85.15±1.07	83.70±1.07	
8wk	89.70±1.05	91.85±1.05	90.65±1.05	
CG, cm				
Initial	76.65±1.25	76.15±1.25	74.05±1.25	0.66
4wk	82.35±1.31	82.55±1.31	80.70±1.31	
8wk	91.05±2.21	91.85±2.21	90.40±2.21	

Although initial LWs were similar among groups ($P = 0.99$), calves in the G3 achieved a higher average LW (68.65 kg) compared to the control group (64.30 kg). This indicates a non-significant positive effect of probiotic supplementation on growth performance, consistent with previous studies suggesting that probiotics enhance digestion and nutrient utilization by modulating gut microbiota (Frizzo *et al.*, 2011; Grigore *et al.*, 2020). Probiotics accelerate the absorption of nutrients by increasing production. In addition, probiotics produce antimicrobial peptides that prevent pathogen growth and enzymes that inhibit bacterial toxins (Makav and Kaya, 2023). However, probiotics modulate the intestinal microflora, inhibiting the growth of pathogenic microorganisms and improving digestive tract health. Furthermore, probiotics promote colonization by attaching to the intestinal epithelial surface (İduğ and Hızlı Güldemir, 2024). Probiotics support musculoskeletal growth by improving the

absorption of nutrients such as calcium, promoting bone mineral density, and enhancing protein synthesis (Collins *et al.*, 2017; Ilesanmi-Oyelere and Kruger, 2020).

BL, CG and BD were also non-significantly higher in probiotic groups by 8wk, indicating improved skeletal and muscular development. This improvement is attributed to probiotics' ability to optimize the gut-bone axis and modulate gut microbiota, enhancing nutrient absorption and reducing inflammation (Sire *et al.*, 2022; Harahap *et al.*, 2024). Additionally, probiotics positively influenced HH and WH, reflecting their role in supporting skeletal growth. Enhanced rumen fermentation, improved feed efficiency, and increased secretion of growth-related hormones are potential mechanisms underlying these effects (El-Tawab *et al.*, 2016; Nalla *et al.*, 2022). These findings emphasize the multifaceted benefits of probiotics in promoting growth, skeletal development, and overall health in calves.

Table 3 presents the gains in total LW (TLW), daily LW gain (DLWG), and BMs of calves.

Table 3: Live weight and body measurement gain of calves

	G1 (Mean±S.E.)	G2 (Mean±S.E.)	G3 (Mean±S.E.)	P
DLWG, kg	0.520±0.08	0.587±0.05	0.632±0.08	0.52
TLWG, cm	23.30±3.48	26.40±1.74	29.55±3.26	0.34
TBLG, cm	14.60±2.67	15.00±1.52	14.80±1.29	0.99
TWHG, cm	9.95±0.44	11.40±0.57	10.35±0.75	0.23
THHG, cm	10.80±0.76	11.90±0.83	12.00±0.86	0.52
TBDG, cm	7.75±0.71	9.10±0.69	9.70±1.00	0.24
TCGG, cm	14.40±1.93	15.70±1.15	16.35±2.43	0.77

TBLG Total BL gain, TWHG Total WH gain, THHG Total HH gain, TBDG Total BD gain, TCGG Total CG gain

The probiotic-supplemented groups exhibited a non-significant increase in DLWG and TLW, with the G3 achieving the highest values (29.55 kg TLW and 0.53 kg DLWG). Probiotics are known to enhance gut microbiota balance, improve the activity of digestive enzymes, and increase nutrient absorption, leading to better feed utilization and energy extraction from the diet (Zhou *et al.*, 2024; Oliphant and Allen-Vercoe, 2019). Additionally, probiotics promote the production of short-chain fatty acids, optimize nutrient metabolism, and support microbial ecosystem stability, further enhancing growth performance and feed conversion efficiency (Ayyat *et al.*, 2023; Nalla *et al.*, 2022).

BMs, including TBLG, THHG, TCGG, showed non-significant increases in the probiotic-supplemented groups. The G3 demonstrated the most notable improvements, particularly in skeletal growth parameters such as HH and CG, which are indicative of enhance bone development and metabolic capacity. Probiotics support calcium and phosphorus absorption by improving gut health and mineral metabolism, contributing to bone mineralization and skeletal system development (Ayyat *et al.*, 2023). Furthermore, increased BD and CG suggest enhanced rumen volume, digestive capacity, and respiratory efficiency, highlighting the multifaceted role of probiotics in promoting growth and overall health in calves.

Health Parameters

The data presented in Table 4 summarize the effects of probiotic supplementation on the health and growth performance of calves, including weaning age, disease incidence, diarrhea, FI and FCR.

Table 4: Health parameters of calves

	G1 (Mean±S.E.)	G2 (Mean±S.E.)	G3 (Mean±S.E.)	P
Weaning age	47.80±2.60	44.70±2.26	41.80±3.25	0.32
NDD	5.40±1.89A	2.00±0.58AB	0.80±0.42B	0.03
NDiaD	5.50±0.48A	1.70±0.67B	1.00±0.56B	0.00
Fecal score	1.45±0.05	1.30±0.01	1.01±0.01	0.35
GA	1.05±0.03	1.03±0.01	1.01±0.01	0.35
Pulse rate	84.54±3.48	81.89±7.29	80.53±6.89	0.90
RT, ^{o}C	38.67±0.07	38.30±0.37	38.59±0.14	0.51
RR	58.69±7.06	50.98±2.48	54.53±3.91	0.54
TFC, kg	14.16±1.35	13.49±0.67	13.53±1.10	0.89
DFC, kg	0.294±0.02	0.313±0.03	0.338±0.03	0.48
FCR	0.57±0.07	0.55±0.09	0.69±0.12	0.58

NDD Number of diseased days, NDiaD Number of diarrhea days, GA General appearance, RT Rectal temperature, RR Respiratory rate, TFC Total feed consumption, FC Daily feed consumption, FCR Feed conversion ratio, AB indicates the difference between means in the same row

While the reduction in weaning age across probiotic groups was not statistically significant, the observed decrease suggests that probiotics support early rumen development. Probiotics enhance microbial activity and nutrient absorption, promoting rumen papillae growth and pH stabilization through increased volatile fatty acid production, thereby facilitating earlier weaning transitions (Diao *et al.*, 2019; Hao *et al.*, 2021). Additionally, probiotic supplementation significantly reduced the NDD and diarrhea incidence, particularly in the G3. These findings indicate that probiotics improve gut health by modulating microbiota composition, suppressing pathogenic bacteria, and enhancing immune function, which collectively reduce gastrointestinal disorders and promote healthier growth (Cangiano *et al.*, 2020; Wu *et al.*, 2021). Calves with strong immune systems had a lower incidence of disease (Yüceer ve Özbeyaz, 2010). Probiotics have been reported to reduce the risk of infection and strengthen the immune system by regulating the gut microbiota. These effects may contribute to lower disease incidence in calves (Horasan and Çelikyüz, 2024). Probiotic use has been shown to be effective in reducing the incidence of disease in calves (Küçükoflaz *et al.*, 2024).

Fecal consistency and GA showed no significant differences between groups, aligning with previous studies suggesting that while probiotics improve gut

health and reduce disease risk, these benefits may not manifest as observable changes in fecal consistency or GA (Guo *et al.*, 2022; Fan *et al.*, 2021). Similarly, physiological parameters, including RT, pulse rate and RR, were not significant affected, suggesting limited impact of probiotics on these metrics under normal conditions (Al-Shawi *et al.*, 2020). FI and FCR also showed no statistically significant differences; however, literature indicates that probiotics enhance nutrient metabolism, microbial protein synthesis, and energy utilization, which could lead to improved long-term feed efficiency and growth performance (Wang *et al.*, 2023; Riaz *et al.*, 2024). These findings collectively highlight the potential of probiotics to improve early growth performance and reduce disease burden, particularly by supporting gut health and immune function in calves.

Hematological and Biochemical Blood Parameters

The use of probiotics did not have a significant effect on hematological and biochemical parameters. Therefore, the results were not tabulated. No significant differences were observed in WBC, RBC, Hgb, Hct, MCV, MCH, and MCHC values. Minor fluctuations were recorded but all values remained within normal ranges (Ayyat *et al.*, 2023; Dixon, 2024).

ALT, GGT, and ALP levels exhibited minor fluctuations, but no significant differences were found between groups. These findings suggest that probiotics have minimal direct effects on liver functions (Schnabl and Brenner, 2014; Fan *et al.*, 2021). No significant differences were observed in CREA and BUN levels or in the BUN/CREA ratio, indicating that probiotics do not adversely affect kidney functions (Kuo *et al.*, 2023; Antanaitis *et al.*, 2024). Ca and IP levels showed slight fluctuations, but not statistically significant differences were noted among the groups (Oliveira and Soares, 2024). TP, ALB, and GLOB levels did not show significant changes and remained within normal physiological ranges in all groups (Talha *et al.*, 2009; Santos *et al.*, 2024). No significant differences were observed in TC and TG levels among the groups (Grigore *et al.*, 2020; Kumar *et al.*, 2023). Fluctuations were observed in TBil, GLU, and other kidney-liver related indicators, but no significant changes were recorded (Hussein *et al.*, 2020).

Probiotics were found to have no significant direct effect on the hematological and biochemical parameters of healthy calves. However, there is evidence of indirect benefits, such as improving gut health and reducing pathogen load (Eslamparast *et al.*, 2013; Wang *et al.*, 2023).

Oxidative Stress, Antioxidative Defence Mechanism and Immune Response

Table 5 summarizes the oxidative stress, and antioxidant defense mechanism parameters in calves subjected to different probiotic.

Table 5: Oxidative stress and antioxidant defense mechanism

	T	G1 (Mean±S.E.)	G2 (Mean±S.E.)	G3 (Mean±S.E.)	P
TAS mmol/L	0	1.04±0.07	1.09±0.01	0.98±0.06	0.55
	1	0.86±0.02	0.91±0.02	0.89±0.10	
	2	1.01±0.99	0.88±0.06	0.92±0.07	
TOS µmol/L	0	4.51±0.55	2.54±1.15	5.72±2.19	0.54
	1	7.47±2.70	2.78±0.99	2.49±0.08	
	2	2.95±0.38	5.66±3.08	5.18±1.21	
OSI	0	0.44±0.06	0.23±0.11	0.53±0.16	0.51
	1	0.85±0.29	0.30±0.10	0.29±0.04	
	2	0.30±0.05	0.61±0.30	0.54±0.11	
PON-1 U/L	0	39.67±1.45	27.33±3.48	37.33±7.17	0.00
	1	261.70±33.0B	445.00±30.0A	262.70±52.9AB	
	2	321.00±33.0B	580.70±22.3A	496.00±13.0AB	
TTL µmol/L	0	656.00±32.80	635.60±71.10	615.40±20.10	0.63
	1	779.10±57.30	567.00±37.30	614.40±28.70	
	2	532.90±19.90	695.00±121.00	616.30±56.40	
NTL µmol/L	0	580.70±12.60	548.40±38.40	565.02±9.67	0.41
	1	684.70±36.00	545.90±32.80	579.90±26.50	
	2	476.70±12.70	550.90±55.40	550.10±39.30	
TDH	0	50.89±0.75	66.30±28.50	25.17±5.33	0.04
	1	73.70±13.90A	60.40±24.30A	17.23±4.26B	
	2	28.10±14.90B	72.20±34.20A	33.10±8.53AB	
CAT kU/L	0	189.60±13.40	88.10±20.20	133.30±20.00	0.24
	1	80.30±35.80	104.70±36.50	52.30±11.40	
	2	70.90±45.90	94.40±40.20	135.30±43.10	
SOD U/mL	0	273.90±12.80	232.30±25.30	293.00±26.70	0.21
	1	633.00±20.80	809.00±277.00	1304.00±375.00	
	2	959.10±47.00	1210.00±119.0	1066.00±131.00	
GPx U/L	0	162.20±31.50	165.00±26.00	170.20±22.60	0.63
	1	163.00±16.93	167.30±21.65	171.50±24.47	
	2	163.80±27.10	168.15±25.56	172.80±26.35	
MDA µmol/mL	0	15.96±2.97	4.57±0.81	5.41±0.89	0.37
	1	9.33±2.98	9.67±1.12	7.97±0.72	
	2	19.37±8.15	10.95±5.40	10.35±3.10	

AB indicates the difference between means in the same row, 1: Initial of experiment, 2: 28-day-old, 3: Final of experiment

While TOS and TAS exhibited fluctuations, no statistically significant differences were observed among the groups at most points (Aydilek *et al.*, 2024; Özbek and Özkan, 2020). Notably, at specific intervals, particularly 28-day-old, TOS levels were non-significantly lower in the probiotic groups compared to the control group, indicating reduced oxidative stress due to probiotic supplementation. Aydilek *et al.* (2014) and Özbek and Özkan (2020) reported that reduced TOS levels in probiotic-supplemented animals, suggesting a potential protective effect of probiotics against oxidative damage. Similarly, the OSI decreased non-significantly in G2 and G3 at certain time points, further supporting the antioxidative role of probiotics. PON-1 enzyme activity showed significant increases, particularly at the final time point, indicating enhanced antioxidant defense mechanisms (Aydilek *et al.*, 2024). Markers reflecting

the thiol antioxidant system, such as TTL and NTL, showed variability without statistically significant differences among the groups, suggesting that this system might not be directly influenced by probiotic under normal conditions (Aydilek *et al.*, 2014). Similarly, while CAT and SOD activities fluctuated, no significant group differences were observed, consistent with findings by Maccarro *et al.* (2021) and Gusti *et al.* (2021). GPx activity also remained unaffected, which aligns with research by Birmingham *et al.* (2014), indicating that probiotics alone may not directly influence GPx activity. MDA, a marker of lipid peroxidation, showed a trend toward lower levels in the G2 and G3, particularly in the G3 group at 28-day-old, suggesting reduced oxidative damage and improved cellular membrane integrity. These findings are consistent with studies by Wang *et al.* (2023) and Zhang *et al.* (2023), which highlight the potential protective effects of probiotics against lipid peroxidation. In addition, probiotics, especially *Lactobacillus* and *Bifidobacterium* species, have been shown to reduce oxidative stress by increasing the activity of antioxidant enzymes (Horasan and Çelikyürek, 2024; Rezaie *et al.*, 2025).

Fluctuations in oxidative stress markers may result from environmental stressors, nutritional status and individual differences. In particular, nutritional factors such as high-fat diets have been shown to influence oxidative stress levels (Rezaie *et al.*, 2025).

The effect of probiotics on immune parameters, particularly immunoglobulin levels, were also assessed (Table 6).

Table 6: Immune response

	T	G1 (Mean±S.E.)	G2 (Mean±S.E.)	G3 (Mean±S.E.)	P
IgE	0	25.65±9.00	30.13±3.00	27.65±4.32	0.58
mg/dL	1	26.57±7.16	33.20±2.57	28.90±5.53	
	2	24.70±11.60	27.07±3.63	26.40±10.10	
IgA	0	1.35±0.42	2.47±0.42	1.40±0.70	0.43
mg/dL	1	1.33±1.22	3.63±0.20	5.57±1.92	
	2	1.93±0.44	4.02±3.03	4.67±2.02	
IgG	0	56.70±17.70	19.29±4.93	18.08±4.52	0.71
ug/dL	1	37.92±5.80	73.41±8.28	83.74±4.07	
	2	116.40±28.20	135.10±31.30	169.90±41.40	
IgM	0	5.90±0.77	8.04±2.31	7.85±3.23	0.33
Mg/dL	1	7.58±0.99	10.05±3.11	9.88±1.24	
	2	5.83±0.98	8.69±1.78	12.31±1.75	

1: Initial of experiment, 2: 28-day-old, 3: Final of experiment

Research has shown that probiotics generally do not significantly alter immunoglobulin (Ig) levels such as IgA, IgG, IgM and IgE under normal conditions (Zheng *et al.*, 2023). However, in this study, a general increase in Ig levels was observed in the G2 and G3, particularly in the final period. The G3 group exhibited significantly higher Ig levels compared to the G1, suggesting enhanced immune function and increased protection against pathogens. These findings are supported by Wang *et al.* (2023) and Zheng *et al.* (2023), who reported similar trends in livestock, highlighting the potential of probiotics to modulate immune response.

While the effects of probiotics on specific immune markers remain variable, their ability to strengthen the immune system through gut health modulation is widely recognized (Wang *et al.*, 2023; Zheng *et al.*, 2023). Overall, probiotics demonstrated potential benefits in reducing oxidative stress and enhancing immune response, although their effects on specific biomarkers appear context-dependend and influenced by experimental conditions and probiotic dosages.

Probiotics are known to have regulatory effects on the immune system. For example, *Lactobacillus* and *Bifidobacterium* strains have been shown to reduce the incidence and duration of infections by supporting immune function (Horasan and Çelikyürek, 2024). Changes in immune parameters can be influenced by individual genetic differences, stress levels and environmental conditions. These factors may caused the effects of probiotics on the immune system to differ between individuals (Akarsu and Yıldırım, 2024; Wang *et al.*, 2024).

This study is one of the first to specifically investigate the effects of *Lactobacillus casei* 7K-2L, *L. acidophilus* TRk 09, *L. helveticus* TRK 03, *L. paracasei,* and *L. lactis* strains on growth performance, oxidative stress markers, and immune response in calves. While previous studies have primarily focused on the general effects of probiotics on growth performance (Frizzo *et al.*, 2011; Wang *et al.*, 2023), this study provides novel insights by evaluating oxidative stress and immune system parameters, contributing to a deeper understanding of the potential mechanisms of probiotic action. Additionally, probiotic dosage in this study was adjusted according to body weight, and the role of probiotics in reducing the incidence of respiratory diseases was also assessed. The findings offer valuable data for optimizing the use of probiotics in calf health management and contribute to the existing literature.

Conclusion

This study evaluated the effects of *Lactobacillus casei* 7K-2L*, L. acidophilus* TRK 09*, L. helveticus* TRK 03*, L.paracasei*, and *L. lactis* strains on growth performance, oxidative stress markers, and immune response of calves. Our results show that probiotic supplementation significantly reduced disease incidence, modulated the immune system and may play a regulatory role on oxidative stress markers.

In particular, reduced calf disease rates were observed in probiotic-treated groups, suggesting that probiotics may support the immune response through the gut microbiota. However, significant improvement trends were observed in growth performance and feed

conversion ratio, but no statistically significant difference was found.

While our study supports the potential benefits of probiotics on calf health and performance, it emphasizes the need for larger sample sizes and long-term studies. Future research may reveal how probiotics can be used more effectively in calf rearing by evaluating different probiotic doses and combinations.

These findings provide important practical insights into the use of probiotics in dairy cattle production and suggest the integration of probiotic supplements into diets, especially to support early calf health.

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Author's Contributions

Raya Myktybayeva: Funding acquisition and project administration.

Serkan Özkaya: Advised the project, organized the study with calves, interpreted the results, and wrote the first draft of the manuscript.

Elif Rabia Şanlı: Collected blood samples from calves and analyzed the blood.

Hatice İlkay Yaşar: Collected daily data from calves.

Cevdet Gökhan Tüzün: Calculated feed intake and feed conversion ratio, and contributed to result interpretation.

Özgür Koşkan: Performed the statistical analysis of the study data.

Togzhan Boranbayeva: Isolated, identified, dried, and researched probiotic microorganisms.

Otebayev Zhassulan: Contributed to investigation and provided resources.

Shokhan Alpeisov: Designed the research methodology and contributed to data interpretation.

Ethics

All procedures applied in the study were performed with the permission of the animal ethics committee.

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