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The impact of multistrain probiotics (*Leuconostoc mesenteroides*, *Lactococcus lactis*) on African catfish (*Clarias gariepinus*) gut microbiota, immunological response, and growth performance

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Introduction: Aquaculture is globally recognized as an effective means of supporting economic growth and livelihood security. However, the sector continues to fall short of projected production targets due to the use of low-quality inputs and inadequate culture techniques. The use of probiotics in aquaculture has been proposed as a nutritional strategy to enhance fish growth and immune responses. This study investigated the effects of dietary supplementation with a multistrain probiotic combination—*Leuconostoc mesenteroides* and *Lactococcus lactis*—on growth performance, immune parameters, and gut microbiota in African catfish (*Clarias gariepinus*).

Methods: Fish were fed either a basal diet or an experimental diet containing *L. mesenteroides* a 95 nd *L. lactis* at 10⁶ CFU/g for 8 weeks.

Results and discussion: The probiotic-supplemented group showed significant improvements in feed utilization, alternative complement pathway activity, lactic acid bacterial populations, mucus secretion, and peroxidase activity compared with the control group ($p < 0.05$). Serum lysozyme activity was also significantly

higher in the probiotic-fed group. Furthermore, fish receiving the supplemented diet exhibited superior growth metrics, including weight gain, final body weight, and specific growth rate ($p < 0.05$). Enhanced superoxide dismutase activity was also observed in the probiotic group. These results suggest that dietary inclusion of *L. mesenteroides* and *L. lactis* may serve as an effective immunostimulant feed additive for African catfish aquaculture.

KEYWORDS

immunity, growth

African clarify catfish, *Leuconostoc mesenteroides*, *Lactococcus lactis*, probiotic,

Introduction

Over the past two decades, aquaculture has expanded significantly and now plays a crucial role in meeting global protein demands. However, the intensification of aquaculture systems has introduced challenges related to fish health, including growth disorders, increased mortality, and disease outbreaks (1–3).

In response to these issues, antibiotics have often been used to control infections and improve productivity (4). Nevertheless, the widespread use of antibiotics poses environmental and biological risks, including the development of antimicrobial-resistant bacteria and disruption of the intestinal microbiota in aquatic organisms (5).

To address these concerns, recent studies have focused on dietary alternatives such as probiotics, which are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host (6–10). In aquaculture, probiotics have been shown to increase nutrient absorption, stimulate immune responses, and modulate the gut microbiota of fish (11–15). These benefits are achieved through multiple mechanisms, including enzyme secretion, microbial competition, and improved intestinal integrity (16).

Probiotics can be delivered through various routes, including incorporation into feed, direct addition to water, or injection (17–21). Research has shown that specific strains, including *Leuconostoc mesenteroides* and *Lactococcus lactis*, have immunostimulatory effects when administered to Nile tilapia (*Oreochromis niloticus*) (2).

On the basis of these findings, the present study investigated the effects of dietary supplementation with *L. mesenteroides* and *L. lactis* on the growth performance, innate immunity, and gut microbial composition of African catfish (*Clarias gariepinus*). These lactic acid bacteria were originally isolated from the intestinal tracts of freshwater fish and were selected based on their probiotic potential.

This study evaluated the efficacy of multistrain probiotic supplementation as a sustainable, health-promoting feed additive in freshwater aquaculture systems.

Materials and methods

Animal ethics

All experimental procedures were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (14). The protocol was approved by the Ethics Committee of Seifullin Kazakh Agrotechnical University (permit number: 2).

Experimental design

A total of 256 healthy juvenile African catfish (*C. gariepinus*) with an average weight of 498 ± 2 g were obtained from a local commercial hatchery. The fish were randomly assigned to control and treatment groups (128 fish per group) and distributed into six 100 L tanks (32 fish per tank), with 2 replicate tanks per group. All tanks were equipped with a continuous flow-through freshwater system, aeration, and appropriate inlet and outlet systems. Prior to the experiment, the fish were acclimated for 14 days in a 500 L tank with aerated water (dissolved oxygen: 5 mg/L). Water quality was monitored throughout the trial. The temperature was maintained at $27^\circ\text{C} \pm 0.5^\circ\text{C}$ using a digital thermometer, and pH was measured using a portable pH meter (Fisherbrand AP115). The fish were fed a control diet (without probiotics) twice daily at 09:00 and 17:00 during the acclimation period. After the 40-day feeding trial, fish were fasted for 24 hours prior to sampling. Growth performance, survival, immune parameters, and the composition of the microbiota were assessed.

Isolation of probiotics

L. mesenteroides and *L. lactis* strains were isolated from the intestinal contents of common carp (*Cyprinus carpio*) fingerlings, as previously described (2). Briefly, 50 μL of intestinal content was

TABLE 1 Formulation of the basal diet for African catfish, *Clarias gariepinus*.

Feed ingredients	g kg ⁻¹	%
Fishmeal	30	3
Soybean meal	30	3
Blood meal	10	1
Milled wheat	7	0.7
Extruded peas	8	0.8
Potato starch	6.7	0.67
Fish oil	4	0.4
Vitamin/Mineral mixture	1.1	0.11
Premix	2.0	0.2
Yeast	0.2	0.02
Gelatin	1	0.1
Proximate composition %		
Moisture	12.1	
Crude protein	44.2	
Crude lipid	8.20	
Crude ash	7.54	

diluted in distilled water at a 1:10 ratio, and 100 µL of the dilution was plated onto MRS agar using sterile pipettes. The plates were incubated at 37°C for 48 hours. Uniform colony distribution was achieved using sterilized glass beads. The colonies were further purified, and the isolates were confirmed by PCR.

PCR confirmation

Genomic DNA was extracted using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA), following the manufacturer's instructions. The reference strains *L. mesenteroides* (NCTC AB023246) and *L. lactis* (NCTC M58837) were used as positive controls. Multiplex PCR (mPCR) targeting the 16S rRNA gene was performed as described by Ali and Kot (15) using an Eppendorf Mastercycler. The amplification parameters were as follows: initial denaturation at 95°C for 2.5 min; 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min 20 sec; and a final extension at 72°C for 2 min. PCR products were analyzed by 1% agarose gel electrophoresis stained with ethidium bromide.

Preparation of experimental diets

The basal diet consisted of fishmeal, soybean meal, blood meal, milled wheat, extruded peas, potato starch, fish oil, premix, yeast,

and gelatin (Table 1). The experimental diet was prepared by adding 1×10^7 CFU/g of both *L. mesenteroides* and *L. lactis* via surface spraying. A 165 g portion of gelatin was dissolved in 500 mL of distilled water and applied to the diet pellets to ensure bacterial adhesion. The feed was air-dried overnight and stored at -4°C until use.

Growth performance

Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated as described by Allameh and Yusoff (19). The following formulas were used:

$$\text{WG (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{SGR (\%)} = 100 \times (\ln W_2 - \ln W_1)/T$$

$$\text{FCR} = \text{Feed intake/Weight gain}$$

$$\text{PER} = \text{Weight gain/protein intake}$$

where W_1 and W_2 are the initial and final weights, respectively, and T is the number of feeding days.

Determination of intestinal bacteria activities

Thirteen fish per group were randomly selected and euthanized after a 24-hour fasting period. Euthanasia was performed in accordance with the Animal Care and Use Committee (ACUC) Guidelines and the Standard Operating Procedure for MS-222 (tricaine methanesulfonate) for euthanasia. Fish were euthanized via anesthetic overdose (MS-222 at 250 mg/L) followed by decapitation. To ensure complete euthanasia, fish were placed in the MS-222 buffer solution for at least 10 min after gill movement had ceased (respiratory arrest), before extraction and decapitation.

The external surfaces of the fish were sterilized with 70% ethanol. The intestinal tracts were excised aseptically, rinsed with phosphate-buffered saline (PBS), and homogenized. Serial dilutions were prepared in PBS, and 100 µL aliquots were plated onto tryptic soy agar (TSA) for total bacterial counts and MRS agar for lactic acid bacteria. Plates were incubated at 25°C for 3–5 days. Colony-forming units (CFUs) were enumerated as described by Ramos and Batista (17).

Immune responses assays

Alternative complement pathway activity (ACH_{50}) was measured following the method of Yano (20). Briefly, 0.1 mL of serum was mixed with barbitone buffer to reach a final volume of

0.25 mL. Then, 0.1 mL of rabbit red blood cells (RaRBCs) was added, and the mixture was incubated for 1 h at 20°C. After incubation, 3.15 mL of 0.9% NaCl solution was added, and the tubes were centrifuged at $1,600 \times g$ for 10 min at 4°C. The optical density (OD) of the supernatant was measured at 414 nm using a spectrophotometer, and a lysis curve was constructed. The serum dilution resulting in 50% hemolysis was used to determine complement activity. The bactericidal activity of the serum and mucus was evaluated as described by Dawood and Koshio (12). Samples were diluted 1:1 in Tris buffer (pH 7.5) and incubated with a suspension of *Escherichia coli* (strain IAM1239; RIBSP, Kazakhstan) at a concentration of 0.001 mg/mL for 12 h at room temperature. Bacterial viability was assessed by calculating the number of colony-forming units (CFUs) on agar plates.

Lysozyme activity in the serum and mucus was determined via turbidimetric assay. A 10 μ L aliquot of each sample was mixed with 190 μ L of *Micrococcus lysodeikticus* suspension (0.2 mg/mL in PBS, pH 7.4) in a 96-well microplate and incubated at 24°C for 5 min. The decrease in absorbance at 450 nm was measured using a microplate reader (Biotek 800 TS, Agilent Technologies, USA). One unit of lysozyme activity was defined as a decrease in OD of 0.001 per minute.

Peroxidase activity was assessed as described by Salinas and Abelli (21). A mixture of 15 μ L of serum and 35 μ L of Hank's buffered salt solution was added to a 96-well plate. Then, 50 μ L of 3,3',5,5'-tetramethylbenzidine (TMB; Thermo Scientific, USA) was added to each well. After 20 min of incubation at room temperature, the reaction was stopped by adding 50 μ L of 2 M sulfuric acid. The absorbance was read at 450 nm using a microplate reader.

Superoxide dismutase (SOD) activity in the serum was determined using the Ransod assay kit (Randox, Crumlin, UK) as

described by Sun and Yang (22). Enzyme activity was expressed in units per milligram of protein, where one unit was defined as the amount of enzyme required to inhibit the reduction of nitroblue tetrazolium (NBT) by 50% at 550 nm.

Statistical analysis

Data were analyzed using Prism 9 (GraphPad Software, San Diego, CA, USA). Student's *t* test and one-way ANOVA followed by Tukey's *post hoc* test were applied. Results are expressed as means \pm standard errors of the mean (SEMs). Differences were considered statistically significant at $p < 0.05$.

Results

Growth performance, nutrient utilization, and fish survival

The effects of probiotic supplementation on growth performance, nutrient utilization, and fish survival are presented in Table 2. Compared with the control group, fish fed a diet supplemented with *L. mesenteroides* and *L. lactis* exhibited significantly greater weight gain (WG), final body weight (FBW), and specific growth rate (SGR) ($p < 0.05$).

In addition, the protein efficiency ratio (PER) and feed efficiency ratio (FER) were significantly improved in the probiotic-fed group ($p < 0.05$), indicating increased nutrient utilization.

A significant increase in protein gain (PG) was also observed in fish fed the diet containing *L. mesenteroides* and *L. lactis* compared with the control group ($p < 0.05$). Although feed intake (FI) was slightly higher in the control group, the difference was not significant ($p > 0.05$). Survival remained at 100% in all experimental groups, with no mortality recorded throughout the trial.

Microbiological analyses of the gut microbiota

Prior to the feeding trial, no detectable levels of lactic acid bacteria (LAB) were detected in the intestinal microbiota of fish in either group. However, compared with the control group, fish fed diets supplemented with *L. mesenteroides* and *L. lactis* exhibited significantly greater intestinal LAB counts ($p < 0.05$; Figure 1). In contrast, no LAB colonies were recovered from the intestines of the fish in the control group.

Immune response development

Compared with the control group, alternative complement pathway (ACP) activity was lowest in the first control group of fish fed the ordinary diet. ACP activity increased in all groups of fish

TABLE 2 Growth performance parameters of African catfish (*Clarias gariepinus*) fed diets supplemented with *L. mesenteroides* and *L. lactis* for 40 days.

Growth performance parameters	Diets		P-value
	Control	Mixture of <i>Leuconostoc mesenteroides</i> and <i>Lactococcus lactis</i> probiotics	
¹ IBW (g)	501.3 \pm 1.23	506.2 \pm 1.26	0.902
² FBW (g)	649 \pm 2.12	821 \pm 3.37*	0.034
³ WG (%)	147.7 \pm 0.89	314.8 \pm 2.11*	0.042
⁴ SGR (%)	3.69 \pm 0.18	7.87 \pm 0.2*	0.03
⁵ FI (g)	15.6 \pm 0.21	15.2 \pm 0.8	0.998
⁶ FER	4.2 \pm 0.3	1.93 \pm 0.3*	0.024
⁷ PER	0.95 \pm 0.2	1.67 \pm 0.02*	0.037
⁸ PG	156.12 \pm 6.7	188.3 \pm 4.13*	0.012

¹IBW, initial body weight; ²FBW, final body weight; ³WG, weight gain; ⁴SGR, specific growth rate; ⁵FI, feed intake; ⁶FER, feed efficiency ratio; ⁷PER, protein efficiency ratio; ⁸PG, protein gain. P \leq 0.05 indicates statistically significant difference. Data are presented as means \pm standard error of the mean (SEM) from triplicate groups.

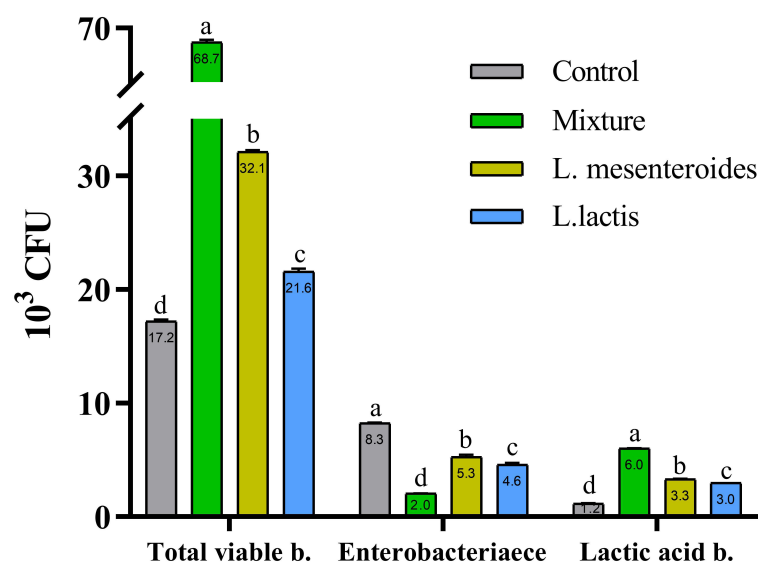


FIGURE 1

Total intestinal bacterial and lactic acid bacteria (LAB) counts in African catfish from control and probiotic-supplemented groups after 8 weeks of feeding. Data are presented as mean \pm SEM from two independent experiments ($n = 20$ fish per group). Bars with different letters indicate statistically significant differences ($p \leq 0.05$); bars sharing the same letter are not significantly different ($p \geq 0.05$). L. mesenteroides – *Leuconostoc mesenteroides*; L. lactis – *Lactococcus lactis*.

supplemented with probiotics. The highest ACP level was observed in the second group receiving a mixture of *Lactobacillus mesenteroides* and *Lactobacillus lactis*. ACP levels in the third and fourth groups fed with mono-probiotic diets (*L. mesenteroides* alone and *L. lactis* alone, respectively) were lower (Figure 2). Similarly, other innate immune parameters were significantly increased in the probiotic-supplemented groups.

Serum and mucus bactericidal activity (Figure 3), lysozyme activity (Figure 4), and peroxidase activity (Figure 5) were all markedly elevated compared with the control group. In addition, SOD activity was significantly higher in fish fed the probiotic mixture ($p \leq 0.05$; Figure 6). Mucus secretion was also significantly increased in this group, indicating an improvement in the mucosal immune response ($p \leq 0.05$; Figure 7).

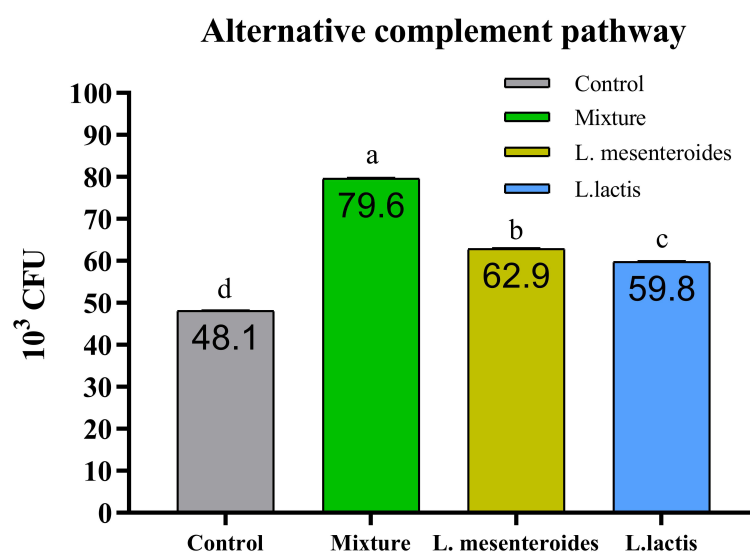


FIGURE 2

Alternative complement pathway (ACP) activity in African catfish (*Clarias gariepinus*) from control and probiotic-supplemented groups. Data are presented as mean \pm SEM from two independent experiments ($n = 20$ fish per group). Bars with different letters indicate statistically significant differences ($p \leq 0.05$). Bars with different letters indicate statistically significant differences ($p \geq 0.05$). L. mesenteroides – *Leuconostoc mesenteroides*; L. lactis – *Lactococcus lactis*.

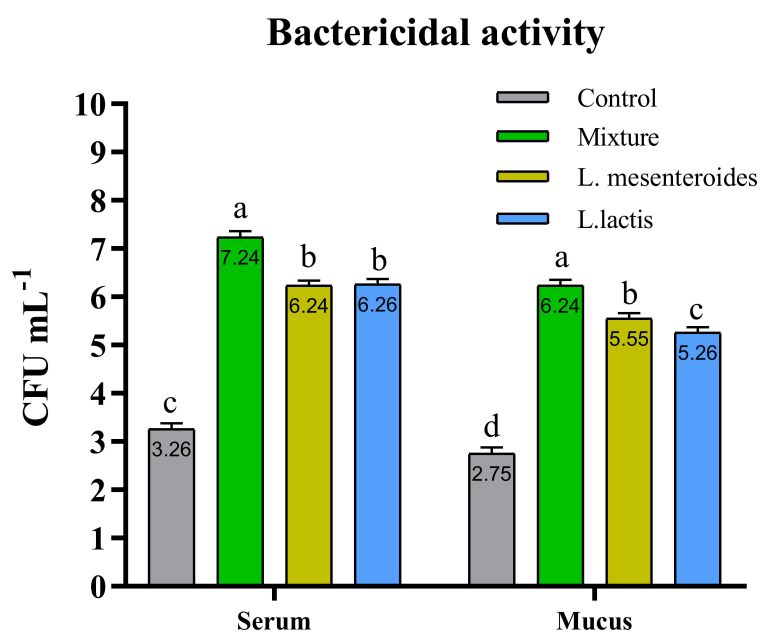


FIGURE 3

Serum and mucus bactericidal activity in African catfish (*Clarias gariepinus*) from control and probiotic-treated groups. Data are presented as mean \pm SEM from two independent experiments ($n = 20$ fish per group). Bars with different letters indicate statistically significant differences ($p \leq 0.05$); bars sharing the same letter are not significantly different ($p \geq 0.05$). *L. mesenteroides* – *Leuconostoc mesenteroides*; *L. lactis* – *Lactococcus lactis*.

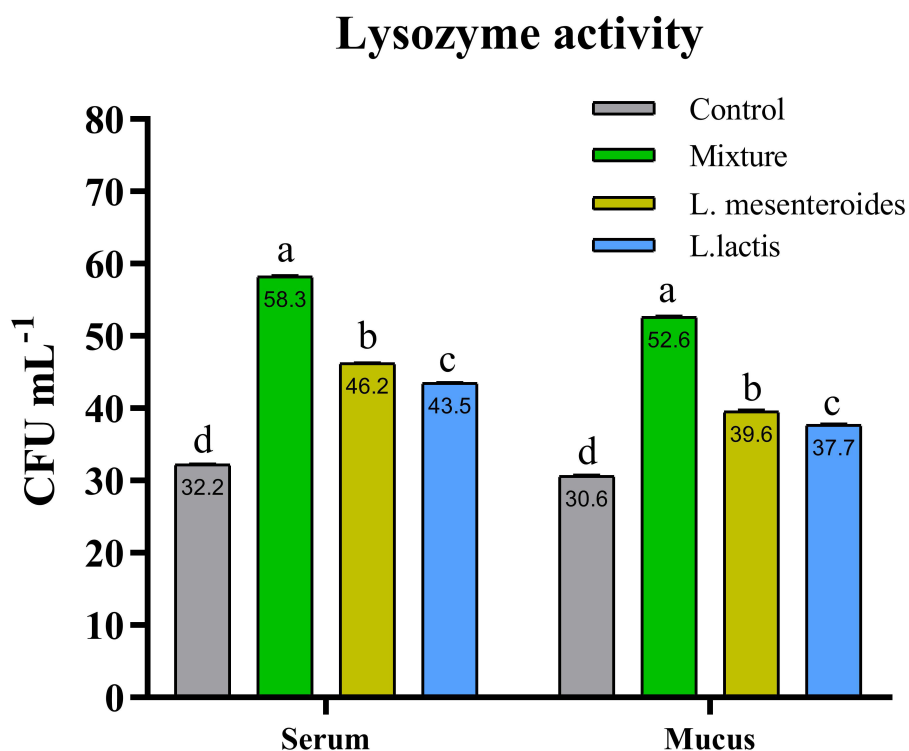


FIGURE 4

Serum and mucus lysozyme activity in African catfish (*Clarias gariepinus*) from control and experimental groups. Data are shown as the mean \pm SEM from two independent experiments ($n = 20$ fish/group). Bars with different letters are varied significantly ($p \leq 0.05$) and with the same alphabet have an insignificant difference ($p \geq 0.05$). *L. mesenteroides* – *Leuconostoc mesenteroides*; *L. lactis* – *Lactococcus lactis*.

Peroxidase activity

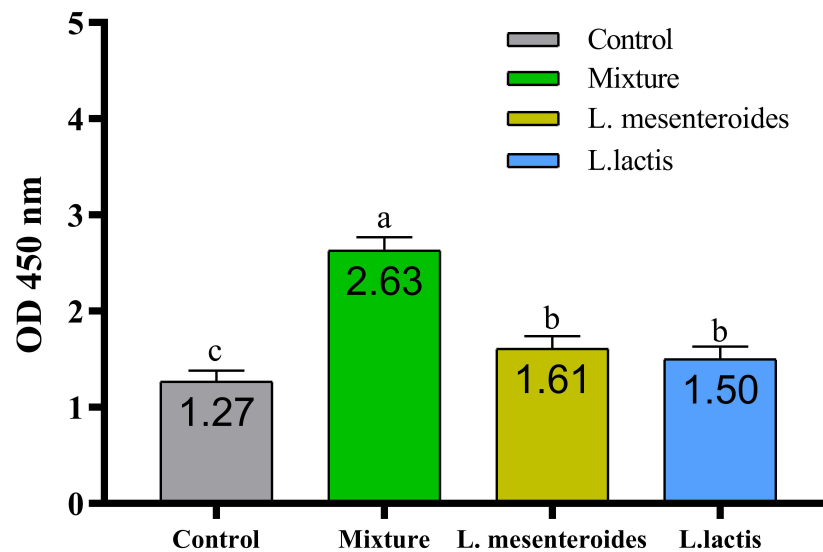


FIGURE 5

Serum peroxidase activity in African catfish (*Clarias gariepinus*) from control and probiotic-treated groups. Data are presented as mean \pm SEM from two independent experiments ($n = 20$ fish per group). Bars with different letters indicate statistically significant differences ($p \leq 0.05$); bars sharing the same letter are not significantly different ($p \geq 0.05$). L. mesenteroides – *Leuconostoc mesenteroides*; L. lactis – *Lactococcus lactis*.

Discussion

Numerous studies have investigated the application of LAB individual strains as probiotics to enhance growth performance, immune responses, and gut microbiota composition in fish species

(22–26). However, comparatively less attention has been given to evaluating the synergistic effects of multistrain LAB probiotics on fish health and physiology (2, 27–30). The present study assessed the effects of dietary supplementation with *L. mesenteroides* and *L. lactis* on the growth performance, immune function, and gut

Superoxide dismutase activity

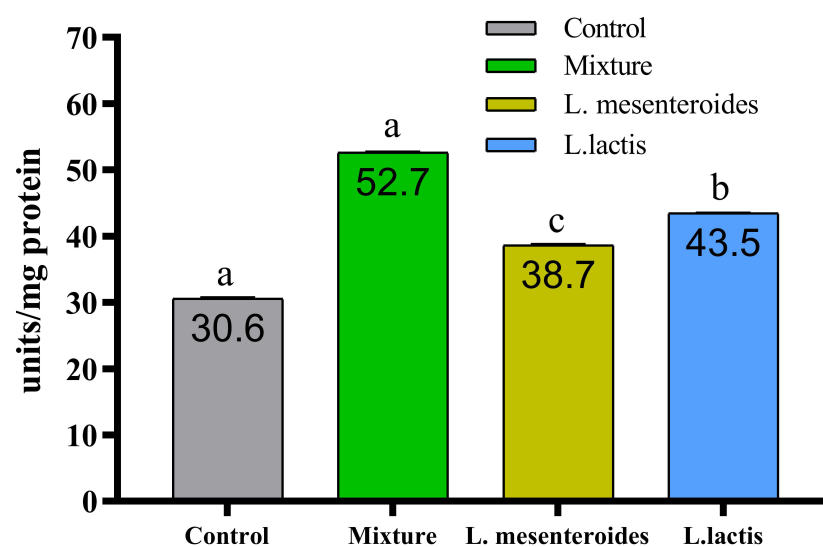


FIGURE 6

Superoxide dismutase (SOD) activity in African catfish (*Clarias gariepinus*) from control and probiotic-treated groups. Data are presented as mean \pm SEM from two independent experiments ($n = 20$ fish per group). Bars with different letters indicate statistically significant differences ($p \leq 0.05$); bars sharing the same letter are not significantly different ($p \geq 0.05$). L. mesenteroides – *Leuconostoc mesenteroides*; L. lactis – *Lactococcus lactis*.

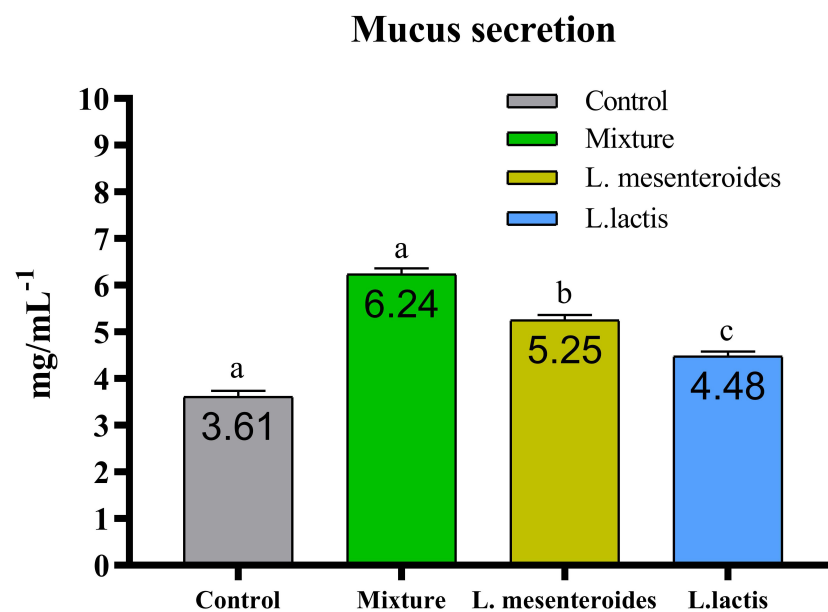


FIGURE 7

Effect of probiotic-supplemented diets on mucus secretion in African catfish (*Clarias gariepinus*). Data are presented as mean \pm SEM from two independent experiments ($n = 20$ fish per group). Bars with different letters indicate statistically significant differences ($p \leq 0.05$); bars sharing the same letter are not significantly different ($p \geq 0.05$). *L. mesenteroides* - *Leuconostoc mesenteroides*; *L. lactis* - *Lactococcus lactis*.

microbiota of African catfish (*C. gariepinus*). The results demonstrated that compared with the control, the 8-week probiotic combination significantly improved both growth rates and innate immune responses.

Similarly, the use of various *Lactobacillus* species as dietary supplements has been shown to enhance growth performance in several fish species, including olive flounder (*Paralichthys olivaceus*) (27), Nile tilapia (*Oreochromis* spp.) (29), and gilthead sea bream (*Sparus aurata*) (30). Moreover, improved growth performance has also been reported in olive flounder (*Paralichthys olivaceus*) (31) and grouper (*Epinephelus coioides*) (32) following probiotic supplementation. Our findings in African catfish support the hypothesis that dietary inclusion of a multistrain combination of *L. mesenteroides* and *L. lactis* results in higher final body weight, weight gain, and specific growth rate than in the control group. Similarly, the use of multiple probiotic strains—including *L. mesenteroides*—has been shown to increase growth performance in Javanese carp (*Puntius gonionotus*) (19). In the present study, fish receiving the probiotic-supplemented diet also exhibited improvements in feed intake and nutrient utilization. These results suggest that probiotic supplementation may increase feed palatability, thereby stimulating appetite and promoting growth.

Previous studies in Nile tilapia have demonstrated that probiotics can enhance growth and feed conversion by stimulating digestive enzyme activity and increasing nutrient bioavailability (33).

Probiotic colonization of the intestinal tract is known to promote microbial balance, improve gut morphology, and facilitate nutrient absorption (34–36). In our study, the enhanced growth performance and feed utilization observed in the probiotic

group may be attributable to improved digestive function mediated by increased enzymatic activity. Supplementation with *Lactobacillus* species has been shown to increase feed digestibility and nutrient extraction (7). Digestive enzyme activity is closely linked to the digestive capacity of fish and can be rapidly affected by dietary composition (37–39).

The introduction of beneficial microbial strains may also contribute to protease production and enhanced protein hydrolysis into amino acids, thereby improving the nutritional status of fish. Furthermore, bacterial enzymatic activity can increase the availability of lipids, proteins, and dry matter (40), ultimately supporting the improved growth and nutrient utilization observed in this study.

The results of this study demonstrated an increase in both total bacterial and LAB counts in the intestinal microbiota of fish in the probiotic-treated groups. This increase is likely a contributing factor to the improved growth performance and feed utilization observed in these groups. Similarly, a significant increase in total bacterial abundance has been reported in fish fed probiotic-supplemented diets (13). The dietary concentration of probiotic bacteria directly influences their colonization efficiency in the gastrointestinal tract. This colonization process is believed to support enhanced immunity, overall welfare, and growth in fish (41).

Therefore, the improvements in growth and nutrient assimilation observed in this study may be attributable to increased colonization and adherence of probiotic microorganisms introduced through dietary supplementation. Several studies have shown that probiotic bacteria colonizing the intestinal tract produce a variety of growth-promoting metabolites and nutrients (19, 42). Furthermore, recent publications have emphasized the critical interaction between

probiotic strains and intestinal epithelial cells in regulating mucosal immunity. This interaction enhances immune responses by modulating both the physical and immunological barrier functions of the intestinal lining (36, 43–46).

Our results also demonstrated that the immune response of fish was significantly enhanced following dietary supplementation with a probiotic mixture containing *L. mesenteroides* and *L. lactis*. Specifically, increased activities of lysozyme and bactericidal components were observed in both serum and mucus, along with elevated activities of the alternative complement pathway, SOD, and peroxidase. Similar immunostimulatory effects have been reported in fish such as rainbow trout (*Oncorhynchus mykiss*) (47), Labeo rohita (48), and olive flounder (*P. olivaceus*) (27) following probiotic supplementation.

The use of bacterial strains with complementary functional properties has been shown to enhance host immunity and promote intestinal health under experimental conditions (49). Additionally, external factors such as probiotics, vitamins, and nutrients have been shown to influence the activity of the alternative complement pathway in fish (50, 51). In the present study, the immune parameter most strongly affected was alternative complement pathway activity, which was significantly elevated in the probiotic-treated group. This observation is consistent with previous findings in studies involving probiotic supplementation in fish (26, 32).

It is well known that bactericidal activity plays a crucial role in the host's defense system against pathogenic microorganisms (7, 52). In our study, the highest levels of bactericidal activity in both serum and mucus were observed in fish fed a diet supplemented with a combination of *L. mesenteroides* and *L. lactis*. According to the literature, fish lysozyme activity is influenced by various probiotic bacterial species. For example, dietary supplementation with *L. mesenteroides* in brown trout (*Salmo trutta*) has been shown to positively affect lysozyme activity (45). Similarly, *L. lactis*-enriched diets modulated lysozyme activity in grouper (*E. coioides*) (32). Our findings are consistent with these reports, demonstrating that supplementation with a mixture of *L. mesenteroides* and *L. lactis* enhanced serum lysozyme activity in African catfish.

When *L. mesenteroides* and *L. lactis* were administered separately, both increased serum peroxidase levels; however, their combination did not lead to a significant change in peroxidase content. Similar findings have been reported in sea bream (*Sparus aurata*) (20).

SOD is a key enzyme involved in the primary antioxidant defense system and plays a critical role in converting highly reactive superoxide radicals into the less harmful hydrogen peroxide. In our study, SOD activity was significantly elevated in all experimental groups. Similar findings have been reported in *L. rohita*, where dietary supplementation with *Bacillus subtilis* in combination with *Pseudomonas aeruginosa* and *Lactobacillus plantarum* resulted in increased antioxidant enzyme activity (26).

The epidermal mucus and skin of fish serve as essential components of the nonspecific defense mechanisms, forming a physical barrier that protects the organism from environmental stressors and pollutants (53). In the present study, fish fed diets

containing *L. mesenteroides* and *L. lactis* exhibited significantly increased mucus secretion compared with the control group. Furthermore, supplementation with these probiotic strains had a more pronounced effect on the innate immune system of African catfish. Compared with single-strain formulations, diets containing multiple probiotic strains appear to modulate the host immune response more effectively.

These findings support the conclusion that well-designed multispecies or multistrain probiotic preparations may exert greater health-promoting effects in the vertebrate gastrointestinal tract than single-strain probiotics (54, 55).

The effects of intestinal probiotics vary widely and depend on multiple factors, including microbial species, dosage, origin, and duration of administration. Therefore, selecting appropriate probiotic strains is essential for formulating effective combinations capable of exerting synergistic effects. In this study, the combination of *L. mesenteroides* and *L. lactis* demonstrated strong potential to enhance the growth performance and immune response of African catfish. Further research is recommended to optimize administration protocols and evaluate the practical applications of such probiotic formulations in aquaculture production systems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The animal study was approved by S.Seifullin Kazakh Agrotechnical Research University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AP: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization. AU: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization. RA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. ZA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. GK: Conceptualization, Data

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