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ORIGINAL RESEARCH

Investigating the impact of *Lactobacillus lactis* TISTR 1464 on growth performance, hematological parameters, digestive enzyme activity, immune response, and post-probiotic status in Nile tilapia (*Oreochromis niloticus*)

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Abstract This study examined the effects of different levels of supplementation with *Lactobacillus lactis* TISTR 1464 on the growth performance, hematological parameters, digestive enzymes, and immune response in of Nile tilapia (*Oreochromis niloticus*) one week post-cessation. Tilapia were fed diets supplemented with *L. lactis* at concentrations of 0 (control), 10^4, 10^6, and 10^8 CFU/g of diet in three replicates. The initial average weight of tilapia was 8.08±0.01 g. Throughout the 10-week supplementation period, the growth of tilapia was not significantly affected by different concentrations of *L. lactis* (P >0.05). However, tilapia on a diet supplemented with 10^8 CFU/g of *L. lactis* had higher whole-body crude lipid levels than the other groups (P <0.05). Additionally, they showed elevated lipase activity (P <0.05). Fish fed diets supplemented with 10^6 and 10^8 CFU/g of *L. lactis* demonstrated significantly higher levels of hematocrit, hemoglobin, and white blood cells (WBC) compared to those fed the control diet. No significant differences were found in cholesterol, albumin, globulin, and liver function biomarkers among the dietary groups. Fish on a diet supplemented with 10^8 CFU/g of *L. lactis* also exhibited increased levels of catalase (CAT), superoxide dismutase, and lysozyme (P <0.05), with no difference in myeloperoxidase. After one week of cessation, CAT levels in fish fed *L. lactis* supplemented diet remained considerably higher than those in the control group (P <0.05), despite no noticeable differences in red blood cells (RBC) and WBC. In conclusion, adding 10^8 CFU/g of *L. lactis* to the Nile tilapia diet boosted lipase activity and stimulated immune responses. However, one week post-cessation, the immune response diminished, suggesting that continuous dietary supplementation with *L. lactis* is necessary for enhancing immune response in Nile tilapia.

Keywords *Lactobacillus lactis* . Probiotic . Immunostimulant . Cessation probiotic

Introduction

Nile tilapia has emerged as an important fish species in aquaculture. It is the third largest aquaculture in the world, after the grass carp and silver carp (FAO 2020). The aquaculture of Nile tilapia tends to expand continuously. Various challenges associated with the aquaculture of tilapia are often linked to disease re-

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sulting from intensive cultivation, water quality management, and improper feeding practices, leading to economic losses (Arumugam et al. 2023). The prevailing treatment for most infections in tilapia is the use of antibiotics. However, the indiscriminate use of antibiotics, especially in cases where the pathogenic microorganisms are unknown and appropriate dosages are not clear, has led to the emergence of drug-resistant bacteria (Chitmanat et al. 2016). Furthermore, the presence of antibiotic residues in fish after treatment and their potential transmission through the food chain to consumers raise concerns about food safety (Chen et al. 2020). In light of these challenges, probiotics have emerged as an alternative method for disease management in tilapia, with potential benefits in improving fish immunity and reducing the dependency on antibiotics (Arumugam et al. 2023). Probiotic microorganisms have gained interest in commercial aquaculture due to their ability to promote fish health, stimulate growth, and boost immunity while remaining safe for both hosts and consumers (Phinyo et al. 2024; El-Saadony et al. 2021). However, the excessive and rapid application of probiotics in aquatic animals may induce microbial dysbiosis and adversely affect the health of susceptible hosts (Xia et al. 2018). Probiotic microorganisms selected for supplementation in aquatic or fish diets yielded different results in growth, health promotion, and immune response outcomes (Assan et al. 2022; Phinyo et al. 2021). Therefore, selecting the appropriate type and level of probiotics for supplementation in diets is crucial to ensure their effective use in aquatic animals.

Lactic acid bacteria (LAB) are commonly found as part of the gut microbiota (Tang et al. 2023). Both spherical and rod-shaped bacteria can produce bacteriocin, a peptide with antimicrobial activity (Tang et al. 2022). Previous studies have shown that LAB can inhibit various pathogens, including *Salmonella typhi*, *Staphylococcus aureus,* and *Pseudomonas putida* (Amarantini et al. 2019). For example, *L. plantarum* AH78 inhibits pathogenic bacteria such as *P. aeruginosa, Klebsiella pneumonia,* and *Streptococcus iniae* (Hamdan et al. 2016). Moreover, *Lactococcus* (*Lc.*) *lactis* can inhibit aquatic pathogens such as *Aeromonas hydrophila*, *A. salmonicida, Lc garviae, Vibrio anguillarum*, *Yersinia ruckeri*, and *S. parauberis* (Balcázar et al. 2008; Kim et al. 2013). Among the LAB species, *Lactobacillus* (*L.*) has been a popular choice for probiotic supplementation in fish. Previously, *L. acidophilus* was administered to common carp at concentrations of 10^4 and 10^6 CFU/mL to improve growth (Adeshina et al. 2020). Other studies on Nile tilapia involved the administration of *L. plantarum* at 10^{10} CFU/g of diet (Jatoba´ et al. 2011), *L. plantarum* AH 78 at 0.5-1% (w/w) (Hamdan et al. 2016), and *Lc. lactis* subsp. *lactis* JCM5805 at 10^8 CFU/g of diet (Xia et al. 2018). In addition, LAB supplementation improved immunity and resistance to pathogens in fish. Examples include introducing *L. sakei* at a dosage of 10^6 CFU/g of diet in Common carp (*Cyprinus carpio*) (Salih et al. 2023), *Lc. rhamnosus* ATCC 7469 at a concentration of 10^8 CFU/g of diet in Rainbow trout (Hooshyar et al. 2020), *Lc. lactis* BFE 920 at a concentration of 2.5×10^7 CFU/g of diet in Olive flounder (*Paralichthys olivaceus*) (Kim et al. 2013), and *L. plantarum* AH 78 at a concentration of 0.5-1% (w/w) in Nile tilapia (Hamdan et al. 2016). Moreover, the administration of *Lactobacillus* in fish has been shown to influence gut microbiota diversity (Foysal et al. 2020), digestive enzyme (Mohammadian et al. 2017), feed utilization (Salih et al. 2023), immune response, and disease resistance in fish (Feng et al. 2019). While probiotics have demonstrated promising effects on fish health, it is equally important to study the effect of probiotic cessation. Previous studies showed that post-cessation of probiotics resulted in changes in gut microbiota, immune response, and digestive enzyme activity (Xia et al. 2018; Zhang et al. 2019b; Sagada et al. 2023). Therefore, understanding the effects of probiotic supplementation and its subsequent cessation on growth, immune response, and enzymatic activity of the digestive tract is essential in planning appropriate probiotic regimes for Nile tilapia culture. In the context of *L. lactis* supplementation in Nile tilapia, studies on its impact on immune response are limited. Therefore, this research aimed to explore the effects of varying levels of *L. lactis* supplementation in tilapia diets on growth performance, digestive enzymes, and immune response in Nile tilapia. This study also investigated post-cessation effects on fish enzymes and immunity to determine appropriate probiotic supplementation and cessation periods for tilapia culture.

Materials and methods

Ethical approval

The experimental procedures adhered to the requirements of the Institute of Animal for Scientific Purposes Development in Thailand. This research was approved by the Animal Ethics Committee of Naresuan Uni-

versity (Approval No. 0005/2564, 2021).

In vitro antibacterial activity against pathogenic bacteria of *Lactobacillus* TISTR 1464

The *L. lactis* was derived from Thailand Institute of Scientific and Technological Research (TISTR) bacteria code no. 1464. The antimicrobial activity of *L. lactis* was tested using the agar well diffusion method. *L. lactis* was inoculated into de Man, Rogosa & Sharpe (MRS) broth (Himedia) and incubated at 37 °C for 18-24 h. Pathogenic bacteria such as *Aeromonas hydrophila*, and *Pseudomonas aeruginosa* were cultured in Luria Bertani (LB) broth (Himedia) while *S. agalactiae* were cultured in Tryptic soy broth (TSB, Himedia). The pathogenic bacteria were incubated at 37 °C for 18-24 h. The turbidity of the cell culture was adjusted using standard McFarland no. 0.5 before swabbing onto media agar. The *L. lactis* was transferred into wells with a diameter of 0.5 mm on the agar plate. The negative control used microorganism-free of LB and TSB medium. The plate was incubated at 37 °C for 24 h. The inhibition zone was measured and calculated using the formula; diameter of clear zone/diameter of the well.

Experimental diet preparation

The basal diet preparation consisted of 36.17% crude protein, 8.13% crude lipid, and 18.71 MJ/kg of gross energy diet. The composition of diet ingredients is shown in Table 2. All the ingredients were finely ground using a 600 µm sieve and thoroughly mixed using a food mixer (SKU00038, Watchara Food and Stating Co., Ltd., Thailand). To this mixture, 400 mL /kg of deionized water was added. After thorough mixing, the resulting mixture was passed through a 3 mm sieve of a meat grinder (Bae Heab Thye, Bangkok, Thailand), and then dehydrated at 60 °C for 24 h. The experimental diet was stored at -20 °C until utilized.

The experimental diet preparation involved supplementing the basal diet with four different formulations of *L. lactis* at concentrations of 0, 10^4, 10^6, and 10^8 CFU/g of diet, respectively. The *L. lactis* TISTR 1464 was cultured in MRS broth. The cells were harvested by centrifuging at 5,000 rpm for 10 min at room temperature. The cell pellets were suspended in phosphate buffer saline (PBS) pH 7.0 (Liu et al. 2013). The cell suspension was adjusted based on the optical density at 600 nm compared with the standard curve. After that, the cell suspension was sprayed onto the feed daily.

Fish preparation, and experimental procedure

Nile tilapia with an average body weight of 0.81±0.09 g/fish were purchased from a local farm in Phitsanulok Province in Thailand. Fish were acclimatized in 500 L fiberglass tanks and fed twice daily on a commercial diet containing not less than 40% protein and 4% fat for 4 weeks. Subsequently, the fish were randomly placed into 12 glass tanks with each tank holding 20 fish (3 tanks per replicate) and having a volume of 120 L of water (0.90 x 0.45 x 0.45 m^3). The experimental tanks were exposed to 12 h of darkness/12 h of light. During the acclimatization period, fish were fed *L. lactis*-free basal diet formulation for seven days. Fish were fed at 5% (w/w) of their body weight twice daily (9.00 AM. and 17.00 PM). Following the acclimatization period, the fish were provided with a specialized diet containing varying amounts of *L. lactis*, specifically at concentrations of 0, 10^4, 10^6, and 10^8 CFU/g. The feeding regimen involved providing the experimental diet at 5% (w/w) of the fish's body weight per day for 10 weeks, with adjustments to the feed quantity made every two weeks based on the fish's weight. After that, the fish were switched back to a control diet for one week. The water quality parameters were monitored throughout the feed trial. Temperature was maintained at 29.23±0.45 °C, pH at 7.92±0.16, dissolved oxygen (DO) at 6.18±0.08 mg/L, and ammonia-N at 1.17±0.09 mg/L. About 70% of the water was replaced daily with dechlorinated water.

Growth performance and index values

Evaluation of growth performance metrics and index values in Nile tilapia was conducted following a 10-week feeding regimen involving varying levels of *L. lactis.* The parameters were computed using the following formulas: weight gain (WG, g/fish) = [final body weight; FBW (g) − initial body weight; IBW (g)]∕number of fish; specific growth rate (SGR, %∕day) = 100 × [Ln FBW (g) − Ln IBW (g)]∕duration (70

days); average daily gain (ADG, g/day) = weight gain/duration (70 days); protein efficiency ratio (PER, %) = 100 × [weight gain/(total intake of dry feed × protein content in feed]; feed conversion ratio (FCR) = total intake of dry feed ⁄weight gain; survival rate (%) = 100 × [final number of fishes ⁄initial number of fishes]; viscerosomatic index (VSI, %) = 100 × [visceral weight/body weight]; hepatosomatic index (HSI, %) = 100 × [liver weight (g)/body weight (g)].

Chemical composition analysis

The chemical composition of the experimental diet and the fish's whole-body (where two fish per tank were pooled, $N = 3$) were analyzed using AOAC's official analysis methods. (2000). Moisture content was determined through a hot air oven at 105°C for 48 h. Crude protein was analyzed by the Kjeldahl method using a semi-automatic Kjeldahl apparatus (Gerhardt VAPODEST, Germany). Crude lipid was extracted from petroleum ether using the classic Soxhlet apparatus (Gerhardt, Germany). For crude fiber analysis, samples were digested with 1.25% (v/v) of H_2SO_4, and 1.25% (w/v) of NaOH using a fiber bag, following Hemamalini et al.'s method (2020). For the ash analysis, samples were calcined in a muffle furnace at 550 °C for 5 h.

Blood and tissue collection

Following 10 weeks of feeding the experimental diet, and one week post-cessation, fish were transitioned to a control diet and were anesthetized with a clove oil solution. The clove oil solution was prepared from clove oil and ethanol in a ratio of 1:9. Blood was collected from the caudal vein ($N = 6$) using a 1 mL sterile syringe. The fish blood was divided into two parts. First, it was collected into an anticoagulant-containing blood collection tube (K_3 EDTA Blood collection tube, Fushino, China) for analysis of red blood cells (RBC), white blood cells (WBC), hemoglobin, and for a blood smear analysis to identify WBC (stained followed by Wright's-stain protocol). The second part was used to extract serum for blood chemistry and immune response. Blood samples were placed in anticoagulant-free tubes, allowed to clot, and then stored overnight at 4 °C. These samples were then centrifuged at 3,000 rpm for 15 min at 4 °C, and stored at -80 °C until utilized (Di et al. 2019). For tissue collection, visceral organs and the liver were weighed to compute indices as outlined in section 2.4. Samples of mid-intestines (2 fish/tank) were collected and stored in a cryotube at -80 °C until analyses of enzyme activity were needed.

Hematological parameter

Blood hematological parameters were assessed as follows: hematocrit was determined using the micro-hematocrit centrifugation technique at 12,000 rpm for 5 min. RBC and WBC counts were obtained by diluting samples with Thoma pipettes, and using a hemacytometer. Hemoglobin (Hb) levels were determined using the cyanmethemoglobin methods as outlined by Blaxhall and Daisley (1973), employing a spectrophotometer at 540 nm wavelength.

Serum biochemistry profiles (two fish per tank were pooled, $N = 3$) were determined as follows; total protein was assessed using the Biuret method (Zheng et al. 2017), while albumin levels were determined using the Bromocresol-green method (Speicher et al. 1978). Total cholesterol and creatinine levels were estimated using the enzymatic colorimetric method. Blood urea nitrogen (BUN) was measured by the Urease UV-kinetic method. For liver function parameters, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and albumin were analyzed using Cobas c 311 analyzers manufactured by Roche Diagnostics (Switzerland). Globulin levels were obtained by subtracting albumin from total protein concentration (Coull et al. 1991).

Digestive enzyme activity assay

For the digestive enzymes, the mid-intestine (two fish per tank of each period, $N = 6$) was homogenized with ice-cold PBS, pH 7.4 (1:4 parts of tissues) chilled on ice. The homogenate was centrifuged at 8,000 rpm for 15 min at 4 °C, with the supernatant collected for analysis. The protease activity was assessed

using 0.65% (w/v) casein in 50 mM potassium phosphate buffer as the substrate, measured at OD 660 nm compared to the tyrosine standard curve (Zhang et al. 2017). Lipase activity was determined using 0.01 M of *p*-nitrophenyl palmitate (*p*-NPP) as the substrate, measured at an OD of 410 nm, and compared to the standard curve of *p*-nitrophenol (*p*-NP). For the hydrolysis of starch, α-amylase activity was assessed using 1% (w/v) of soluble starch as the substrate and measured reducing sugar using the DNS (3,5-dinitrosalicylic acid) assay (Miller 1959) at OD 540 nm wavelength and compared to the standard chart of maltose.

The sample's protein concentration was measured using the Lowry method, at OD 650 nm, compared to a Bovine serum albumin (BSA) standard curve. The enzyme activity assay and protein reaction measurements were performed using a microplate reader (BioTek Synergy HT, USA) in 96-well microplates.

Serum antioxidant enzyme activity and immune response

The assessment of antioxidant enzyme activity in fish serum (two fish per tank for each period, $N = 6$) involved the following procedures: catalase activity (CAT) was measured following the protocol established by Wangkahart et al. (2022). Initially, 10 µL of serum was added to 0.05% freshly cold H_2O_2 in phosphate-buffered saline (PBS), pH 7.4, as the substrate. Enzyme reactions were assayed in 96-well plates, with CAT activity measured at 20 s and 80 s. Absorbance was recorded at 450 nm using a microplate reader (BioTek Synergy HT, USA). Superoxide dismutase (SOD) activity was analyzed using a SOD determination kit from Sigma-Aldrich, following the manufacturer's guidelines, and absorbance was measured at 450 nm using a microplate reader. Myeloperoxidase (MPO) activity was determined by adding 35 µL of 3,3′-5,5′-tetramethyl benzidine hydrochloride to 20 µL serum solution (dilute in PBS, pH 7.4), followed by the addition of 5 mM of H_2O_2. The reaction was incubated for 1 min at room temperature, and the reaction was stopped by adding 2M H_2SO_4. The color of the enzyme reaction was measured at OD 450 nm (Pulli et al. 2013).

Lysozyme activity (LZM) was assessed by adding 25 µL of serum to 100 µL of 0.2 mg/mL of *Micrococcus lysodeikticus* in PBS, pH 7.4. The microplate reader was gently shaken for 3 s, and a decrease in turbidity was measured at OD of 450 nm at 30 s and 180 s, respectively (Luck 1974).

Statistical analysis

The normality of the data was assessed using the Shapiro-Wilk test. Data were analyzed using a one-way analysis of variance (ANOVA) with significance set at $P < 0.05$: mean values were significantly different when analyzed with IBM SPSS statistics (version 19.0, IBM Corporation, USA). *Post-hoc* or pairwise comparisons were made using Duncan's multiple range tests (DMRT). All data were presented as mean ± SEM.

Results

Inhibition of pathogenic microorganisms in aquatic animals

The test results showing the ability of *L. lactis* to inhibit aquatic pathogens are presented in Table 1. The *L. lactis* effectively inhibited the growth of three pathogenic bacteria that infect aquatic animals: *A. hydrophila*, *P. aeruginosa,* and *S. agalactiae*. The inhibition ratios for each pathogen, ranging from 2.02±0.29 to 2.10±0.20, were not significantly different ($P > 0.05$). In contrast, the negative control did not show any inhibition zones. Notably, *L. lactis* displayed substantial efficacy in inhibiting the growth of *A. hydrophila* and *S. agalactiae*.

Growth performance and body condition indices

The growth performance of tilapia after being fed a supplemented diet with different levels of *L. lactis* (0, 10^4, 10^6 and 10^8 CFU/g) for 10 weeks is shown in Table 3. We found that growth performance and feed utilization parameters, such as final body weight (FBW), weight gain (WG), specific growth rate (SGR), average daily gain (ADG), and protein efficiency ratio (PER) exceeded those of the control group ($P > 0.05$).

However, *L. lactis* supplementation at different concentrations did not affect the survival rate (P >0.05). There was no statistically significant difference between the VSI and HSI values (P >0.05).

Whole-body analysis

After being fed the experimental diet, the comprehensive analysis of whole-body composition is shown in Table 4. The crude lipid contents in fish supplemented with *L. lactis* at 10^8 CFU/g were significantly higher compared to those fed other formulas (P <0.05). In contrast, no significant differences were observed in the levels of moisture content, crude protein and ash found in the whole-body composition following the consumption of the experimental diet (P >0.05).

Table 1 Inhibition activity against the aquatic pathogens by *L. lactis* TISTR 1464

Pathogen	Quality	Ratio*
A. hydrophila	+++	2.10±0.20
P. aeruginosa	+	2.03±0.20
S. agalactiae	+++	2.02±0.29

Values shown are mean± standard error of mean (SEM) of three replicates (N = 3).
Efficiency of antibacterial; mild (+), strong (++), and very strong (+++)

Table 2 Formulation and chemical composition of basal diet

Diet ingredients (g/kg)	Basal diet
Fish meal	220
Soybean meal	300
Corn meal	150
Rice bran	200
Wheat flour	30
Corn gluten meal	50
Corn oil	30
[1]Mineral and vitamin premix	20
Chemical composition (dry basis)	
Moisture (%)	6.86
Crude protein (%)	36.17
Crude lipid (%)	8.13
Fiber (%)	5.51
Ash (%)	10.75
[2]GE (MJ/kg)	18.71
[3]NFE (%)	44.95

[1] Mineral and vitamin premix (per kg): calcium, 100 g; phosphorous, 80 g; magnesium, 2.16 g; zinc sulfate, 1.6 g; ferrous sulfate, 1.24 mg; copper sulfate, 1.2 g; manganese sulfate, 1.2 g; iodine, 0.76 g; potassium, 0.23 g; cobalt chloride, 0.2 g; sodium selenite, 0.01 g; vitamin A acetate, 20,000 IU; vitamin D3, 6,000,000 IU; tocopherol, 5,500 IU; nicotinic acid, 47.5 g; L-ascorbic acid, 21 g; methionine, 12 g; riboflavin, 10 g; menadione nicotin-amide bisulfite, 8 g; pantothenic acid, 7.5 g; pyridoxine hydrochloride, 4.5 g; thiamine nitrate, 2.5 g; cyanocobalamin, 2.5 g; folic acid, 1 g.
[2] Gross energy (GE, MJ/kg) calculated from the formula: crude protein = 23.67 kJ/g, crude lipid = 39.54 kJ/g and carbohydrates = 17.57 kJ/g
[3] Nitrogen free extract (NFE, %) calculated from the formula: 100 - [crude protein (%) + crude lipid (%) + ash (%)]

Table 3 Growth performance, feed utilization, and index value of Nile tilapia fed different concentrations of *L. lactis* for 10 weeks

Parameters	*L. lactis* level (CFU/g)				P-value
	0	10^4	10^6	10^8	
IBW (g/fish)	8.06±0.03	8.08±0.02	8.08±0.04	8.09±0.03	0.869
FBW (g/fish)	59.76±2.18	59.64±1.40	62.91±2.57	58.56±1.59	0.489
WG (g/fish)	51.7±2.21	51.56±1.40	54.82±2.54	50.46±1.57	0.485
SGR (%/day)	2.86±0.06	2.85±0.03	2.93±0.05	2.83±0.03	0.465
ADG (g/day)	0.74±0.03	0.74±0.02	0.78±0.04	0.72±0.02	0.545
PER	2.08±0.10	2.14±0.04	2.20±0.10	2.12±0.06	0.762
FCR	1.33±0.07	1.29±0.03	1.26±0.06	1.31±0.04	0.485
Survival rate (%)	98.33±1.67	98.33±1.67	96.67±3.33	100±0.00	0.728
VSI (%)	8.11±0.36	7.88±0.25	8.45±0.20	8.23±0.33	0.610
HSI (%)	1.02±0.06	1.07±0.05	0.96±0.16	1.09±0.12	0.814

Values shown are mean± standard error of mean (SEM) of three replicates.
Abbreviations: IBW: initial body weight (g/fish); FBW: final body weight (g/fish); WG: weight gain (g/fish); SGR: specific growth rate (%/day); ADG: average daily gain (g/day); PER: Protein efficiency ratio ; FCR: feed conversion ratio; VSI: viscerosomatic index (%); HSI: hepatosomatic index (%).

Digestive enzyme activities

Fig. 1 presents the effect of a diet supplemented with *L. lactis* on digestive enzyme activity over a period of 10 weeks and one week following the cessation of *L. lactis*. Supplementation with *L. lactis* at 10^8 CFU/g for 10 weeks resulted in significantly higher lipase activity (Fig. 1A) compared to other treatment groups (P <0.05). In the same vein, no significant differences in amylase (Fig. 1B) and protease activities (Fig. 1C) were observed (P >0.05). Furthermore, one week post-cessation of *L. lactis*, all formulations supplemented with *L. lactis* at 10^4, and 10^8 CFU/g showed statistically higher lipase activity (Fig. 1A) than the control diets (P <0.05). Moreover, the protease activity (Fig. 1C) in formulations supplemented with *L. lactis* at 10^6 and 10^8 CFU/g was significantly higher than in the control diet (P <0.05). However, *L. lactis* supplementation had no significant effect on intestinal amylase activity.

Blood hematology

Upon completion of the *L. lactis*-supplemented diet, the results showed that hematocrit (Ht), and hemoglobin (Hb) were significantly higher than in the control diet (P <0.05) as shown in Table 5. However, no differences were found in mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) (P >0.05). Nevertheless, after the discontinuation

Table 4 Whole-body composition of Nile tilapia fed different levels of *L. lactis* for 10 weeks

Parameters	*L. lactis* level (CFU/g)				P-value
	0	10^4	10^6	10^8	
Moisture (%)	75.88±0.95	75.53±1.14	76.33±1.12	75.01±0.74	0.817
Crude protein (%)	13.90±0.15	14.31±0.15	13.63±0.04	13.97±0.17	0.050
Crude lipid (%)	4.24±0.01^b	4.01±0.02^b	4.03±0.33^b	4.90±0.04^a	0.016
Ash (%)	4.63±0.07	4.88±0.16	4.58±0.07	4.86±0.07	0.134

Values shown are mean± standard error of mean (SEM) of three replicates.
Different superscript letters in the same row represent significantly different values (P <0.05).

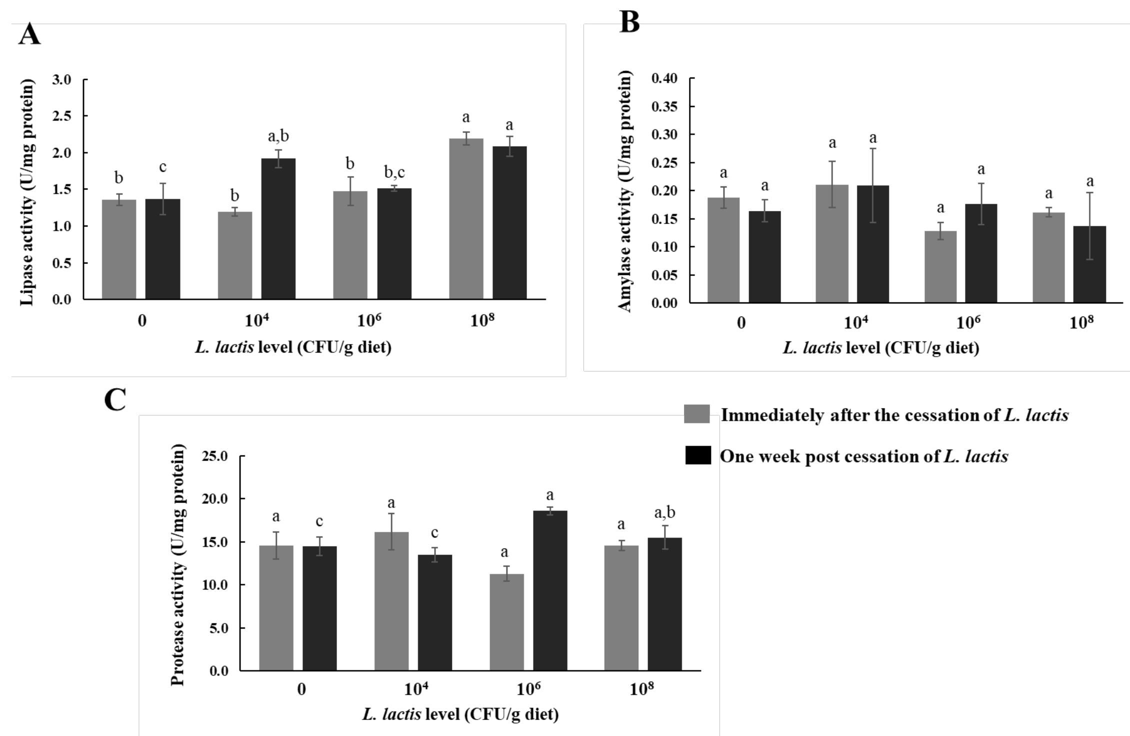

Fig. 1 Digestive enzyme activities including lipase (A), amylase (B), and protease activities (C) of Nile tilapia fed with different concentrations of *L. lactis* for 10 weeks, and one week post-cessation of *L. lactis*. Each bar represents the mean ± SEM (*N* = 6). Different letters above the bars indicate significant differences within the same period (P <0.05).

of the *L. lactis* supplementation diet for one week, there were no noteworthy differences in Ht, RBC, Hb, MCHC, MCH, and MCV (P >0.05).

As shown in Table 6, following the supplementation period, the amount of WBC and lymphocytes of fish fed the *L. lactis* diet at 10^8 CFU/g were significantly higher than those of the control diet (P <0.05). However, one week following the discontinuation of *L. lactis* supplementation, there were no differences in either the amount or type of WBC (P >0.05).

Blood chemistry

The results of blood chemistry analysis conducted on fish serum following the consumption of the experimental feed are shown in Table 7. There were no significant differences in total protein, albumin, globulin, and cholesterol values across all dietary regimens (P >0.05). Similarly, the liver and kidney function indicators such as AST, ALP, creatinine, and BUN were not significantly different (P >0.05).

Serum non-immune response

The ability of *L. lactis* to enhance the immune response in a fish fed with *L. lactis* supplemented diet for 10 weeks and one week post-cessation is displayed in Table 8. It was found that fish exposed to *L. lactis* supplementation at 10^8 CFU/g had significantly higher CAT, SOD, and LZM activities compared to those

Table 5 Blood hematology of Nile tilapia fed different concentrations of *L. lactis* for 10 weeks, and one week post-cessation of *L. lactis*

Parameters	*L. lactis* level (CFU/g diet)				P-Value
	0	10^4	10^6	10^8	
Immediately after the cessation of *L. lactis*					
Ht (%)	25.92±0.36^b	28.83±0.08^a	28.67±1.01^a	28.83±0.65^a	0.028
RBC (× 10^6 cell/mm^3)	1.28±0.03^b	1.44±0.04^a	1.49±0.06^a	1.40±0.02^{a,b}	0.028
Hb (g/dL)	10.59±0.13^b	13.17±0.92^a	12.74±0.44^a	12.86±0.43^a	0.038
MCHC (g/dL)	40.89±1.09	45.69±3.24	44.56±2.21	44.59±0.83	0.433
MCH (pg/cell)	82.65±0.92	91.49±6.53	85.74±4.45	91.85±3.71	0.422
MCV (fL)	202.47±6.49	200.36±5.26	192.39±0.63	205.83±4.97	0.319
One week post-cessation of *L. lactis*					
Ht (%)	30.17±1.59	29.83±1.21	27.83±0.83	28.17±0.60	0.407
RBC (× 10^6 cell/mm^3)	1.13±0.18	0.96±0.08	1.01±0.16	1.07±0.18	0.611
Hb (g/dL)	12.92±0.36	13.09±0.18	13.37±0.13	12.99±0.33	0.670
MCHC (g/dL)	33.33±1.76	31.56±1.15	34.82±0.47	36.43±0.50	0.068
MCH (pg/cell)	89.40±3.23	97.83±4.45	96.75±5.50	97.66±7.03	0.637
MCV (fL)	270.44±21.73	309.85±6.17	277.94±16.23	268.41±20.79	0.361

Values shown are mean± standard error of mean (SEM) of three replicates (*N* = 6).
Different superscript letters in the same row represent significantly different values (P <0.05).
Abbreviations (Unit) and formula: Ht: hematocrit (%); RBC: red blood cells (× 10^6 cell/mm^3); Hb: Hemoglobin (g/dL). Blood parameters calculated according to Witeska et al. (2022) using the formula; MCHC: mean corpuscular hemoglobin concentration (g/deciliter; dL) = (Hb × 100)/Ht; MCH: mean corpuscular hemoglobin (pg/cell) = Hb/RBC; MCV: mean corpuscular volume (fL) = 10 × [Ht/RBC]

Table 6 White blood cells in Nile tilapia fed different concentrations of *L. lactis* for 10 weeks, and one week post-cessation of *L. lactis*

Type of WBC	*L. lactis* level (CFU/g diet)				P-Value
	0	10^4	10^6	10^8	
Immediately after the cessation of *L. lactis*					
WBC (× 10^3, cell/mm^3)	8.71±0.51^b	9.31±1.01^b	11.51±0.60^a	12.31±0.31^a	0.013
Neutrophile (%)	31.00±1.04	30.33±1.30	27.83±0.06	25.67±2.09	0.085
Lymphocyte (%)	66.00±0.29^b	66.83±1.59^b	69.83±0.33^{a,b}	71.67±1.92^a	0.043
Monocyte (%)	1.83±0.44	1.83±0.33	2.00±0.29	2.33±0.17	0.672
One week post cessation of *L. lactis*					
WBC (× 10^3, cell/mm^3)	11.81±1.22	13.81±0.49	12.08±0.74	13.76±0.46	0.220
Neutrophile (%)	30.83±2.89	33.17±3.09	28.67±0.93	31.17±1.17	0.591
Lymphocyte (%)	66.67±2.35	64.33±3.49	66.00±2.00	66.50±1.76	0.905
Monocyte (%)	1.00±0.29	0.83±0.33	1.83±0.33	0.67±0.17	0.083

Values shown are mean± standard error of mean (SEM) of three replicates (*N* = 6).
Different superscript letters in the same row represent significantly different values (P <0.05).
Abbreviation (Unit): WBC: white blood cells (× 10^3 cell/mm^3)

on the control diet (P <0.05). However, no significant differences were noted in MPO levels among fish fed all experimental diets (P >0.05). One week post-cessation, fish fed with 10^4, 10^6 and 10^8 CFU/g of *L. lactis* still had significantly higher CAT levels than the control group (P <0.05). Nevertheless, values of SOD, MPO, and LZM values were not significantly different (P >0.05).

Discussion

The growing concern about disease in tilapia culture has led to a focus on probiotic supplementation for immune enhancement, serving as an alternative to antibiotics in fish farming (Ouwehand et al. 2016). *Lactobacillus* is an effective probiotic for aquatic animals and can inhibit the growth of pathogenic microbes (Reid and Bruce 2001). Studies investigating the fish immune system's response following cessation of probiotics are essential for optimal supplementation timing. In this study, *L. lactis* TISTR 1464 displayed inhibitory effects against three species of aquatic pathogens, namely *A. hydrophila*, *P. aeruginosa,* and *S. agalactiae*. This is consistent with studies indicating *L. lactis*'s capacity *to* produce inhibitory molecules, such as bacteriocin, which is effective against various pathogens. Bacteriocin is produced during the primary phase of bacterial growth. It is produced by *Lactobacilli* and can inhibit pathogens such as Lactacin G, Nisin, and Lactococcin M (Zacharof and Lovitt 2012; Chen and Hoover 2003; Hasan et al. 2023). In addition, it is safe for the host and is sensitive to degradation by proteolytic enzymes (Meade et al. 2020). Similar studies showed LAB's ability to inhibit pathogens such as *E. coli*, *S. aureus*, *S. enteritidis*, and *A. hydrophila* (Feng et al. 2019). *L. lactis* can inhibit both gram-positive and gram-negative bacteria, including *S. aureus*, *S. iniae*, *S. parauberis*, *A. hydrophila*, *Escherichia coli*, *Salmonella* spp., and *V. anguillarum* (Brashears and Durre 1999; Brashears et al. 1998; Nguyen et al. 2017, Feng et al. 2019). Moreover, *Lactobacillus* can inhibit pathogenic fungi, including *Aspergillus niger*, *A. flavus*, and *A. nidulans* (Al-Dhabi et al. 2020).

Table 7 Blood chemistry measurements in Nile tilapia fed different concentrations of *L. lactis* for 10 weeks

Parameters	*L. lactis* level (CFU/g diet)				P-Value
	0	10^4	10^6	10^8	
Total protein (g/dL)	2.23±0.03	2.03±0.15	2.27±0.09	2.40±0.10	0.154
Albumin (g/dL)	0.87±0.03	0.77±0.07	0.80±0.00	0.90±0.06	0.250
Globulin (g/dL)	1.37±0.03	1.27±0.09	1.47±0.09	1.50±0.06	0.163
Cholesterol (g/dL)	126.33±9.49	120.67±10.74	127.33±4.67	137.00±19.97	0.829
AST (U/L)	11.00±3.06	23.33±8.41	39.00±12.29	21.00±1.00	0.153
ALP (U/L)	25.00±3.06	23.67±3.18	22.67±0.33	28.33±1.76	0.412
Creatinine	0.14±0.02	0.14±0.03	0.14±0.01	0.12±0.01	0.855
BUN (mg/dL)	1.40±0.21	1.63±0.33	1.20±0.06	1.27±0.12	0.497

Values shown are mean± standard error of mean (SEM) of three replicates ($N = 3$).
Different superscript letters in the same row represent significantly different values (P <0.05).
Abbreviations: AST: aspartate aminotransferase (U/L); ALP: alkaline phosphatase (U/L); BUN: blood urea nitrogen (mg/dL)

Table 8 Non-specific immunity and antioxidant enzyme in serum of Nile tilapia fed different levels of *L. lactis* for 10 weeks, and one week post-cessation of *L. lactis*

Parameters	*L. lactis* level (CFU/g diet)				P-value
	0	10^4	10^6	10^8	
Immediately after the cessation of *L. lactis*					
CAT (U/mL)	3.96±0.38^b	5.63±0.95^b	10.83±1.50^a	11.25±1.02^a	0.000
SOD (U/mL)	24.32±3.72^b	26.49±4.04^b	31.59±3.46^{a,b}	34.52±3.94^a	0.037
MPO (OD 450 nm)	2.33±0.18	2.34±0.15	2.57±0.11	2.54±0.07	0.545
LZM (U/mL)	3.87±0.61^c	3.93±0.33^{b,c}	5.13±0.38^b	6.87±0.71^a	0.001
One week post cessation of *L. lactis*					
CAT (U/mL)	5.63±1.50^c	12.71±0.87^b	14.58±1.79^a	14.17±1.88^{a,b}	0.000
SOD (U/mL)	30.51±4.20	28.58±2.35	32.60±3.36	28.17±3.62	0.412
MPO (OD 450 nm)	2.82±0.28	2.56±0.24	2.36±0.26	2.33±0.21	0.611
LZM (U/mL)	5.27±0.89	5.80±0.52	8.00±0.52	6.33±1.22	0.093

Values shown are mean± standard error of mean (SEM) of three replicates ($N = 6$).
Different superscript letters in the same row represent significantly different values (P <0.05).
Abbreviations: CAT: catalase activity (U/mL); SOD: superoxide dismutase (U/mL); MPO: myeloperoxidase activity (Optical density, OD measured at 450 nm); LZM: lysozyme activity (U/mL)

Immune response in fish after exposure to probiotic can be gauged by assessing antioxidant enzymes, MPO, and LZM. Antioxidant enzymes such as SOD, and CAT protect cells against oxidative stress in aerobic organisms. CAT is an enzyme that breaks down hydrogen peroxide, which is harmful to cells (Zhu et al. 2021; Nandi et al. 2019). SOD is an enzyme that responds to oxidative stress in cells and protects them from free radicals (Fukai and Ushio-Fukai 2011). In addition, MPO and LZM activities act as immune responses against pathogen attacks and protect cells against microbial threats (Khan et al. 2018; Biller-takahashi and Urbinati 2014; Ferraboschi et al. 2021). Our study found that supplementing tilapia feeds with *L. lactis* at concentrations of 10^8 CFU/g for 10 weeks significantly increased the activity of antioxidant enzymes (CAT, and SOD), and LZM. However, no discernible impact on MPO was detected. Previous research has indicated that probiotics can boost immunity in fish (Lazado and Caipang 2014). For example, increased antioxidant activity was observed in the following: Japanese eel (*Anguilla japonica*) fed with *L. pantosus* PL11 supplemented diet (Lee et al. 2013), hybrid grouper (*Epinephelus lanceolatus x E. fuscoguttatus*) fed with *L. acidophilus* LAG01 supplemented diet (He et al. 2017), and pond loach (*Misgurnus anguillicaudatus*) fed with *L. helveticus* supplemented diet (Yang et al. 2021). LZM augmentation has been witnessed in various instances, including *L. plantarum* supplementation in tilapia (Gewaily et al. 2021), a blend of *L. rhamnosus* and *Lc. latis* in red sea bream (*P. major*) (Dawood et al. 2016), and *L. fermentum* in common carp (*C. carpio*) (Ahmadifar et al. 2019). Supplementation with probiotics induces alterations in the gut microbiota, leading to enhanced intestinal health and improved immunity in fish. This enhancement is associated with the reduction in pathogenic load facilitated by bacteriocin production (Shija et al. 2023). In conclusion, for optimal immune benefits, *L. lactis* supplementation in tilapia feed is recommended at a concentration of 10^8 CFU/g.

For growth performance, we found that supplementing diet with *L. lactis* did not affect tilapia growth. These findings are consistent with previous studies which found that LAB supplementation in aquaculture did not promote fish growth. For example, similar results were noted for *L. brevis* JCM 1170 and *L. acidophilus* JCM 1132 in hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) (Liu et al. 2013), *Lc. lactis* subsp. *lactis* I2 in olive flounder (*Paralichthys olivaceus*) (Heo et al. 2013) *L. acidophilus* in Siberian sturgeon (*Acipenser baerii*) (Mocanu et al. 2022), and *L. plantarum* in Sobaity (*Sparidentex hasta*) (Agh et al. 2022) and gold fish (*Carassius auratus gibelio*) (Hosseini et al. 2016). On the other hand, some studies have reported growth enhancement from LABs. For instance, *Lc. lactis* Z-2 in *Cyprinus carpio* improved digestive enzymes, including amylase, lipase and protease, and feed utilization (Wang et al. 2021). Others included, *Lc. lactis* in mandarin fish (*Siniperca chuatsi*) (Zhu et al. 2021), and *L. plantarum* in clownfish (*Amphiprion ocellaris*) (Paixão et al. 2020), *Labeo rohita* (Giri et al. 2013), and yellowtail (*Seriola quinqueradiata*) (Fukada et al. 2023). In a study by Xia et al. (2018), a combination of two microbial strains, *Lc. lactis* subsp. and *Lc. lactis* subsp. *lactis* JCM 5805 did not improve growth in Nile tilapia (*O. niloticus*). However, a growth increase was noted when combined with *L. rhamnosus* JCM 1136. The effect of probiotics on fish growth depends on various determinants. Specifically, the ability of probiotics to multiply within fish intestines (Allameh et al. 2017) is crucial. Moreover, the sustainable survival of probiotics in the gut requires prebiotic supplementation (Agh et al. 2022). The feeding methods and the concentration of probiotics also contribute to this effect. In this experiment, a feeding regimen equivalent to 5% of the fish's body weight was used. The amount of probiotics and feed that the fish receive will be different from feeding until satiation.

The chemical composition of fish is related to feed efficiency and post-feeding growth (Abdel-Tawwab et al. 2015). This study found that fish supplemented with 10^8 CFU/g of *L.lactis* had significantly higher whole body crude lipid content than fish fed an alternative formulation. Similarly, lipase activity within the digestive tract also increased after 10 weeks of supplementation. One week post-cessation, we found that fish previously fed *L. lactis* at 10^8 CFU/g had higher protease and lipase enzyme activity. A recent study found that *Lactobacillus* supplementation improved lipid utilization in fish (Dawood et al. 2016). Probiotic supplementation has a significant influence on the gut microbiome resulting in a balanced intestinal microbial environment and improved feed utilization. These effects extend to intestinal morphology, including an increased epithelial length, width, and thickness (Mohammadian et al. 2022). These changes result in increased nutrient accumulation in fish (Suzer et al. 2008; Krishnaveni et al. 2021). Furthermore, the influence of probiotics on the digestive system is evidenced by the increase in various digestive enzymes, such as amylase, lipase, and protease, as well as nutrient absorption enzymes, including Na+, K+-ATPase, γ-glu-

tamyl transferase (γ-GT), and creatine kinase (CK) (Zhang et al. 2019a). A study conducted by Renuka et al. (2013) on common carp (*C. carpio*) revealed that diet supplementation with *Lactobacillus* sp. at $2x10^7$ CFU/g resulted in heightened protease, amylase, and lipase activity in the digestive tract. Therefore, the increased gastrointestinal enzyme activity as reported by Mirghaed et al. (2018) highlights that probiotic supplementation significantly improves feed utilization in fish. Moreover, LAB probiotics also increase the overall chemical components of fish. For example, a study by Dawood et al. (2015b) demonstrated that β-glucan combined with *L. plantarum* (HK-LP), *L. rhamnosus* and *Lc. latis* at 10^6 cells/g in red sea bream (*Pagrus major*) increased whole-body crude lipid. Similarly, supplementation of diet with $2x10^8$ CFU/g *L. fermentum* in fish (Krishnaveni et al. 2021), and *S. faecium* and *L. acidophilus* in Nile tilapia (*O. niloticus*) diet resulted in higher whole-body crude protein and lipid content (Lara-Flores et al. 2003).

Blood parameters offer insights into fish stress and health stemming from environmental and nutritional responses (Witeska et al. 2022; Dawood et al. 2015a). WBC especially lymphocytes and monocytes play important roles in the immune system (Biller-takahashi and Urbinati 2014). Our study found that fish subjected to *L. lactis* supplementation had higher levels of Ht, RBC, and hemoglobin. Specifically, WBC and lymphocyte counts were higher in fish supplemented with *L. lactis* at 10^8 CFU/g compared to those on the control diet. However, hematology parameters did not differ significantly one week post-cessation. Previous studies have suggested the involvement of *Lactobacilli* in the production of vitamins A, B12, and RBC, where RBC and Hb play an important role in transporting oxygen to cells (Krishnaveni et al. 2021; Thompson et al. 2020). This aligns with a previous study assessing supplementation of *L. plantarum* in Sturgeon fish (*A. baerii*), which demonstrated an increase in RBC, Hb, and WBC, although no differences were observed in MCV, MCH, and MCHC. Additionally, a decrease in monocytes was reported (Pourgholam et al. 2017). Similarly, supplementation of diet with heat-killed *L. plantarum* L-137 at 20-50 mg/kg in catfish (*Clarias macrocephalus*) (Hien et al. 2021), and *L. fermentum* at $2x10^8$ CFU/g in the common carp (*C. carpio*) diet resulted in a higher WBC count, and an increased survival rate post-infection with *A. hydrophila* (Krishnaveni et al. 2021). In addition, *L. acidophilus* supplementation in striped catfish (*Pangasianodon hypophthalmus*) increased WBC, lymphocyte, and granulocyte counts. A 10^7 CFU/g supplement of *L. acidophilus* increased immunoglobulin levels (Akter et al. 2023). Therefore, the increase in white blood cell counts in fish was influenced by both *Lactobacillus* supplementation and the concentration used. Probiotics stimulate iron uptake by releasing organic acids in the intestines of the hosts, leading to increased WBCs, lymphocytes, and macrophages, thereby fostering immune activation in fish and influencing their resistance to pathogenic microorganisms (Hossain et al. 2022).

Various probiotics have different effects on the health of different fish species. Therefore, it is crucial to assess blood chemistry measurements to determine fish health following feeding AST and ALP serve as biomarkers for liver damage, playing a role in fatty acid and amino acid metabolism associated with glycolysis, citrate, and urea cycles (Yang et al. 2009; Sookoian and Pirola 2012). ALP, produced mainly in the liver, is a marker for liver disease, particularly cholestatic disease (Newsome et al. 2018; Fernandez and Kidney 2007). In addition, BUN and creatinine, by-products of amino acid and muscle metabolism are considered biomarkers of renal function (Ferguson and Waikar 2012; Nankivell 2001). Our results showed that *L. lactis* supplementation in Nile tilapia diets did not affect liver and kidney function indicators. This trend aligns with studies on *L. acidophilus* in Siberian sturgeon (*Acipenser baerii*) (Mocanu et al. 2022), and *L. fermentum* in common carp (*C. carpio*) (Ahmadifar et al. 2019), which observed no impact on cholesterol, AST, and alanine transaminase (ALT) values, while observing an elevation in immune response via increased immunoglobulin (IgM). Additionally, probiotic supplementation decreased cholesterol and triglycerides. For instance, *L. rhamnosus* supplementation in red sea bream (*P. major*) (Dawood et al. 2017) and zebrafish (Falcinelli et al. 2015) induced changes in the microbiome that produce short-chain fatty acids (SCFAs), resulting in reduced cholesterol and triglycerides (Falcinelli et al. 2015). Therefore, it is evident that *L. lactis* supplementation does not have a negative effect on the liver and kidneys of tilapia. This is consistent with findings from supplementation with other *Lactobacillus* species in fish.

In our investigation, cessation of *L. lactis* supplementation at a concentration of 10^8 CFU/g in tilapia resulted in higher lipase and protease activities one week later, compared to the control group. However, no significant effect was observed on amylase activity. Probiotic supplementation is known to influence changes in microflora. Probiotics possess the capability to produce bacteriocins, reducing the abundance of pathogens (Feng et al. 2019). Additionally, they generate SCFAs, delivering energy to epithelial cells,

and influencing the production of lipolytic and proteolytic enzymes in the intestines, thereby positively affecting gut health (Asaduzzaman et al. 2018). Previous studies have reported an increase in *Rhizobium* and *Achromobacter* one week following the cessation of *L. rhamnosus* and *Lc. lactis* supplementation. These bacteria are known for their ability to produce cellulolytic and pectolytic enzymes (Xia et al. 2018). Regarding antioxidant enzymes and non-specific immune responses, our study revealed that one week post-cessation of *L. lactis* supplementation, tilapia previously fed *L. lactis* had elevated CAT levels, while LZM, SOD, and MPO showed no significant differences compared to the control group. Even in fish with continuous *L. lactis* supplementation, CAT, SOD, and LZM increased significantly, suggesting an immune-stimulating effect. It can be inferred that *L. lactis* exerts an immune-stimulating effect in tilapia when administered continuously. This phenomenon is linked to the survival of probiotics in the intestinal environment, thereby influencing the growth of microorganisms in the fish's intestines (Al-Hisnawi et al. 2019). Previous studies have indicated a decrease in the abundance of probiotics following their cessation. For instance, Xia et al. (2018) reported the absence of detectable probiotics one week after the cessation of *Lc. lactis* JCM5805 supplementation in tilapia, a trend also observed in rainbow trout (*Oncorhynchus mykiss*) supplemented with *Pediococcus acidilactici* (Al-Hisnawi et al. 2019), and *Carnobacterium* sp. (Robertson et al. 2000) after three to six days, respectively. Additionally, investigations into the immune response of fish post-cessation of probiotic administration have been conducted. In a recent study, Sharifuzzaman and Austin (2010) observed a decline in LZM activity and serum peroxidase activity after cessation of *Kocuria* SM1 supplementation in rainbow trout (*O. mykiss*). Furthermore, Mohammadian et al. (2019) documented a decrease in WBC count 15 days after cessation of *L. casei* and oligosaccharide supplementation in common carp (*C. carpio*). Additionally, a two weeks cessation of *L. casei* YYL3 and *L. plantarum* YYL5 supplementation in Channel catfish resulted in reduced *Lactobacilli* levels and increased *Pseudomonas* abundance compared to the control group, which exhibited higher *Aeromonas* pathogen levels (Mohammadian et al. 2022). Therefore, supplementing prebiotics along with probiotics may contribute to the prolonged viability of the probiotics (Alves-Santos et al. 2020).

Conclusions

In conclusion, supplementing tilapia feeds with *L. lactis* TISTR 1464 at a concentration of 10^8 CFU/g led to increased lipase activity in the digestive tract. Furthermore, one week post-cessation, heightened lipase and protease activities were observed. Interestingly, supplementation with *L. lactis* at 10^8 CFU/g of diet improved non-specific immune responses and antioxidant enzyme activities. Importantly, supplementation in tilapia did not adversely affect liver and kidney function and fish growth. Nevertheless, to sustain immune stimulation, continuous supplementation of *L. lactis* in the tilapia diet is advisable during culture.

Competing interests The authors declare no conflict of interest.

Authors' contributions MP, designing, conceptualizing, formal analysis, investigation, resources, data collection, data analysis, writing the original draft, review and editing, visualization, supervision; TC and PT, data collection, data analysis; TB and JM, data analysis, review and editing the manuscript. All authors read and approved the final version the manuscript.

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References

Abdel-Tawwab M, Hagras AE, Elbaghdady HAM, Monier MN (2015) Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.):growth performance, whole-body composition, and innate immunity. Aquac Int 23:261-1274. https://doi.org/10.1007/s10499-015-9882-y

Adeshina I, Abubakar MIO, Ajala BE (2020) Dietary supplementation with *Lactobacillus acidophilus* enhanced the growth, gut morphometry, antioxidant capacity, and the immune response in juveniles of the common carp, *Cyprinus carpio*. Fish Physiol Biochem 46(4):1375-1385. https://doi.org/10.1007/s10695-020-00796-7

Agh N, Morshedi V, Noori F, Ghasemi A, Pagheh E, Rashidian G (2022) The effects of single and combined use of *Lactobacillus plantarum* and xylooligosacharide on growth, feed utilization, immune responses, and immune and growth related genes of sobaity (*Sparidentex hasta*) fingerlings. Aquac Rep 25:101271. https://doi.org/10.1016/j.aqrep.2022.101271

Ahmadifar E, Moghadam MS, Dawood MA, Hoseinifar SH (2019) *Lactobacillus fermentum* and/or ferulic acid improved the immune responses, antioxidative defence and resistance against *Aeromonas hydrophila* in common carp (*Cyprinus carpio*) finger-

lings. Fish Shellfish Immunol 94:916-923. https://doi.org/10.1016/j.fsi.2019.10.019

Akter MN, Hashim R, Sutriana A, Nor SAM, Janaranjani M (2023) *Lactobacillus acidophilus* supplementation improves the innate immune response and disease resistance of Striped catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) Juveniles against *Aeromonas hydrophila*. Trends Sci 20(7):4932-4932.

Al-Dhabi NA, Valan Arasu M, Vijayaraghavan P, Esmail GA, Duraipandiyan V, Kim YO, Kim H, Kim HJ (2020) Probiotic and antioxidant potential of *Lactobacillus reuteri* LR12 and *Lactobacillus lactis* LL10 isolated from pineapple puree and quality analysis of pineapple-flavored goat milk yoghurt during storage. Microorganisms 8(10):1461. https://doi.org/10.3390/microorganisms8101461

Al-Hisnawi A, Rodiles A, Rawling MD, Castex M, Waines P, Gioacchini G, Carnevali O, Merrifield DL (2019) Dietary probiotic *Pediococcus acidilactici* MA18/5M modulates the intestinal microbiota and stimulates intestinal immunity in rainbow trout (*Oncorhynchus mykiss*). J World Aquac Soc 50(6):1133-1151

Allameh SK, Noaman V, Nahavandi R (2017) Effects of probiotic bacteria on fish performance. Advanced techniques in clinical microbiology 1(2):11. http://www.imedpub.com/advanced-techniques-in-clinical-microbiology

Alves-Santos AM, Sugizaki CSA, Lima GC, Naves MMV (2020) Prebiotic effect of dietary polyphenols: A systematic review. J Funct Foods 74:104169. https://doi.org/10.1016/j.jff.2020.104169

Amarantini C, Satwika D, Budiarso TY, Yunita ER, Laheba EA (2019) Screening of antimicrobial-producing lactic acid bacteria isolated from traditional fish fermentation against pathogenic bacteria. J Phys Conf Ser 1397(1):012045. https://doi.org/10.1088/1742-6596/1397/1/012045

AOAC (2000) Official methods of analysis of AOAC international, 17th ed. The Association, Gaithersburg, MD

Arumugam M, Jayaraman S, Sridhar A, Venkatasamy V, Brown PB, Abdul Kari Z, Tellez-Isaias G, Ramasamy T (2023) Recent advances in Tilapia production for sustainable developments in Indian aquaculture and its economic benefits. Fishes 8(4):176. https://doi.org/10.3390/fishes8040176

Asaduzzaman MD, Iehata S, Akter S, Kader MA, Ghosh SK, Khan MNA, Abol-Munafi AB (2018) Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian Mahseer *Tor tambroides*. Aquac Rep 9:53-61. https://doi.org/10.1016/j.aqrep.2017.12.003

Assan D, Kuebutornye FKA, Hlordzi V, Chen H, Mraz J, Mustapha UF, Abarike ED (2022) Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: a mini review. Comp Biochem and Physiol B: Biochem Mol Biol 257:110653. https://doi.org/10.1016/j.cbpb.2021.110653

Balcázar JL, Vendrell D, de Blas I, Ruiz-Zarzuela I, Muzquiz JL, Girones O (2008) Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquaculture 278(1-4):188-191. https://doi.org/10.1016/j.aquaculture.2008.03.014

Biller-Takahashi JD, Urbinati EC (2014) Fish immunology. The modification and manipulation of the innate immune system: Brazilian studies. An Acad Bras Cienc 86: 1484-1506. https://doi.org/10.1590/0001-3765201420130159

Blaxhall PC, Daisley KW (1973) Routine haematological methods for use with fish blood. J Fish Biol 5(6):771-781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Brashears MM, Reilly SS, Gilliland SE (1998) Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157: H7 on refrigerated raw chicken meat. J Food Prot 61(2):166-170. https://doi.org/10.4315/0362-028X-61.2.166

Brashears MM, Durre WA (1999) Antagonistic action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherichia coli* O157: H7 during growth and refrigerated storage. J Food Prot 62(11):1336-1340. https://doi.org/10.4315/0362-028X-62.11.1336

Chen H, Hoover DG (2003) Bacteriocins and their food applications. Compr Rev Food Sci Food Saf 2(3):82-100. https://doi.org/10.1111/j.1541-4337.2003.tb00016.x

Chen J, Sun R, Pan C, Sun Y, Mai B, Li QX (2020) Antibiotics and food safety in aquaculture. J Agric Food Chem 68(43):11908-11919. https://doi.org/10.1021/acs.jafc.0c03996

Chitmanat C, Lebel P, Whangchai N, Promya J, Lebel L (2016) Tilapia diseases and management in river-based cage aquaculture in northern Thailand. J Appl Aquac 28(1):9-16. https://doi.org/10.1080/10454438.2015.1104950

Coull BM, Beamer N, De Garmo P, Sexton G, Nordt F, Knox R, Seaman GV (1991) Chronic blood hyperviscosity in subjects with acute stroke, transient ischemic attack, and risk factors for stroke. Stroke 22(2):162-168

Dawood MA, Koshio S, Ishikawa M, Yokoyama S (2015a) Effects of partial substitution of fish meal by soybean meal with or without heat-killed *Lactobacillus plantarum* (LP20) on growth performance, digestibility, and immune response of amberjack, *Seriola dumerili* juveniles. Bio Med Res Int 514196:1-11. https://doi.org/10.1155/2015/514196

Dawood MA, Koshio S, Ishikawa M, Yokoyama S (2015b) Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. Fish Shellfish immunol 45(1):33-42. https://doi.org/10.1016/j.fsi.2015.01.033

Dawood MA, Koshio S, Ishikawa M, Yokoyama S, El Basuini MF, Hossain MS, Nhu TH, Dossou S, Moss AS (2016) Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish Shellfish Immunol 49:275-285. https://doi.org/10.1016/j.fsi.2015.12.047

Dawood MA, Koshio S, Ishikawa M, El-Sabagh M, Yokoyama S, Wang WL, Yukun Z, Olivier A (2017) Physiological response, blood chemistry profile and mucus secretion of red sea bream (*Pagrus major*) fed diets supplemented with *Lactobacillus rhamnosus* under low salinity stress. Fish Physiol Biochem 43:179-192. https://doi.org/10.1007/s10695-016-0277-4

Di J, Chu Z, Zhang S, Huang J, Du H, Wei Q (2019) Evaluation of the potential probiotic *Bacillus subtilis* isolated from two ancient sturgeons on growth performance, serum immunity and disease resistance of *Acipenser dabryanus*. Fish Shellfish Immunol 93:711-719. https://doi.org/10.1016/j.fsi.2019.08.020

El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MA, Dhama K, Abdel-Latif HM (2021) The functionality of probiotics in aquaculture: An overview. Fish Shellfish Immunol 117:36-52. https://doi.org/10.1016/j.fsi.2021.07.007

Falcinelli S, Picchietti S, Rodiles A, Cossignani L, Merrifield DL, Taddei AR, Maradonna F, Olivotto I, Gioacchini G, Carnevali O (2015) *Lactobacillus rhamnosus* lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. Sci Rep 5(1):9336. https://doi.org/10.1038/srep09336

FAO (food and agriculture organization of the united nations) (2020) The state of world fisheries and aquaculture 2020, sustainability in action, Rome. https://doi.org/10.4060/ca9229en

Feng J, Chang X, Zhang Y, Yan X, Zhang J, Nie G (2019) Effects of *Lactococcus lactis* from *Cyprinus carpio* L. as probiotics on growth performance, innate immune response and disease resistance against *Aeromonas hydrophila*. Fish Shellfish Immunol 93:73-81. https://doi.org/10.1016/j.fsi.2019.07.028

Ferguson MA, Waikar SS (2012) Established and emerging markers of kidney function. Clin Chem 58(4):680-689. https://doi.org/10.1373/clinchem.2011.167494

Fernandez NJ, Kidney BA (2007) Alkaline phosphatase: beyond the liver. Vet Clin Pathol 36(3):223-233. https://doi.org/10.1111/j.1939-165X.2007.tb00216.x

Ferraboschi P, Ciceri S, Grisenti P (2021) Applications of lysozyme, an innate immune defense factor, as an alternative antibiotic. Antibiotics 10(12):1534. https://doi.org/10.3390/antibiotics10121534

Foysal MJ, Alam M, Kawser AR, Hasan F, Rahman MM, Tay CY, Prodhan SH, Gupta SK (2020) Meta-omics technologies reveals beneficiary effects of *Lactobacillus plantarum* as dietary supplements on gut microbiota, immune response and disease resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 520:734974. https://doi.org/10.1016/j.aquaculture.2020.734974

Fukada H, Senzui A, Kimoto K, Tsuru K, Kiyabu Y (2023) Evaluation of the in vivo and in vitro interleukin-12 p40 and p35 subunit response in yellowtail (*Seriola quinqueradiata*) to heat-killed *Lactobacillus plantarum* strain L-137 (HK L-137) supplementation, and immersion challenge with *Lactococcus garvieae*. Fish Shellfish Immunol Reports 4:100095. https://doi.org/10.1016/j.fsirep.2023.100095

Fukai T, Ushio-Fukai M (2011) Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal 15(6):1583-1606. https://doi.org/10.1089/ars.2011.3999

Gewaily MS, Shukry M, Abdel-Kader MF, Alkafafy M, Farrag FA, Moustafa EM, Doan HV, Abd-Elghany MF, Abdelhamid AF, Eltanahy A, Dawood MA (2021) Dietary *Lactobacillus plantarum* relieves Nile tilapia (*Oreochromis niloticus*) juvenile from oxidative stress, immunosuppression, and inflammation induced by deltamethrin and *Aeromonas hydrophila*. Front Mar Sci 8:621558. https://doi.org/10.3389/fmars.2021.621558

Giri SS, Sukumaran V, Oviya M (2013) Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. Fish Shellfish Immunol 34(2):660-666. https://doi.org/10.1016/j.fsi.2012.12.008

Hamdan AM, El-Sayed AFM, Mahmoud MM (2016) Effects of a novel marine probiotic, *Lactobacillus plantarum* AH 78, on growth performance and immune response of Nile tilapia (*Oreochromis niloticus*). J Appl Microbiol 120(4):1061-1073. https://doi.org/10.1111/jam.13081

Hasan I, Rimoldi S, Saroglia G, Terova G (2023) Sustainable fish feeds with insects and probiotics positively affect freshwater and marine fish gut microbiota. Animals 13(10):1633. https://doi.org/10.3390/ani13101633

He RP, Feng J, Tian XL, Dong SL, Wen B (2017) Effects of dietary supplementation of probiotics on the growth, activities of digestive and non-specific immune enzymes in hybrid grouper (*Epinephelus lanceolatus*♂× *Epinephelus fuscoguttatus*♀). Aquac Res 48(12):5782-5790. https://doi.org/10.1111/are.13401

Hemamalini C, Patro TSSK, Anuradha N, Triveni U, Jogarao P (2020) Estimation of nutritive composition of seven small millets. J Pharmacogn Phytochem 9(3):1871-1874.

Heo WS, Kim YR, Kim EY, Bai SC, Kong IS (2013) Effects of dietary probiotic, *Lactococcus lactis* subsp. *lactis* I2, supplementation on the growth and immune response of olive flounder (*Paralichthys olivaceus*). Aquaculture 376:20-24. https://doi.org/10.1016/j.aquaculture.2012.11.009

Hien TTT, Hoa TTT, Liem PT, Onoda S, Duc PM (2021) Effects of dietary supplementation of heat-killed *Lactobacillus plantarum* L-137 on growth performance and immune response of bighead catfish (*Clarias macrocephalus*). Aquac Rep 20:100741. https://doi.org/10.1016/j.aqrep.2021.100741

Hooshyar Y, Abedian Kenari A, Paknejad H, Gandomi H (2020) Effects of *Lactobacillus rhamnosus* ATCC 7469 on different parameters related to health status of rainbow trout (*Oncorhynchus mykiss*) and the protection against *Yersinia ruckeri*. Probiotics Antimicrob Proteins 12:1370-1384. https://doi.org/10.1007/s12602-020-09645-8

Hossain MK, Hossain MM, Mim ZT, Khatun H, Hossain MT, Shahjahan M (2022) Multi-species probiotics improve growth, intestinal microbiota and morphology of Indian major carp mrigal *Cirrhinus cirrhosus*. Saudi J Biol Sci 29(9):103399. https://doi.org/10.1016/j.sjbs.2022.103399

Hosseini M, Miandare HK, Hoseinifar SH, Yarahmadi P (2016) Dietary *Lactobacillus acidophilus* modulated skin mucus protein profile, immune and appetite genes expression in gold fish (*Carassius auratus gibelio*). Fish Shellfish Immunol 59:149-154. https://doi.org/10.1016/j.fsi.2016.10.026

Jatobá A, Vieira FDN, Buglione-Neto CC, Mouriño' JLP, Silva BC, Seiftter WQ, Andreatta ER (2011) Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. Fish Physiol Biochem 37:725-732. https://doi.org/10.1007/s10695-011-9472-5

Khan AA, Alsahli MA, Rahmani AH (2018) Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives. Med Sci 6(2):33. https://doi.org/10.3390/medsci6020033

Kim D, Beck BR, Heo SB, Kim J, Kim HD, Lee SM, Kim Y, Oh SY, Lee K, Do H, Lee K, Holzapfel WH, Song SK (2013) *Lactococcus lactis* BFE920 activates the innate immune system of olive flounder (*Paralichthys olivaceus*), resulting in protection against *Streptococcus iniae* infection and enhancing feed efficiency and weight gain in large-scale field studies. Fish Shellfish Immunol 35(5):1585-1590. http://dx.doi.org/10.1016/j.fsi.2013.09.008

Krishnaveni G, Vignesh S, Vidhyalakshmi N, Vijay V, Ramesh U (2021) Effects of dietary supplementation of *Lactobacillus fermentum* URLP18 on growth, innate immunity and survival against *Aeromonas hydrophila* ATCC 7966 challenge in freshwater fish *Cyprinus carpio* (common carp). Aquac Res 52(3):1160-1176. https://doi.org/10.1111/are.14974

Lara-Flores M, Olvera-Novoa MA, Guzmán-Méndez BE, López-Madrid W (2003) Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216(1-4):193-201. https://doi.org/10.1016/S0044-8486(02)00277-6

Lazado CC, Caipang CMA (2014) Mucosal immunity and probiotics in fish. Fish Shellfish Immunol 39(1):78-89. https://doi.org/10.1016/j.fsi.2014.04.015

Lee JS, Cheng H, Damte D, Lee SJ, Kim JC, Rhee MH, Suh J, Park SC (2013) Effects of dietary supplementation of *Lactobacillus*

pentosus PL11 on the growth performance, immune and antioxidant systems of Japanese eel *Anguilla japonica* challenged with *Edwardsiella tarda*. Fish Shellfish Immunol 34(3):756-761. https://doi.org/10.1016/j.fsi.2012.11.028

Liu W, Ren P, He S, Xu L, Yang Y, Gu Z, Zhou Z (2013) Comparison of adhesive gut bacteria composition, immunity, and disease resistance in juvenile hybrid tilapia fed two different *Lactobacillus* strains. Fish Shellfish Immunol 35(1):54-62. https://doi.org/10.1016/j.fsi.2013.04.010

Luck H (1974) Method of enzymatic analysis. In:Bergmayer MV (Eds.) Catalase, Verlag Chemic Academic Press, New York, USA

Meade E, Slattery MA, Garvey M (2020) Bacteriocins, potent antimicrobial peptides and the fight against multi drug resistant species: resistance is futile? Antibiotics 9(1):32. https://doi.org/10.3390/antibiotics9010032

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3):426-428. https://doi.org/10.1021/ac60147a030

Mirghaed AT, Yarahmadi P, Hosseinifar SH, Tahmasebi D, Gheisvandi N, Ghaedi A (2018) The effects singular or combined administration of fermentable fiber and probiotic on mucosal immune parameters, digestive enzyme activity, gut microbiota and growth performance of caspian white fish (*Rutilus frisii kutum*) fingerlings. Fish Shellfish Immunol 77:194-199. https://doi.org/10.1016/j.fsi.2018.02.007

Mocanu EE, Savin V, Popa MD, Dima FM (2022) The Effect of probiotics on growth performance, haematological and biochemical profiles in siberian sturgeon (*Acipenser baerii* Brandt, 1869). Fishes 7(5):239. https://doi.org/10.3390/fishes7050239

Mohammadian T, Alishahi M, Tabandeh MR, Ghorbanpoor M, Gharibi D (2017) Effect of *Lactobacillus plantarum* and *Lactobacillus delbrueckii* subsp. *bulgaricus* on growth performance, gut microbial flora and digestive enzymes activities in *Tor grypus* (Karaman 1971). Iran J Fish Sci 16(1):296-317. http://hdl.handle.net/1834/12160

Mohammadian T, Nasirpour M, Tabandeh MR, Mesbah M (2019) Synbiotic effects of β-glucan, mannan oligosaccharide and *Lactobacillus casei* on growth performance, intestine enzymes activities, immune-hematological parameters and immune-related gene expression in common carp, *Cyprinus carpio*: An experimental infection with *Aeromonas hydrophila*. Aquaculture 511:634197. https://doi.org/10.1016/j.aquaculture.2019.06.011

Mohammadian T, Monjezi N, Peyghan R, Mohammadian B (2022) Effects of dietary probiotic supplements on growth, digestive enzymes activity, intestinal histomorphology and innate immunity of common carp (*Cyprinus carpio*): a field study. Aquaculture 549:737787. https://doi.org/10.1016/j.aquaculture.2021.737787

Nandi A, Yan LJ, Jana CK, Das N (2019) Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxid Med Cell Longev 2019:1-19. https://doi.org/10.1155/2019/9613090

Nankivell BJ (2001) Creatinine clearance and the assessment of renal function. Aust Prescr 24(1):1-3. https://doi.org/10.18773/austprescr.2001.009

Newsome PN, Cramb R, Davison SM, Dillon JF, Foulerton M, Godfrey EM, Hall R, Harrower U, Hudson M, Langford A, Mackie A, Mitchell-Thain R, Sennett K, Sheron NC, Verne J, Walmsley M, Yeoman A (2018) Guidelines on the management of abnormal liver blood tests. Gut 67(1):6-19. http://dx.doi.org/10.1136/gutjnl-2017-314924

Nguyen TL, Park CI, Kim DH (2017) Improved growth rate and disease resistance in olive flounder, *Paralichthys olivaceus*, by probiotic *Lactococcus lactis* WFLU12 isolated from wild marine fish. Aquaculture 471:113-120. https://doi.org/10.1016/j.aquaculture.2017.01.008

Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B (2016) Probiotic approach to prevent antibiotic resistance. Ann Med 48(4):246-255. https://doi.org/10.3109/07853890.2016.1161232

Paixão PEG, do Couto MVS, da Costa Sousa N, Abe HA, Reis RGA, Dias JAR, Menesesa JO, Cunhaa FS, Santosa TBR, Silvac ICA, Medeirosd ES, Fujimoto RY (2020) Autochthonous bacterium *Lactobacillus plantarum* as probiotic supplementation for productive performance and sanitary improvements on clownfish *Amphiprion ocellaris*. Aquaculture 526:735395. https://doi.org/10.1016/j.aquaculture.2020.735395

Phinyo M, Thaikosa A, Eakapol W (2021) Effect of dietary papaya peel and baker's yeast supplementation on growth performance, hematological value of silver barb (*Barbodes gonionotus*) and its resistance to *Aeromonas hydrophila*. J Sustain Sci Manag 16(2):11-21. https:// doi.org/10.46754/jssm.2021.02.003

Phinyo M, Pumma S, Thinjan P, Wangkahart E, Soonthornchai W (2024) Effects of dietary fermented soybean meal with Thua nao starter on the growth performance, body composition, and disease resistance against *Aeromonas hydrophila* of Nile tilapia (*Oreochromis niloticus*). Aquac Rep 34:101890. https://doi.org/10.1016/j.aqrep.2023.101890

Pourgholam MA, Khara H, Safari R, Sadati MAY, Aramli MS (2017) Influence of *Lactobacillus plantarum* inclusion in the diet of Siberian sturgeon (*Acipenser baerii*) on performance and hematological parameters. Turkish J Fish Aquat Sci 17(1):1-5. https://doi.org/10.4194/1303-2712-v17_1_01

Pulli B, Ali M, Forghani R, Schob S, Hsieh KL, Wojtkiewicz G, Linnoila JJ, Chen JW (2013) Measuring myeloperoxidase activity in biological samples. PLoS One 8 (2013):e67976. https://doi.org/10.1371/journal.pone.0067976

Reid G, Bruce AW (2001) Selection of *Lactobacillus* strains for urogenital probiotic applications. J Infect Dis 183(1):S77-S80. https://doi.org/10.1086/318841

Renuka KP, Venkateshwarlu M, Ramachandra NA, Prashantha KS (2013) Influence of probiotics on growth performance and digestive enzyme activity of common carp (*Cyprinus carpio*). Int J Curr Res 5(7):1696-1700. http://www.journalcra.com

Robertson PAW, O'Dowd C, Burrells C, Williams P, Austin B (2000) Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture 185(3-4):235-243. https://doi.org/10.1016/S0044-8486(99)00349-X

Sagada G, Wang L, Xu B, Sun Y, Shao Q (2023) Interactive effect of dietary heat-killed *Lactobacillus plantarum* L-137 and berberine supplementation on intestinal mucosa and microbiota of juvenile Black sea bream (*Acanthopagrus Schlegelii*). Probiotics Antimicrob Proteins 1-13. https://doi.org/10.1007/s12602-023-10153-8

Salih AHM, Patra I, Sivaraman R, Alhamzawi R, Khalikov KM, Al-Qaim ZH, Golgouneh S, Jawad MA, Adhab AH, Vázquez-Cárdenas AL, Abarghouei S (2023) The Probiotic *Lactobacillus sakei* subsp. *Sakei* and Hawthorn extract supplements improved growth performance, digestive enzymes, immunity, and resistance to the pesticide acetamiprid in Common carp (*Cyprinus carpio*). Aquac Nutr 2023. https://doi.org/10.1155/2023/8506738

Sharifuzzaman SM, Austin B (2010) Development of protection in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Vibrio anguilla-*

rum following use of the probiotic Kocuria SM1. Fish Shellfish Immunol 29(2):212-216. https://doi.org/10.1016/j.fsi.2010.03.008

Shija VM, Amoah K, Cai J (2023) Effect of *Bacillus* probiotics on the immunological responses of Nile tilapia (*Oreochromis niloticus*): a review. Fishes 8(7):366. https://doi.org/10.3390/fishes8070366

Sookoian S, Pirola CJ (2012) Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. World J Gastroenterol 18(29):3775

Speicher CE, Widish JR, Gaudot FJ, Hepler BR (1978) An evaluation of the overestimation of serum albumin by bromocresol green. Am J Clin Pathol 69(3):347-350. https://doi.org/10.1093/ajcp/69.1.347

Suzer C, Çoban D, Kamaci HO, Saka Ş, Firat K, Otgucuoğlu Ö, Küçüksari H (2008) *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata,* L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280(1-4):140-145. https://doi.org/10.1016/j.aquaculture.2008.04.020

Tang H, Huang W, Yao YF (2023) The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. Microbial Cell 10(3):49. https://doi.org/10.15698/mic2023.03.792

Tang HW, Phapugrangkul P, Fauzi HM, Tan JS (2022) Lactic acid bacteria bacteriocin, an antimicrobial peptide effective against multidrug resistance: a comprehensive review. Int J Pept Res Ther 28:1-14. https://doi.org/10.1007/s10989-021-10317-6

Thompson HO, Önning G, Holmgren K, Strandler HS, Hultberg M (2020) Fermentation of cauliflower and white beans with *Lactobacillus plantarum*–impact on levels of riboflavin, folate, vitamin B 12, and amino acid composition. Plant Foods Hum Nutr 75:236-242. https://doi.org/10.1007/s11130-020-00806-2

Wang J, Feng J, Liu S, Cai Z, Song D, Yang L, Nie G (2021) The probiotic properties of different preparations using *Lactococcus lactis* Z-2 on intestinal tract, blood and hepatopancreas in *Cyprinus carpio*. Aquaculture 543:736911. https://doi.org/10.1016/j.aquaculture.2021.736911

Wangkahart E, Bruneel B, Chantiratikul A, de Jong M, Pakdeenarong N, Subramani PA (2022) Optimum dietary sources and levels of selenium improve growth, antioxidant status, and disease resistance: re-evaluation in a farmed fish species, Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol 121:172-182. https://doi.org/10.1016/j.fsi.2021.12.003

Witeska M, Kondera E, Ługowska K, Bojarski B (2022) Hematological methods in fish-Not only for beginners. Aquaculture 547:737498. https://doi.org/10.1016/j.aquaculture.2021.737498

Xia Y, Lu M, Chen G, Cao J, Gao F, Wang M, Liu Z, Zhang D, Zhu H, Yi M (2018) Effects of dietary *Lactobacillus rhamnosus* JCM1136 and *Lactococcus lactis* subsp. *lactis* JCM5805 on the growth, intestinal microbiota, morphology, immune response and disease resistance of juvenile Nile tilapia, *Oreochromis niloticus*. Fish Shellfish Immunol 76:368-379. https://doi.org/10.1016/j.fsi.2018.03.020

Yang G, Cui X, Liu S, Lu J, Hou X, Meng W, Wu B, Su Y, Zhang H, Fang Y (2021) Effects of dietary *Lactobacillus helveticus* on the growth rate, disease resistance and intestinal health of pond loach (*Misgurnus anguillicaudatus*). Aquaculture 544:737038. https://doi.org/10.1016/j.aquaculture.2021.737038

Yang RZ, Park S, Reagan WJ, Goldstein R, Zhong S, Lawton M, Rajamohan F, Qian K, Liu L, Gong DW (2009) Alanine aminotransferase isoenzymes: molecular cloning and quantitative analysis of tissue expression in rats and serum elevation in liver toxicity. Hepatology 49(2):598-607. https://doi.org/10.1002/hep.22657

Zacharof MP, Lovitt RW (2012) Bacteriocins produced by lactic acid bacteria a review article. APCBEE Procedia 2:50-56. https://doi.org/10.1016/j.apcbee.2012.06.010

Zhang C, Zhang J, Fan W, Huang M, Liu M (2019a) Effects of dietary *Lactobacillus delbrueckii* on growth performance, body composition, digestive and absorptive capacity, and gene expression of common carp (*Cyprinus carpio* Huanghe var). Aquac Nutr 25(1):166-175. https://doi.org/10.1111/anu.12840

Zhang D, Zhang Y, Liu B, Jiang Y, Zhou Q, Wang J, Wang H, Xie J, Kuang Q (2017) Effect of replacing fish meal with fermented mushroom bran hydrolysate on the growth, digestive enzyme activity, and antioxidant capacity of allogynogenetic crucian carp (*Carassius auratus gibelio*). Turkish J Fish Aquat Sci 17(5):1039-1048. https://doi.org/10.4194/1303-2712-v17_5_20

Zhang H, Wang H, Hu K, Jiao L, Zhao M, Yang X, Xia L (2019b) Effect of dietary supplementation of *Lactobacillus casei* YYL3 and *L. plantarum* YYL5 on growth, immune response and intestinal microbiota in channel catfish. Animals 9(12):1005

Zheng K, Wu L, He Z, Yang B, Yang Y (2017) Measurement of the total protein in serum by biuret method with uncertainty evaluation. Measurement 112:16-21. https://doi.org/10.1016/j.measurement.2017.08.013

Zhu CZ, Li D, Chen WJ, Ban SN, Liu T, Wen H, Jiang M (2021) Effects of dietary host-associated *Lactococcus lactis* on growth performance, disease resistance, intestinal morphology and intestinal microbiota of mandarin fish (*Siniperca chuatsi*). Aquaculture 540:736702. https://doi.org/10.1016/j.aquaculture.2021.736702

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