Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 2855 – 2874 (2025)
www.ejabf.journals.ekb.eg


# Supplementing with Dietary *Lactobacillus acidophilus* Modulates Cold Stress Deterioration of the Antioxidant Activity, Immunological Response, and Growth Indices in the Nile Tilapia

**Salem Almarri, Ahmed AlSaqufi, Hesham Hassanien***

Animal Production and Aquaculture Department, College of Agricultural and Food Science, King Faisal University, P.O. Box 420 Hofuf 31982 Al-Hasa, Saudi Arabia

***Corresponding Author: helsanwey@kfu.edu.sa**

## ARTICLE INFO

**Article History:**
Received: Feb. 18, 2025
Accepted: April 24, 2025
Online: April 29, 2025

**Keywords**:
The Nile tilapia,
*Lactobacillus acidophilus*,
Cold tolerance,
Growth performance

## ABSTRACT

This study sought to show how feeding *Lactobacillus acidophilus* (LA) to the Nile tilapia (*Oreochromis niloticus*) might lessen cold stress (CS) deterioration of the antioxidant activity, immunological response, and growth performance. A preliminary trial was carried out dividing 180 fingerlings (28.6±0.24g) into 6 treatment groups according to the amount of LA supplied to the fish meals (0, $10^2$, $10^4$, $10^6$, $10^8$, and $10^{10}$ CFU/g). Each treatment group consisted of three replicate tanks, each containing 10 fish. To determine the appropriate lactic acid (LA) dosage and supplementation duration for the main study, preliminary testing was conducted. The results showed that supplementing fish feed with $10^6$ CFU/g of LA for four weeks was optimal for achieving significant growth improvements. In the primary experiment, 480 fingerlings (30.1±0.4g) were split up into two LA × two CS factorial treatment groups, each of which included six replicate tanks with 20 fish. During a 4-week feeding period, fish in LA groups were fed a baseline diet with $10^6$ CFU/g LA (+ LA group) or without LA (– LA group). Fish in each feeding group were further distributed into two subgroups either kept at 26°C (– CS subgroup) or subjected to cold stress at 18°C (+ CS subgroup). The findings demonstrated that, compared with the control fish, CS substantially ($P< 0.05$) reduced the antioxidant activity, immunological response, and growth indices in the challenged fish. Conversely, adding LA to fish feeds improved ($P< 0.05$) the antioxidant activity, immunological parameters, feed intake, and growth indices. In fish under cold stress, LA therapy reduced the increased mortality, heterophil to lymphocyte (H/L) ratio, and lipid peroxidation. In conclusion, LA feeding at $10^6$ CFU/g to the Nile tilapia fish might be suggested as a promising way to promote the fish's health and growth, particularly when they are subjected to CS circumstances.

## INTRODUCTION

Farming of the Nile tilapia, *Oreochromis niloticus*, plays a crucial role in meeting the increasing global demand for aquatic products **(Prabu *et al.*, 2019)**. The new FAO

report, titled "**The State of World Fisheries and Aquaculture 2022 | FAO**," predicts that the worldwide tilapia market will reach a value of 9.2 billion dollars by the year 2027. Furthermore, it is expected that the growth rate will increase annually by 2.2% in the upcoming years. **Miao and Wang (2020)** reported that around 26.9% of the world's tilapia output comes from China, with Thailand (3.5%), the Philippines (4.6%), Brazil (5.3%), Egypt (17.4%), and Indonesia (20.3%) making major contributions as well.

The Nile tilapia fish are widely favored for warm-water aquaculture, and they are known for their fast growth, efficient use of feed, ability to reproduce naturally, and delicious flesh **(El-Sayed, 2019)**. Nevertheless, this species exhibits a temperature preference range between 25–30°C and are highly vulnerable to the cold stress (CS), as documented by **Zerai *et al.* (2010)**. Other studies by **Nobrega *et al.* (2017)** and **Hassaan *et al.* (2019)** documented that cold fronts in the winter often result in a significant decline in both tilapia output and survival rates. Other research reported that temperatures lower than 20°C can cause major disruptions to the body's physiological functioning, metabolism, and immune system **(Barton, 2002; Galloway & Kieffer, 2003; Panase *et al.*, 2018)**. As a consequence, a notable reduction in the feed efficiency and growth performance might occur.

Given the rising global demand, putting ideas that augment growth rates and feed efficiency into practice has been an essential priority in the development of aquaculture systems **(Oh & Maran, 2015)**. In particular, using probiotics to enhance nutrient digestion, stress tolerance, fish growth, and production is a practical safe and alternative approach to conventional treatments **(Carnevali *et al.*, 2017; El-Houseiny *et al.*, 2019; Ibrahim *et al.*, 2019; Mansour *et al.*, 2022)**.

*Lactobacillus acidophilus* (LA) is one of the highly significant probiotic group bacteria since it is normally presented in the intestinal microflora of a healthy fish **(Ringø *et al.*, 2010; Wang, 2011)**. LA, when given in sufficient quantities, begin to inhabit and reproduce in the intestines and positively affects the host by influencing their biological systems **(Cross, 2002)**. Furthermore, LA has been successfully evaluated as a growth enhancer in different fish species including the Nile tilapia **(Lara-Flores *et al.*, 2003)**, African catfish **(Al-Dohail *et al.*, 2011)**, rainbow trout **(Faramazi *et al.*, 2011)**, and grass carp **(Wang, 2011)**. Research has also found that probiotic bacteria belonging to the *Lactobacillus* species can stimulate growth and immunological function in the host aquatic animals **(Wang & Gu, 2010; Alishahi *et al.*, 2018)**.

As far as we are aware, the benefits of LA application in aquaculture feeding have not yet been fully understood and require more research, particularly when CS is present. As a result, the present investigation included two experiments. The first experiment was a pilot study intended to determine the ideal LA dose and supplementation period that have a major influence on the Nile tilapia growth performance. A second experiment was then carried out to see if the chosen dietary LA intervention might lessen the detrimental

effects of CS on the Nile tilapia's antioxidant activity, immunological response, and growth performance.

## MATERIALS AND METHODS

### 1. Culture of LA

A commercial source that specializes in microbial media and cultivation (Wisby Gmbh Co. & Kg, Niebüll, Germany) provided the LA as a freeze-dried lyophilized culture. The bacteria were left to grow in a specific MRS broth medium for twenty-four hours at 37 °C. After that, LA cells were harvested by centrifugation (2000 ×g, 4 °C, 10 minutes) and then dried frozen at –20°C. Based on the original colony concentration in the MRS broth, a cornstarch-skimmed milk powder was added to the LA culture until reach a colony forming unit (CFU) of $1 \times 10^9$ cells/g. The LA culture was stored at 4°C in a refrigerator and tested every two weeks for the viability and target concentration. To guarantee the existence of viable LA cells throughout the study, the *Lactobacillus* culture was fed to the fish's food every day.

### 2. Experimental design

Two experiments were conducted in the current investigation. Initially, a pilot experiment was conducted to ascertain the optimal dosage of LA that would stimulate significant growth phenomena in the Nile tilapia. A total of one hundred eighty, 28.6±0.24g, fingerlings of the Nile tilapia (*Oreochromis niloticus*) were acquired from a specific company called Aqua Co. in Riyadh, Saudi Arabia. They were then raised in 18 aquaria of 10 fish each at specified environmental parameters for the Nile tilapia (26°C, 7.5 pH, and 6.5mg/ L dissolved $O_2$). The aquaria were partitioned into 6 treatment groups of 3 replicates in each treatment based on the dose of LA added to the fish meals at 0, $10^2$, $10^4$, $10^6$, $10^8$, and $10^{10}$ CFU/g. Diets were provided twice daily and were determined on a weekly basis using 3% of the fish biomass. The mean body weight was recorded on a weekly basis for a duration of 8 consecutive weeks and subjected to statistical analysis in order to choose the suitable amount of lactic acid and duration of supplementation for the primary experiments in the current investigation.

For the primary experiment, the same aquaculture firm provided 480 fingerlings, weighing an average of 30.1±0.4g. Fish were acclimated for a duration of 2 weeks in a glass aquarium with a size of $1m^3$. Subsequently, the fish were allocated into completely randomized factorial design consisting of 2 LA × 2 CS treatments (6 independent aquariums of 120L each housed with 20 fish per treatment group). The fish in the LA treatments received either a baseline diet enriched with LA at a dosage of $10^6$ CFU/g (+ LA group) or a baseline diet without LA (– LA group). Over the course of four weeks, diets were given to each group at 8:00 a.m. and 3:00 p.m. every day. Three percent of the fish biomass was used to adjust the feed quantity supplied to each tank every week. Fish

in the – LA and + LA feeding groups were either held at 26°C (– CS subgroup) or subjected to a cold stress of 18°C (+ CS subgroup). Following the steps described by **Alrashada *et al.* (2023)**, a thermostat-cooling system was used to create the cold stress by gradually lowering 1°C/12 h of the water temperature until reaching 18°C.

During feed collection, around 25% of the water was daily siphoned off and quickly refilled with pre-equilibrated aerated freshwater at the same temperatures. While every three days, the aquaria were cleaned and aerated with 50% of freshwater to get rid of any waste. Using advanced pellet mill equipment, the nutritional elements were well mixed and compacted into particles of 1.5mm-diameter. After that, the meal pellets were allowed to dry for a full day at room temperature before being kept at 4°C in a refrigerator. The elements of the baseline diet and the nutritional values, analyzed by the AOAC techniques **(Latimer Jr., 2023)**, are displayed in Table (1).

**Table 1.** The nutritional elements and values of the fish baseline diet

| Element | Gram per kilogram (as fed) |
|---|---|
| Yellow corn | 315.0 |
| Fish meal | 180.0 |
| Soybean meal | 320.0 |
| Wheat bran | 60.0 |
| Vegetable oil | 50.0 |
| Corn gluten | 52.0 |
| Calcium mono-hydrogen phosphate | 5.0 |
| Lysin | 2.0 |
| Methionine | 2.0 |
| Premix [1] | 14.0 |
| Nutritional values | Gram per kilogram (DM basis) |
| Dry matter (DM) | 908.0 ± 4.62 |
| Crude protein (CP) | 349.8 ± 1.51 |
| Crude lipids (CL) | 68.3 ± 4.62 |
| Total ash (TA) | 75.0 ± 0.58 |
| Crude fiber (CF) | 30.7 ± 0.32 |
| Nitrogen-free extract (NFE) [2] | 476.2 ± 7.91 |
| Gross energy (GE) [3] | 19.2 ± 0.23 |

[1] Each kilogram of premix contains minerals of 250 mg ferric citrate, 60 mg zinc carbonate, 40 mg manganese sulfate, 40 mg potassium sulfate, 12 mg copper sulfate, 10 mg magnesium oxide, 0.4 mg potassium iodide, 0.2 mg cobalt, and 0.24 mg sodium selenite, and vitamins of 4000 IU cholecalciferol, 40000 IU retinol, 400 mg α-tocopherol acetate, 40 mg riboflavin, 30 mg thiamine, 30 mg pyridoxine, 12 mg menadione, 80 µg cyanocobalamin, 10 mg folic acid, 100 mg pantothenic acid, 500 mg ascorbic acid, and 3 mg biotin.

[2] NFE = 1000 – (CL + CP + TA + CF).

[3] GE (MJ/kg) = (39.52 CL + 23.63 CP + 17.15 NFE) / 1000.

## 3. Growth performance

After feed introducing to the fish, the residual feed was collected, dried, and weighed to determine the feed intake (FI). Fish body weights were determined at the start and at the end of the trial to obtain the initial (IBW), final (FBW), and gained (GBW) body weight. The following formulae were used to calculate the growth indices for each replication within a treatment group: GBW=FBW–IBW; SGR=100×($Ln_{FBW}$ – $Ln_{IBW}$)/D, where SGR representing the specific growth rate, Ln representing the natural logarithm for corresponding subscript, and D representing the days of feeding trial (28 days); FCR=FI/GBW, where FCR representing the feed conversion ratio; and SR=(LF–DF)/LF×100, where SR representing the survival rate, LF representing the starting number of live fish, and DF representing the total number of dead fish throughout the feeding period.

## 4. Antioxidant activity

After four weeks of feeding, 18 blood samples were withdrawn from the fish caudal vein (3 fishes per replicate). According to **Martins *et al.* (2016)**, the fish were put to sleep by immersing them in 100mg/ L solution of MS-222. To aid in coagulation, blood samples were maintained over night at 4°C. The serum was separated by centrifugation for 20 minutes at 1075 ×g then stored at –20°C for further examination. Myeloperoxidase (MPO) activity was evaluated considering the concept of hydrogen peroxide reduction into a compound that interacts with o-dianisidine reagent to finally produce a yellowish color product to be measured at 460nm **(Mihaila *et al.*, 2021)**. Malondialdehyde (MDA) was evaluated based on the concept of thiobarbituric acid (TBA) reaction to generate a red molecule that can be scanned at 532nm **(Arora *et al.*, 2022)**. The content of the reduced glutathione (rGSH) was indirectly obtained through generating a complex from the interaction between the sample and the dinitrobenzoic acid (DNBT) reagent, then scanning the resulted yellow color at 405nm. The superoxide dismutase (SOD) activity was assessed by inhibiting the xanthine-xanthine oxidase reaction system and, as a result, inhibiting the generation of nitrite from hydroxylamine oxidation. A purple color that may be quantified calorimetrically at 550nm is produced when a chromogenic reagent is added to nitrite **(Li *et al.*, 2023)**. The capacity of catalase (CAT) to break down $H_2O_2$ was used to measure the CAT activity. A yellowish compound is created when the remaining $H_2O_2$ reacts with ammonium molybdate and, thereafter, this color can be read at 405nm **(Bartolini *et al.*, 2022)**. All the tests were carried out following the manufacturer's protocols of the Elabscience Biotechnology Inc. colorimetric assay kits (Houston, Texas, USA). A microplate reader from BioTek instruments (model ELx808TM, Vermont, USA) was employed for all the colorimetric detections.

## 5. Immune response

After a 4-week feeding period, blood samples from three fishes per replicate were taken in each treatment group (n=18). In tubes containing 10% ethylenediaminetetraacetate (EDTA), an anticoagulant, the samples were carefully combined. Following the standard procedures outlined by **Rawling *et al.* (2009)**, a drop of the whole blood was placed into a Bright-Line$^{TM}$ hemocytometer slide (American Optical, Buffalo, NY, USA) to quantify the total leukocyte cells (TLCs) count. Additionally, another drop of the whole blood was run gently on a glass slide and stained by Hema-3 dye reagents (Fisher Scientific, Pittsburgh, PA, USA) to count 200 leukocyte cells, including lymphocytes (L) and heterophils (H).

Leukocyte proliferation index (LPI) assays were performed using the rest fraction of the whole blood, somewhat altering the protocols ascribed by **Carvalho *et al.* (2018)**. A Histopaque-1077 gradient medium (Sigma, MA, USA) was covered with blood samples that had been diluted 1:2 with PBS and centrifuged for 30 minutes at room temperature at 400 ×g. After aspirating and twice washing the cell layer at the interface with sterile PBS, the cell layer was centrifuged again at 600 ×g for 10 minutes. The resulting cell pellet was reconstituted in a milliliter of RPMI-1640 medium (Invitrogen Corp., Grand Island, NY, USA), counted, and evaluated with trypan blue dye for vitality more than 95%, lymphocytes were divided into three portions in curved-bottom 96-well microplates after being complemented to 3 million cells per mL. To aid in leukocyte proliferation, a 10µg/ mL lipopolysaccharide solution (Sigma, MA, USA) was appended to the suspension. To serve as control cells, RPMI-1640 was added to a series of wells. After 18 hours of incubation at 27°C, the microplate was incubated for a further 4 hours with the tetrazolium salt MTT and then centrifuged at 110 ×g for five minutes. After formazan crystals were precipitated in the wells, 100mL of dimethyl sulfoxide (DMSO) was added to dissolve it again. A microplate reader (Bio-Rad 550 Laboratories Inc., USA) was used to determine the optical density (OD) of the experimental and control samples at 570nm. The OD of the stimulated experimental cells divided by the OD of the unstimulated control cells was used to compute the LPI.

The turbidimetric technique was applied to evaluate the lysozyme activity (LZA), as ascribed in a previous work **(Bae *et al.*, 2012)**. The basis for this test is the capacity of fish blood's lysozyme proteins to break down Gram-positive bacteria's cell walls. Fifty microliters of the serum (3 samples per replicate, n=18 samples per treatment group) were mixed with one milliliter of *Micrococcus lysodeikticus* suspension obtained from Sigma-Aldrich company. A Cecil-spectrophotometer instrument (model CE1010, Cambridge, United Kingdom) was used to measure the decrease in absorbance at intervals of 0.5 and 4.5 minutes throughout a 30-minute period. The wavelength was 450nm. One unit of LZA was defined as a 0.001 per minute drop in absorbance.

With certain adjustments made to the protocol of **Almarri *et al.* (2023)**, the phagocytic activity (PA) was measured in the blood samples taken from each group (3

fishes per replicate, n=18 samples per the treatment) after the 4-week feeding period. The leukocyte suspension was separated as mentioned before. A yeast *Candida albicans* suspension (Sigma), previously dyed with Tetramethyl-Rhodamine-Isothiocyanate (TRITC), was mixed with the leukocyte solution at a ratio of 1:4. This mixture was cultivated on a 24-well plate covered with gelatine and plasma for 30 minutes at 37°C. A hemacytometer and an inverted microscope were used to count the total number of phagocytes and those that contained at least one yeast. The leucocytes' PA was detected by calculating the percentage of phagocytes presenting ingested yeast from the entire phagocytes.

### 6. Statistical analysis

For the statistical analysis of the pilot experiment and the major trial data, IBM SPSS Statistics version 22 (IBM Corp., NY, USA) was used. Over the course of eight weeks, the pilot experiment examined the growth performance of fish exposed to varying amounts of LA (0, $10^2$, $10^4$, $10^6$, $10^8$, and $10^{10}$ CFU/g). The linear and quadratic trends linked to the elevated LA levels were examined using a one-way-ANOVA in conjunction with a polynomial contrast test. This research used a 2×2 factorial analysis of the main trial data, concentrating on the following key elements: CS treatment at 18 °C (– CS vs. + CS), LA therapy at $10^6$ CFU/g (– LA vs. + LA), and their interactions (LA × CS). Fish growth, antioxidant activity, and immune response variables were explored by the GLM of the 2-way-ANOVA framework. When statistically significant interaction effects were found, a one-way-ANOVA and a Duncan post hoc test were employed to rank the interaction means. At *P*-value < 0.05, the significance threshold was set.

## RESULTS AND DISCUSSION

### 1. Pilot experiment

The findings of the pilot research are shown in Fig. (1). The findings demonstrate that LA levels did not influence the body weights of the fish throughout the first three weeks of LA supplementation ($P > 0.05$). During the $4^{th}$ to $8^{th}$ weeks of LA supplementation, the body weights of the fish in the group receiving $10^6$ CFU/g LA were considerably ($P< 0.05$) greater than those in the other groups. In a similar study on striped catfish supplemented with 0, $10^3$, $10^5$, $10^7$ and $10^9$ CFU/g LA in the diet, the positive effect on growth performance was found at $10^5$ CFU/g compared to the other concentrations **(Akter *et al.*, 2019)**. Based on these results, LA supplementation at $10^6$ CFU/g for 4 weeks was selected for feeding the Nile tilapia challenged with CS in the primary experiment of the current study.

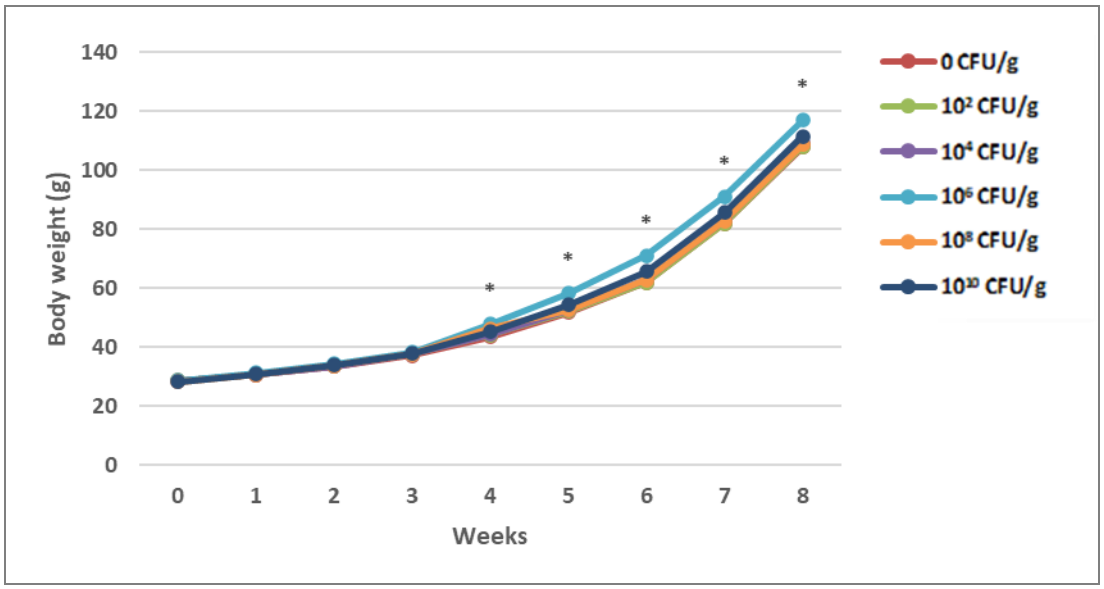


**Fig. 1.** The body weights of the Nile tilapia supplemented with different levels of *Lactobacillus acidophilus* (LA) in the feed for 8 consecutive weeks. The results show a remarkable ($P$< 0.05) elevation in the fish body weights from 4 to 8 weeks of feeding LA at $10^6$ CFU/g (*) compared to the other LA concentrations

## 2. Effect of LA on tilapia antioxidant activity under CS conditions

The antioxidant activity in fish under CS conditions is a crucial physiological process to avert the negative impacts of excessive reactive oxygen species (ROS) generated in the body upon exposing to an oxidative stress **(Birben *et al.*, 2012)**. In this regard, the effect of LA inclusion in feeds of the Nile tilapia challenged by CS on some oxidant indicators, like MPO and MDA, and some antioxidant indicators, like GSH, CAT, and SOD, were investigated in the current study (Table 2). The results illustrated a noticeable interaction ($P$ < 0.05) for the CS×LA on the MDA, GSH, CAT, and SOD, while the MPO did not differ significantly in the interaction groups. It was found that the MDA levels were increased by 43% in the CS group ($P$ < 0.05), and the GSH, CAT, and SOD activity were decreased ($P$ < 0.05) by 20, 30, and 32%, respectively, compared to the control. When LA was included in the feed, the antioxidant activity was improved in comparison with the non-LA treated group ($P$ < 0.05). Furthermore, LA treatment significantly ($P$ < 0.05) alleviated approximately 17, 27, 32, and 37% of the CS negative effects on the MDA, GSH, CAT, and SOD, respectively, in the CS+LA group compared to the CS group.

**Table 2.** Effect of adding *Lactobacillus acidophilus* (LA) to the diet on the antioxidant activity of the Nile tilapia under cold stress (CS) challenge

| Treatment groups | MPO (U/L) | MDA (µmol/L) | rGSH (µmol/L) | CAT (U/mL) | SOD (U/mL) |
|---|---|---|---|---|---|
| Control | 62.7 | 4.2 $^{c}$ | 11.6 $^{b}$ | 57.0 $^{b}$ | 9.3 $^{ab}$ |
| CS | 68.6 | 6.0 $^{a}$ | 9.3 $^{c}$ | 39.7 $^{c}$ | 6.3 $^{c}$ |
| LA | 55.3 | 3.2 $^{d}$ | 14.2 $^{a}$ | 69.7 $^{a}$ | 11.6 $^{a}$ |
| CS+LA | 61.2 | 5.0 $^{b}$ | 11.8 $^{b}$ | 52.5 $^{b}$ | 8.6 $^{b}$ |
| SEM | 6.77 | 0.36 | 1.04 | 4. 25 | 0.90 |
| *P*-value | | | | | |
| CS | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| LA | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| CS×LA | 0.737 | 0.012 | 0.005 | <0.001 | 0.033 |

Data represent the interaction means with pooled standard error of means (SEM) and the probabilities (*P*-value) of cold stress (CS), *Lactobacillus acidophilus* (LA), and their interaction (CS×LA). Uncommon superscripts in the same column denote the significance of the means at $P < 0.05$. Treatment groups: Control, fish were fed on a baseline diet without LA supplementation and exposed to a normal temperature of 26°C; CS, fish were fed on a baseline diet without LA supplementation and exposed to CS of 18°C; LA, fish were fed on a baseline diet supplemented with $10^6$ CFU/g LA and exposed to a normal temperature of 26°C; CS+LA, fish were fed on a baseline diet supplemented with $10^6$ CFU/g LA and exposed to CS of 18°C. Variables: MPO, myeloperoxidase; MDA, malondialdehyde; rGSH, reduced glutathione; CAT, catalase; SOD, superoxide dismutase.

In line with other studies **(Lu *et al.*, 2019)**, the current results indicated that exposure to the cold caused an increase of oxidative stress and a decrease of antioxidant defense in fish. It is suggested that SOD enzyme activity is a crucial process for superoxide elimination which is a highly ROS produced during the cellular oxidation **(Wang *et al.*, 2016)**. While CAT activity is also essential to reduce the $H_2O_2$, a less ROS generated by SOD during the dismutation of $O_2$ **(Birben *et al.*, 2012)**. In contrast, LA treatment decreased the MDA levels in the CS challenged fish due to the alleviation of lipid peroxidation by the presence of free radicals **(Birben *et al.*, 2012)**. Our results agreed with previous studies that shown an elevation in the antioxidant activity by dietary probiotics supplementation in various fish species, such as Roho labeo fish fed *Bacillus subtilis* **(Giri *et al.*, 2013)**, red sea bream fish fed *Lactobacillus rhamnosus* **(Dawood *et al.*, 2016)**, Mozambique tilapia fish fed *Bacillus licheniformis* **(Gobi *et al.*, 2018)**, and black sea bream fish fed *Lactobacillus plantaarum* **(Sagada *et al.*, 2021)**. The beneficial effect of LA on the antioxidant activity could be explained by the high expression of the antioxidant enzymes **(Zhang *et al.*, 2013)** and/or by the regulation of the Nrf2/keap1b signaling pathway **(Qin *et al.*, 2020)**.

### *3. Effect of LA on tilapia immune response under CS conditions*

The dietary LA supplements' effect on the immunological response of CS-challenged the Nile tilapia is shown in Table (3). The fish immunological parameters were significantly ($P< 0.05$) impacted by the CS×LA treatments. The CS challenge caused a substantial ($P< 0.05$) reduction of 18% in the TLCs, 22% in the LPI, 24% in the LZA, and 25% in the PA, and a rise of 18% in the heterophil to lymphocyte (H/L) ratio, as compared to the control fish. Conversely, compared to the control group, LA therapy substantially ($P< 0.05$) reduced the H/L ratio by 16% and raised the TLCs, LPI, LZA, and PA by 20, 21, 13, and 28%, respectively. Additionally, adding LA to the diet of fish with CS considerably ($P< 0.05$) reduced the destructive effect of CS on the fish's immunological markers and brought them back to levels that were comparable to the control.

**Table 3.** Effect of adding *Lactobacillus acidophilus* (LA) to the diet on the immunlogical parameters of the Nile tilapia under cold stress (CS) challenge

| Treatment group | TLCs ($10^3$/µL) | H/L ratio | LPI | LZA (U/mL) | PA (%) |
|---|---|---|---|---|---|
| Control | 5.87 $^{b}$ | 0.50 $^{b}$ | 3.95 $^{b}$ | 28.69 $^{b}$ | 23.06 $^{b}$ |
| CS | 4.81 $^{c}$ | 0.59 $^{a}$ | 3.07 $^{c}$ | 21.76 $^{d}$ | 17.25 $^{c}$ |
| LA | 7.03 $^{a}$ | 0.42 $^{c}$ | 4.78 $^{a}$ | 32.42 $^{a}$ | 29.41 $^{a}$ |
| CS+LA | 5.97 $^{b}$ | 0.51 $^{b}$ | 3.90 $^{b}$ | 25.49 $^{c}$ | 23.60 $^{b}$ |
| SEM | 0.124 | 0.028 | 0.237 | 1.414 | 1.405 |
| *P*-value | | | | | |
| CS | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| LA | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| CS×LA | 0.022 | 0.004 | 0.034 | <0.001 | 0.002 |

Data represent the interaction means with pooled standard error of means (SEM) and the probabilities (*P*-value) of cold stress (CS), *Lactobacillus acidophilus* (LA), and their interaction (CS×LA). Uncommon superscripts in the same column denote the significance of the means at $P < 0.05$. Treatment groups: Control, fish were fed on a baseline diet without LA supplementation and exposed to a normal temperature of 26°C; CS, fish were fed on a baseline diet without LA supplementation and exposed to CS of 18°C; LA, fish were fed on a baseline diet supplemented with $10^6$ CFU/g LA and exposed to a normal temperature of 26°C; CS+LA, fish were fed on a baseline diet supplemented with $10^6$ CFU/g LA and exposed to CS of 18°C. Variables: TLCs, total leukocyte cells; H/L ratio, heterophil to lymphocyte cell ratio; LPI, leukocyte proliferation index; LZA, lysozyme activity; PA, phagocytic activity.

Except for the H/L ratio, which sharply rose in fish exposed to CS, the immunological parameters were drastically lowered. According to **Davis *et al.* (2008)**, a high H/L ratio might be a sign that fish exposed to CS are under stress. Furthermore, CS has been shown to have a detrimental effect on fish immunity, as seen by a notable decrease in humoral response, antibody formation, and lymphocyte proliferation **(Hoseinifar *et al.*, 2015)**. Supplementing LA to the fish feed has been indicated to

promote the immunity in some fish species, including the Nile tilapia **(Lara-Flores *et al.*, 2003; Villamil *et al.*, 2014)**, black swordtail **(Hoseinifar *et al.*, 2015)**, rainbow trout **(Enferadi *et al.*, 2018)**, and stripped catfish **(Akter *et al.*, 2019)** and the common carp **(Adeshina *et al.*, 2020)**.

The current findings revealed that the dietary LA addition had a favorable impact on the tilapia fish's immunological markers, especially those challenged with CS. The increase in the LZA and PA reflected an enhancement in fish resistance to infections and an improvement in fish innate immune response **(Sagada *et al.*, 2021)**. It was reported that some strains of lactobacilli cells or its components, such as lipopolysaccharides and peptidoglycan, could be considered as a continuous alert of the defense system and consequently, as a strong regulator of fishes' immunological responses **(Panigrahi *et al.*, 2005)**. Additionally, *lactobacilli* may enhance immune response by stimulating the mucosal and humoral immunity **(Van Nguyen *et al.*, 2019)**. Furthermore, the LA probiotic effects on the immunomodulatory response can contribute to the ability of aquatic animals to balance the stress and infection through more secretion of cytokines associated with pro- and anti-inflammation **(Foysal *et al.*, 2020)**. In this regard, it is possible that LA can mitigate the detrimental effects of CS on the H/L ratio and the LPI in tilapia fish via mediation of T-helper type 1 (Th1), Th2, and Interferon-gamma (IFN-γ) cytokines **(Torii *et al.*, 2007; Krams *et al.*, 2012; Imani Fooladi *et al.*, 2015)**. In agreement with other studies on LA administration in human **(Mañé *et al.*, 2011)**, mice **(Lee *et al.*, 2013)**, and poultry **(Alaqil *et al.*, 2020)**, the leukocyte proliferation was improved by LA treatment in both control and CS-challenged fish. As a consequence, the increase in leukocyte proliferation causes an increase in the B-lymphocytes which are responsible for the immunoglobulin production and humoral immunity **(Ding *et al.*, 2017)**.

## 4. Effect of LA on tilapia growth indices under CS conditions

Table (4) displays the impact of adding LA to the diet on the growth indices of CS-challenged Nile tilapia. The CS×LA results showed a substantial ($P< 0.05$) interaction influence on every fish growth performance metric. Comparing the FBW, GBW, FI, SGR, FCR, and SR to the control, the exposure to CS substantially ($P< 0.05$) degraded them by around 17, 52, 22, 47, 58, and 37%, respectively. Conversely, compared to the control, the LA therapy substantially ($P< 0.05$) increased the FBW by 3% and the GBW by 10%. Additionally, all growth performance indicators in the CS-treated fish were considerably ($P< 0.05$) improved by the LA therapy, which raised the FBW, GBW, SGR, FI, FCR, and SR in the CS+LA group by about 5, 31, 23, 37, 7, and 38%, respectively, in comparison to the CS group.

**Table 4.** Effect of adding *Lactobacillus acidophilus* (LA) to the diet on the growth indices of the Nile tilapia under cold stress (CS) challenge

| Treatment groups | IBW (g) | FBW (g) | GBW (g) | FI (g) | FCR | SGR (%) | SR (%) |
|---|---|---|---|---|---|---|---|
| Control | 29.9 | 45.2 $^{b}$ | 15.3 $^{b}$ | 28.8 $^{ab}$ | 1.9 $^{c}$ | 1.5 $^{a}$ | 97.0 $^{a}$ |
| CS | 30.0 | 37.4 $^{d}$ | 7.4 $^{d}$ | 22.4 $^{c}$ | 3.0 $^{a}$ | 0.8 $^{c}$ | 61.2 $^{c}$ |
| LA | 29.7 | 46.5 $^{a}$ | 16.8 $^{a}$ | 29.1 $^{a}$ | 1.7 $^{c}$ | 1.6 $^{a}$ | 97.9 $^{a}$ |
| CS+LA | 29.5 | 39.2 $^{c}$ | 9.7 $^{c}$ | 27.5 $^{b}$ | 2.8 $^{b}$ | 1.1 $^{b}$ | 84.3 $^{b}$ |
| SEM | 0.28 | 0.53 | 0.56 | 0.66 | 0.02 | 0.05 | 1.46 |
| *P*-value | | | | | | | |
| CS | 0.503 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| LA | 0.503 | <0.001 | <0.001 | <0.001 | 0.019 | <0.001 | <0.001 |
| CS×LA | 1.000 | 0.003 | 0.012 | <0.001 | 0.219 | 0.036 | <0.001 |

Data represent the interaction means with pooled standard error of means (SEM) and the probabilities (*P*-value) of cold stress (CS), *Lactobacillus acidophilus* (LA), and their interaction (CS×LA). Uncommon superscripts in the same column denote the significance of the means at $P < 0.05$. Treatment groups: Control, fish were fed on a baseline diet without LA supplementation and exposed to a normal temperature of 26°C; CS, fish were fed on a baseline diet without LA supplementation and exposed to CS of 18°C; LA, fish were fed on a baseline diet supplemented with $10^6$ CFU/g LA and exposed to a normal temperature of 26°C; CS+LA, fish were fed on a baseline diet supplemented with $10^6$ CFU/g LA and exposed to CS of 18°C. Variables: IBW, initial body weight; FBW, final body weight; GBW, gained body weight; FI, feed intake; FCR, feed conversion ratio; SGR, specific growth rate; SR, survival rate.

Stress disrupts homeostasis and triggers certain physiological responses in fish to preserve survival and bodily balance **(Barton, 2002)**. Indeed, the Nile tilapia may flourish in an extent of water temperatures from 24 to 32°C, relying upon the age, species, and genetic diversity **(El-Sayed & Kawanna, 2008)**. According to the present research, the Nile tilapia fish's feed efficiency and all growth indices are negatively impacted by the CS-challenge at 18°C. The feed intake decline may be an explicit source of deterioration in the other tilapia growth markers, such as FBW, BWG, SGR, and FCR **(Nobrega *et al.*, 2020)**. Similar results were observed in prior research when tilapia fish were farmed in a low suboptimal temperature (22°C) instead of optimal temperature (28°C) **(Ma *et al.*, 2015; Corrêa *et al.*, 2018)**. Previous research indicates that the Nile tilapia subjected to 18°C for several days exhibited a negative impact on their survival, elevating the death rates by 20–60% **(Ibrahim *et al.*, 2019; Almarri *et al.*, 2023)**.

In general, lactic acid bacteria have been acquainted for its probiotic properties and can be used to enhance the growth performance in aquaculture **(Al-Dohail *et al.*, 2011)**. The beneficial effects of LA on the immunological parameters and antioxidant activity in the CS-treated tilapia should also be evident in their growth performance. The present study's improved fish growth performance may be due to LA's enhancement of intestinal integrity and digestion, which improves nutrient absorption **(Xia *et al.*, 2018; Won *et al.*, 2020)**. It is thought that probiotic bacteria produce bioactive compounds called bacteriocins, which may arrest the development of pathogenic bacteria **(Selle *et al.*,**

**2014)**. Moreover, the extracellular enzymes produced by LA activity in the feeds may provide the fish with fundamental nutrients, including vitamins, fatty acids, and amino acids, which increase the growth aspects **(Balcázar *et al.*, 2006)**. In a constant to other studies on fish received *bacilli* probiotics **(Dawood *et al.*, 2016; Zaineldin *et al.*, 2018; Sagada *et al.*, 2021)**, LA administration to tilapia fish increased the feed intake in both LA and CS×LA groups compared to their controls. The increased feed intake may be referred to the increased levels of proteins and lipids in the fish diet **(Aliyu-Paiko *et al.*, 2010)**. It also could be due to the increase of feed palatability or the mediation of gastrointestinal tract's satiation sensory neurons **(Ritter, 2004)**; however, there is now little proof that probiotics have a major impact on fish feed palatability. The fish body secretes endogenous enzymes, which can be synthesized with the aid of exogenous enzymes, such as protease, amylase, and lipase, released by probiotic cells **(Mohapatra *et al.*, 2012)**. These exogenous enzymes have a wider pH tolerance than endogenous enzymes, which can postpone digestion and guarantee optimal nutrient utilization **(Daboor *et al.*, 2010)**. It was reported that increased lipase activity may be a reason for effective lipid utilization of diets supplemented with *L. acidophilus*, which in turn causes lipids digestion and essential fatty acids assimilation, leading to increased development in fish **(Akter *et al.*, 2019)**.

## CONCLUSION

The antioxidant activity, immunological response, and growth indices of the Nile tilapia fish raised at 26°C were all enhanced by dietary LA addition at a concentration of $10^6$ CFU/g. On the other hand, the Nile tilapia challenged with the CS showed an impairment in all indicators of growth performance, immunological response, and antioxidant activity. The detrimental effects on the feed conversion efficiency, final weights, and specific growth rates, as well as the antioxidant enzyme activity, leukocyte proliferation, and phagocytosis activity were mitigated by dietary LA supplementation in fish challenged with CS. Additionally, LA therapy reduced the H/L ratio and malondialdehyde levels, which are markers of stress, and enhance the survival rates of fish challenged with CS. Thus, adding LA at a level of $10^6$ CFU/g to the Nile tilapia fish diet may be a promising program to improve their health and growth indices, particularly in the face of cold stress.

## ACKNOWLEDGEMENT

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (KFU251169).

## REFERENCES

**Adeshina, I.; Abubakar, M. I. and Ajala, B. E.** (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 46:1375–1385.

**Akter, M. N.; Hashim, R.; Sutriana, A.; Siti Azizah, M. N. and Asaduzzaman, M.** (2019). Effect of *Lactobacillus acidophilus* supplementation on growth performances, digestive enzyme activities and gut histomorphology of striped catfish (*Pangasianodon hypophthalmusSauvage*, 1878) juveniles. Aquac Res 50:786–797.

**Alaqil, A. A.; Abbas, A. O.; El-Beltagi, H. S.; Abd El-Atty, H. K.; Mehaisen, G. M. and Moustafa, E. S.** (2020). Dietary supplementation of probiotic *lactobacillus acidophilus* modulates cholesterol levels, immune response, and productive performance of laying hens. Animals 10:1–12.

**Al-Dohail, M. A.; Hashim, R. and Aliyu-Paiko, M.** (2011). Evaluating the use of *Lactobacillus acidophilus* as a biocontrol agent against common pathogenic bacteria and the effects on the haematology parameters and histopathology in African catfish *Clarias gariepinus* juveniles. Aquac Res 42:196–209.

**Alishahi, M.; Dezfuly, Z. T.; Mohammadian, M. and Mesbah, M.** (2018). Effects of Two Probiotics, *Lactobacillus Plantarum* and *Lactobacillus Bulgaricus* on Growth Performance and Intestinal Lactic Acid Bacteria of *Cyprinus Carpio*. Iran J Vet Med, 12:207-218.

**Aliyu-Paiko, M.; Hashim, R. and Shu-Chien, A. C.** (2010). Influence of dietary lipid/protein ratio on survival, growth, body indices and digestive lipase activity in Snakehead (*Channa striatus*, Bloch 1793) fry reared in re-circulating water system. Aquac Nutr 16:466–474.

**Almarri, S. H.; Khalil, A. A.; Mansour, A. T. and El-Houseiny, W.** (2023). Antioxidant, Immunostimulant, and Growth-Promoting Effects of Dietary Annona squamosa Leaf Extract on Nile Tilapia, *Oreochromis niloticus*, and its tolerance to thermal stress and aeromonas sobria infection. Animals 13:746-758.

**Alrashada, Y. N.; Hassanien, H. A.; Abbas, A. O.; Alkhamis, S. A. and Alkobaby, A. I.** (2023). Dietary propolis improves the growth performance, redox status, and immune response of Nile tilapia upon a cold-stress challenge. PLoS One 18: e0293727.

**Arora, S.; Singh, P.; Tabassum, G.; Dohare, R. and Syed, M. A.** (2022). miR-495–3p regulates sphingolipid metabolic reprogramming to induce Sphk1/ceramide mediated mitophagy and apoptosis in NSCLC. Free Radic Biol Med 189:71–84.

**Bae, J. Y.; Park, G. H.; Lee, J. Y.; Okorie, O. E. and Bai, S. C.** (2012). Effects of dietary propolis supplementation on growth performance, immune responses,

disease resistance and body composition of juvenile eel, *Anguilla japonica*. Aquaculture International 20:513–523.

**Balcázar, J. L.; De Blas, I.; Ruiz-Zarzuela, I.; Cunningham, D.; Vendrell, D. and Múzquiz, J. L.** (2006). The role of probiotics in aquaculture. Vet Microbiol 114:173–186.

**Bartolini, D.; Arato, I.; Mancuso, F.; Giustarini, D.; Bellucci, C.; Vacca, C.; Aglietti, D. M.; Stabile, A. M.; Rossi, R.; Cruciani, G.; Rende, M.; Calafiore, R.; Luca, G. and Galli, F.** (2022). Melatonin modulates Nrf2 activity to protect porcine pre-pubertal Sertoli cells from the abnormal H2O2 generation and reductive stress effects of cadmium. J Pineal Res 73:e12806.

**Barton, B. A.** (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517–525.

**Birben, E.; Sahiner, U. M.; Sackesen, C.; Erzurum, S. and Kalayci, O.** (2012). Oxidative Stress and Antioxidant Defense. World Allergy Organization Journal 2012 5:1 5:9–19.

**Carnevali, O.; Maradonna, F. and Gioacchini, G.** (2017). Integrated control of fish metabolism, wellbeing and reproduction: The role of probiotic. Aquaculture 472:144–155.

**Carvalho, P. L. P. F.; Yamamoto, F. Y.; Barros, M. M. and Gatlin, D. M.** (2018). L-glutamine in vitro supplementation enhances Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) leukocyte function. Fish Shellfish Immunol 80:592–599.

**Corrêa, C. F.; Nobrega, R. O.; Block, J. M. and Fracalossi, D. M.** (2018). Mixes of plant oils as fish oil substitutes for Nile tilapia at optimal and cold suboptimal temperature. Aquaculture 497:82–90.

**Cross, M. L.** (2002). Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. FEMS Immunol Med Microbiol 34:245–253.

**Davis, A. K.; Maney, D. L. and Maerz, J. C.** (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol 22:760–772.

**Dawood, M. A. O.; Koshio, S.; Ishikawa, M.; El-Sabagh, M. M.; Esteban, A. and Zaineldin, A. I.** (2016). Probiotics as an environment-friendly approach to enhance red sea bream, Pagrus major growth, immune response and oxidative status. Fish Shellfish Immunol 57:170–178.

**Ding, Y. H.; Qian, L. Y.; Pang, J.; Lin, J. Y.; Xu, Q.; Wang, L. H.; Huang, D. S. and Zou, H.** (2017). The regulation of immune cells by Lactobacilli: A potential therapeutic target for anti-atherosclerosis therapy. Oncotarget 8:59915–59928.

**El-Houseiny, W.; Khalil, A. A.; Abd-Elhakim, Y. M. and Badr, H. A.** (2019). The potential role of turmeric and black pepper powder diet supplements in reversing cadmium-induced growth retardation, ATP depletion, hepatorenal damage, and testicular toxicity in *Clarias gariepinus*. Aquaculture 510:109–121.

**El-Sayed, A. F. M.** (2019). Tilapia culture: Second edition. Tilapia Culture: Second Edition:1–348.

**El-Sayed, A. F. M. and Kawanna, M.** (2008). Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. Aquac Res 39:670–672.

**Enferadi, M. H. N.; Mohammadizadeh, F.; Soltani, M. A.; Bahri, H. and Sheikhzadeh, N.** (2018). Effects of *Lactobacillus plantarum* on Growth Performance, Proteolytic Enzymes Activity and Intestine Morphology in Rainbow Trout (*Oncorhynchus mykiss*). Turk J Fish Aquat Sci 18:351–356.

**Daboor, M.; Esmael, N. A. and Lall, S. P.** (2010). Effect of Different Dietary Probiotics on Growth, Feed Utilization and Digestive Enzymes Activities of Nile Tilapia, *Oreochromis niloticus* Mohamed A Essa1, Sabry S EL-Serafy2, Magda M El-Ezabi2, Said. Aquaculture, 454, 243-251.

**Faramazi, M.; Kiaalvandi, S.; Lashkarbolooki, M. and Iranshahi, F.** (2011). The Investigation of Lactobacillus acidophilus as probiotics on growth performance and disease resistance of rainbow trout (*Oncorhynkus mykiss*). American-Eurasian Journal of Scientific Research 6:32–38.

**Foysal, M. J.; Fotedar, R.; Siddik, M. A. B. and Tay, A.** (2020). *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). Sci Rep 10.

**Galloway, B. J. and Kieffer, J. D.** (2003). The Effects of an Acute Temperature Change on the Metabolic Recovery from Exhaustive Exercise in Juvenile Atlantic Salmon (*Salmo salar*). Physiological and Biochemical Zoology 76:652–662.

**Giri, S. S.; Sukumaran, V. and Oviya, M.** (2013). Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. Fish Shellfish Immunol 34:660–666.

**Gobi, N.; Vaseeharan, B.; Chen, J. C.; Rekha, R.; Vijayakumar, S.; Anjugam, M. and Iswarya, A.** (2018). Dietary supplementation of probiotic Bacillus licheniformis Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against Aeromonas hydrophila in tilapia *Oreochromis mossambicus*. Fish Shellfish Immunol 74:501–508.

**Hassaan, M. S.; EL Nagar, A. G.; Salim, H. S.; Fitzsimmons, K. and El-Haroun, E. R.** (2019). Nutritional mitigation of winter thermal stress in Nile tilapia by propolis-extract: Associated indicators of nutritional status, physiological responses and transcriptional response of delta-9-desaturase gene. Aquaculture 511.

**Hoseinifar, S. H.; Roosta, Z.; Hajimoradloo, A. and Vakili, F.** (2015). The effects of Lactobacillus acidophilus as feed supplement on skin mucosal immune

parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*). Fish Shellfish Immunol 42:533–538.

**Ibrahim, R. E.; El-Houseiny, W.; Behairy, A.; Abo-Elmaaty, A. and Al-Sagheer, A. A.** (2019). The palliative role of *Eruca sativa* leaves dietary supplementation against oxidative stress, immunosuppression, and growth retardation in temperature-stressed *Oreochromis niloticus*. J Therm Biol 84:26–35.

**Imani Fooladi, A. A.; Yazdi, M. H.; Pourmand, M. R.; Mirshafiey, A.; Mohammad Hassan, Z.; Azizi, T.; Mahdavi, M. and Soltan Dallal, M. M.** (2015). Th1 Cytokine Production Induced by Lactobacillus acidophilus in BALB/c Mice Bearing Transplanted Breast Tumor. Jundishapur J Microbiol 8:17354.

**Krams, I.; Vrublevska, J.; Cirule, D.; Kivleniece, I.; Krama, T.; Rantala, M. J.; Sild, E. and Hõrak, P.** (2012). Heterophil/lymphocyte ratios predict the magnitude of humoral immune response to a novel antigen in great tits (*Parus major*). Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology 161:422–428.

**Lara-Flores, M.; Olvera-Novoa, M. A.; Guzmán-Méndez, B. E. and 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 216:193–201.

**Lee, J.; Bang, J. and Woo, H. J.** (2013). Effect of orally administered Lactobacillus brevis HY7401 in a food allergy mouse model. J Microbiol Biotechnol 23:1636–1640.

**Li, M.; Liu, J.; Shi, L.; Zhou, C.; Zou, M.; Fu, D.; Yuan, Y.; Yao, C.; Zhang, L.; Qin, S.; Liu, M.; Cheng, Q.; Wang, Z. and Wang, L.** (2023). Gold nanoparticles-embedded ceria with enhanced antioxidant activities for treating inflammatory bowel disease. Bioact Mater 25:95–106.

**Lu, D. L.; Ma, Q.; Sun, S. X.; Zhang, H.; Chen, L. Q.; Zhang, M. L. and Du, Z. Y.** (2019). Reduced oxidative stress increases acute cold stress tolerance in zebrafish. Comp Biochem Physiol A Mol Integr Physiol 235:166–173.

**Ma, X. Y.; Qiang, J.; He, J.; Gabriel, N. N. and Xu, P.** (2015). Changes in the physiological parameters, fatty acid metabolism, and SCD activity and expression in juvenile GIFT tilapia (*Oreochromis niloticus*) reared at three different temperatures. Fish Physiol Biochem 41:937–950.

**Mañé, J.; Pedrosa, E.; Lorén, V.; Gassull, M. A.; Espadaler, J.; Cuñé, J.; Audivert, S.; Bonachera, M. A. and Cabré, E.** (2011). A mixture of *Lactobacillus plantarum* CECT 7315 and CECT 7316 enhances systemic immunity in elderly subjects. A dose-response, double-blind, placebo-controlled, randomized pilot trial. Nutr Hosp 26:228–235.

**Mansour, A. T.; Mahboub, H. H.; Elshopakey, G. E.; Aziz, E. K.; Alhajji, A. H. M.; Rayan, G.; Ghazzawy, H. S. and El-Houseiny, W.** (2022). Physiological

Performance, Antioxidant and Immune Status, Columnaris Resistance, and Growth of Nile Tilapia That Received *Alchemilla vulgaris* Supplemented Diets. Antioxidants 2022, Vol. 11, Page 1494 11:1494.

**Martins, T.; Valentim, A. M.; Pereira, N. and Antunes, L. M.** (2016). Anaesthesia and analgesia in laboratory adult zebrafish: a question of refinement. Lab Anim 50:476–488.

**Miao, W. and Wang, W.** (2020). Trends of aquaculture production and trade: Carp, tilapia, and shrimp. Asian Fish Sci 33:1–10.

**Mihaila, A. C.; Ciortan, L.; Macarie, R. D.; Vadana, M.; Cecoltan, S. M.; Preda, B.; Hudita, A.; Gan, A. M.; Jakobsson, G.; Tucureanu, M. M.; Barbu, E.; Balanescu, S.; Simionescu, M.; Schiopu, A. and Butoi, E.** (2021). Transcriptional Profiling and Functional Analysis of N1/N2 Neutrophils Reveal an Immunomodulatory Effect of S100A9-Blockade on the Pro-Inflammatory N1 Subpopulation. Front Immunol 12:708770.

**Mohapatra, S.; Chakraborty, T.; Prusty, A. K.; Das, P.; Paniprasad, K. and Mohanta, K. N.** (2012). Use of different microbial probiotics in the diet of rohu, Labeo rohita fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquac Nutr 18:1–11.

**Van Nguyen, N.; Onoda, S.; Van Khanh, T.; Hai, P. D.; Trung, N. T.; Hoang, L. and Koshio, S.** (2019). Evaluation of dietary Heat-killed Lactobacillus plantarum strain L-137 supplementation on growth performance, immunity and stress resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 498:371–379.

**Nobrega, R. O.; Banze, J. F.; Batista, R. O. and Fracalossi, D. M.** (2020). Improving winter production of Nile tilapia: What can be done? Aquac Rep 18:100453.

**Nobrega, R. O.; Corrêa, C. F.; Mattioni, B. and Fracalossi, D. M.** (2017). Dietary α-linolenic for juvenile Nile tilapia at cold suboptimal temperature. Aquaculture 471:66–71.

**Latimer Jr., G. W.** (2023). Official Methods of Analysis of AOAC INTERNATIONAL, 22 (GW Latimer Jr., Ed.). Oxford University Press, New York, USA.

**Oh, S. Y. and Maran, B. A. V.** (2015). Feeding frequency influences growth, feed consumption and body composition of juvenile rock bream (*Oplegnathus fasciatus*). Aquaculture International 23:175–184.

**Panase, P.; Saenphet, S. and Saenphet, K.** (2018). Biochemical and physiological responses of Nile tilapia Oreochromis niloticus Lin subjected to cold shock of water temperature. Aquac Rep 11:17–23.

**Panigrahi, A.; Kiron, V.; Puangkaew, J.; Kobayashi, T.; Satoh, S. and Sugita, H.** (2005). The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. Aquaculture 243:241–254.

**Prabu, E.; Rajagopalsamy, C. B. T.; Ahilan, B.; Jeevagan, I. J. M. A. and Renuhadevi, M.** (2019). Tilapia – An Excellent Candidate Species for World Aquaculture: A Review. Annu Res Rev Biol:1–14.

**Qin, L.; Xiang, J.; Xiong, F.; Wang, G.; Zou, H.; Li, W.; Li, M. and Wu, S.** (2020). Effects of Bacillus licheniformis on the growth, antioxidant capacity, intestinal barrier and disease resistance of grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol 97:344–350.

**Rawling, M. D.; Merrifield, D. L. and Davies, S. J.** (2009). Preliminary assessment of dietary supplementation of Sangrovit® on red tilapia (*Oreochromis niloticus*) growth performance and health. Aquaculture 294:118–122.

**Ringø, E.; Olsen, R. E.; Gifstad, T.; Dalmo, R. A.; Amlund, H.; Hemre, G. I. and Bakke, A. M.** (2010). Prebiotics in aquaculture: a review. Aquac Nutr 16:117–136.

**Ritter, R. C.** (2004). Gastrointestinal mechanisms of satiation for food. Physiol Behav 81:249–273.

**Sagada, G.; Gray, N.; Wang, L.; Xu, B.; Zheng, L.; Zhong, Z.; Ullah, S.; Tegomo, A. F. and Shao, Q.** (2021). Effect of dietary inactivated Lactobacillus plantarum on growth performance, antioxidative capacity, and intestinal integrity of black sea bream (*Acanthopagrus schlegelii*) fingerlings. Aquaculture 535.

**Selle, K. M.; Klaenhammer, T. R. and Russell, W. M.** (2014). LACTOBACILLUS | Lactobacillus acidophilus.Pages 412–417 in Encyclopedia of Food Microbiology. Robinson, R.K., Batt, C.A., Patel, P.D., eds. second edition. Academic Press, San Diego, CA.

**The State of World Fisheries and Aquaculture** (2022). FAO. Available at https://www.fao.org/family-farming/detail/en/c/1565527/ (verified 14 September 2023).

**Torii, A.; Torii, S.; Fujiwara, S.; Tanaka, H.; Inagaki, N. and Nagai, H.** (2007). *Lactobacillus acidophilus* strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. Allergology International 56:293–301.

**Villamil, L.; Reyes, C. and Martínez-Silva, M. A.** (2014). In vivo and in vitro assessment of Lactobacillus acidophilus as probiotic for tilapia (*Oreochromis niloticus, Perciformes:Cichlidae*) culture improvement. Aquac Res 45:1116–1125.

**Wang, Y.** (2011). Use of probiotics *Bacillus coagulans, Rhodopseudomonas palustris* and *Lactobacillus acidophilus* as growth promoters in grass carp (*Ctenopharyngodon idella*) fingerlings. Aquac Nutr 17:e372–e378.

**Wang, Y. and Gu, Q.** (2010). Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. Marine Biology Research 6:327–332.

**Wang, L.; Wu, Z. Q.; Wang, X. L.; Ren, Q.; Zhang, G. S.; Liang, F. F. and Yin, S. W.** (2016). Immune responses of two superoxide dismutases (SODs) after

*lipopolysaccharide* or *Aeromonas hydrophila* challenge in pufferfish, Takifugu obscurus. Aquaculture 459:1–7.

**Won, S.; Hamidoghli, A.; Choi, W.; Park, Y.; Jang, W. J.; Kong, I. S. and Bai, S. C.** (2020). Effects of *Bacillus subtilis* WB60 and *Lactococcus lactis* on Growth, Immune Responses, Histology and Gene Expression in Nile Tilapia, *Oreochromis niloticus*. Microorganisms 2020, Vol. 8, Page 67 8:67.

**Xia, Y.; Lu, M.; Chen, G.; Cao, J.; Gao, F.; Wang, M.; Liu, Z.; Zhang, D.; Zhu, H. and 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.

**Zaineldin, A. I.; Hegazi, S.; Koshio, S.; Ishikawa, M.; Bakr, A.; El-Keredy, A. M.; Dawood, M. A.; Dossou, S.; Wang, W. and Yukun, Z.** (2018). *Bacillus subtilis* as probiotic candidate for red sea bream: Growth performance, oxidative status, and immune response traits. Fish Shellfish Immunol 79:303–312.

**Zerai, D. B.; Fitzsimmons, K. M. and Collier, R. J.** (2010). Transcriptional response of delta-9-desaturase gene to acute and chronic cold stress in Nile Tilapia, *Oreochromis niloticus*. J World Aquac Soc 41:800–806.

**Zhang, C. N.; Li, X. F.; Xu, W. N.; Jiang, G. Z.; Le Lu, K.; Wang, L. N. and Bin Liu, W.** (2013). Combined effects of dietary *fructooligosaccharide* and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*). Fish Shellfish Immunol 35:1380–1386.