Aquaculture Studies, 23(SI), AQUAST1228
http://doi.org/10.4194/AQUAST1228

AQUACULTURE STUDIES

RESEARCH PAPER

Combined Effects of Dietary Probiotic *Lactobacillus* sp. and Prebiotic Glycyrrhizic Acid on Growth Performance, Feed Utilization and Digestive Enzymes Activities of Nile Tilapia (*Oreochromis niloticus*) Juveniles

Ebru Yılmaz[1,*], Deniz Çoban[1], Cüneyt Suzer[2], Murat Er[3], Onurkan Antepli[2], İbrahim Köse[2]

[1]Aydın Adnan Menderes University, Faculty of Agriculture, Department of Aquaculture and Fisheries, Aydın, Turkey
[2]Ege University, Faculty of Fisheries, Aquaculture Department, 35100, Bornova, Izmir, Turkey
[3]İzmir Bakırçay University, Menemen Vocational School, Laboratory and Veterinary Health, İzmir, Turkey

How to Cite

Yılmaz, E., Çoban, D., Suzer, C., Er, M., Antepli, O., Köse, İ. (2023). Combined Effects of Dietary Probiotic *Lactobacillus* Sp. and Prebiotic Glycyrrhizic Acid on Growth Performance, Feed Utilization and Digestive Enzymes Activities of Nile Tilapia (*Oreochromis niloticus*). *Aquaculture Studies*, *23(SI),*

Article History
Received 01 December 2022
Accepted 23 February 2023
First Online 03 March 2023

Corresponding Author
Tel.: +902562206482
E-mail: ebruyilmaz@adu.edu.tr

Keywords
Nutrition
Growth promoter
Immunostimulant

Abstract

The present study investigated the effects of dietary commercial probiotic *Lactobacillus* sp. and commercial prebiotic glycyrrhizic acid on the growth performance, feed utilization and digestive enzymes activities of Nile tilapia. Seven diets with or without probiotics and prebiotics supple-mentation namely Control (without probiotics and prebiotics), P10, P20 and P30 (Control + Pro-aqua) and V2, V4 and V6 (Control +Viusid) were fed to juvenile Nile tilapia (5.22±0.05 g) for 60 days in triplicate tanks (30 fish per tank). All fish were fed ad libitum three times a day for 60 days. At the end of the experiment, the weight gain and specific growth rate of the fish fed with probiotic content group was found to be higher than the other groups (P<0.05). In the experiment, there was no change in the nutritional value of tilapia fed with a diet containing all feeding groups (P>0.05). On the other hand, significant changes were observed in alkaline and acid protease enzyme activities depending on the days (P<0.05). Especially, after the first 15-day period of the experiments, increases in both supplemented groups enzyme activities were determined. The increase in enzyme activity in the probiotic supplemented groups showed higher activity than the prebiotic administered groups (P<0.05). It can be concluded that probiotic can act as a growth promoter and increase digestive enzymes activity in tilapia.

Introduction

Oreochromis niloticus (L.), or the Nile tilapia, among the most important species of it genus because it growths rapidly, tolerantto diverse environmental conditions, is resistant to diseases and stress, and has high rates of survival in high density production (El-Sayed, 2019). However, death and economic losses are observed with the increase of stock density in aquaculture practices (Ridha & Azad, 2012; Pohlenz & Gatlin III, 2014; Chauhan & Singh, 2019). Therapeutic substances including chemicals and antibiotic medications are used to treat microbial infections that occur with increased stock density (Cabello, 2006; Chauhan & Singh, 2019). However, the widespread use of these chemotherapeutic drugs may allow of drug-resistant microorganisms to develop, with the death of beneficial microbes in the gastrointestinal tract, and the subsequent occurrence of harmful consequences for both aquatic environments and consumer health (Munoz-Atienzal *et al.*, 2013; Reverter *et al.*, 2014; Azevedo *et al.*, 2015). In 2006, the European Union banned applications of antibiotics as growth promoters. This ban reduced the growth of antibiotic-resistant microorganisms, but at the same time, fishery infections rate increased (Casewell *et al.*, 2003; European

Parliament and Council 2003; Bywater *et al*., 2005). Therefore, there is a need for, non-toxic antibiotic environmentally friendly alternative treatments for diseases among fish (Chauhan & Singh, 2019). In recent years, immunostimulants such as beta-glucan, honeybee pollen, chitin, peptidoglycan, lipopolysaccharide, nucleotide, plant extracts, probiotics and prebiotics have been widely used as alternatives to antibiotics (Sakai, 1999; Hoseinifar et al., 2015; Hoseinifar et al., 2016; Nowosad *et al*., 2023).

The microorganisms known as probiotics may be introduced to fish feed or water. At appropriate dosages, they stimulate the growth of the fish, support the animals' digestive systems, strengthen their immunity, prevent diseases, and improve quality of the water by positively influencing the microbial life of both the water and its sediments (Verschuere et al., 2000; Ringo *et al*., 2010; Gobi *et al*., 2018; Meidong *et al*., 2018; Deng *et al*., 2018). In the production of tilapia, lactic acid bacteria (*Bacillus* and *Lactobacillus*) are most often used as probiotics (El-Sayed, 2019). Researchers have confirmed that including *Lactobacillus* spp. in the diets of farmed tilapia supports the performance and general well-being of the fish. (Amer & El-Tawil, 2011; Ridha & Azad, 2012; Ramos *et al.,* 2017; Rahman, 2019; Andriani *et al*., 2019; Khunrang *et al*., 2021; Muaddama & Putri, 2021).

Research on the use of glycyrrhizic acid, a prebiotic with a wide range of applications in aquaculture, and its effects on reducing pathological bacterial load and supporting, live weight gain, feed utilization, and the immune system has also been increasing in recent years (Ocampo *et al*., 2014; Xu *et al*., 2015; Harikrishnan *et al*., 2021; Li *et al*., 2022). Glycyrrhizic acid is a prebiotic with antioxidant, anti-inflammatory, antineoplastic, immunomodulatory, hepatoprotective antihepatotoxic, neuroprotective, antiarthritic, antiallergic, antimicrobial, and antiviral, antiestrogenic, anticholinergic, antileukemic and anticarcinogenic activities. It is obtained from the root of the licorice plant and is widely used in the pharmaceutical and chemical industries (Xu *et al*., 2015; Harikrishnan *et al*., 2021).

The present study was undertaken with the aim of exploring the meristic and physiological effects of *Lactobacilllus* probiotics, are now being used extensively in aquaculture, together withglycyrrhizic acid, which is considered to be a growth promoter and prebiotic, on juvenile Nile tilapia.

Materials and Methods

Experimental Diet

In this study, commercial probiotic and prebiotic supplementation was performed with the liquid form of Pro-aqua, contains spores of four bacterial species (*Lactobacillusplantarum,* L. casei, *Rhodopseudomonas palustris,* and *Saccharomyces cerevisiae),* and the liquid form of Viusid (Catalysis Inc., Spain), which contains glycyrrhizic acid, glycerin, vitamins, B-12 and C, folic acid, calcium, malic acid, arginine, and glucosamine. Viusid was added to commercial fish feed by spraying method at rates of 2 mL/kg, 4 mL/kg and 6 mL/kg, respectively, while Pro-aqua was added at rates of 10 mL/kg, 20 mL/kg and 30 mL/kg. The fish of the control group received the standard diet without the addition of probiotics and prebiotics.

Fish and Experimental Design

The fish used in this study were acquired from the Faculty of Fisheries of Çukurova University in Adana, Turkey. At the start of the feeding trial, fish were stocked in 200-L glass aquaria. Fish weighing a mean of 5.21±0.05 g (± standard deviation *SD*, *n*=630) were subsequently distributed to 21 aquaria with (30 fish), in each of the tanks. Each treatment protocol was applied to three tanks, creating a triplicate research design. The water was exchanged once every 24 hours at a level corresponding to approximately 10% of the tank's water. The values of each tank received continuous aeration. Temperature, pH, and dissolved oxygen, usingalso recorded on a daily basis; these measurements were performed with a WTW Multi 3420 water analyzer. The mean values of these parameters over the full course of the study were as follows: temperature of 28.0±0.4°C, pH of 7.2±0.1, and dissolved oxygen of 7.7±0.2 mg/L.

Diet Preparation

Table 1 presents information about the basal diet provided to the fish. All of the experimental animals received ad libitum standard commercial diet (Aqua Norm 53% protein, 15% lipid, 1.5 mm, Turkey) for 2 weeks to ensure that they were acclimatized to study condition before the experimentation began. Subsequently, the tilapia received ad libitum feed three times a day at (8.00, 12.30 and 17.00) an experimental duration of for 60 days.

Growth Performance and Proximate Composition

Four Fish from Each Aquarium were randomly selected and anesthetized using eugenol (clove oil) at a dose of 0.2 mL L^{-1}.

Calculations for weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival were performed using the equations given below (Paray et al., 2020):

Weight gain (g)=Final weight - Initial weight

Specific growth rate (SGR;%/d)=100 x (ln final fish weight) - (ln initial fish weight)/experimental days

Feed conversion ratio (FCR)=Feed intake (g) /Weight gain (g);

Survival (%)=(initial number of fish/final number of fish) x 100

The standard methods described in the relevant literature were applied to perform proximate analysis of both the feeds and whole-body fish. Measurements of moisture were conducted samples were dried to constant weights for 24 hours at 105°C in an oven. Values of crude protein were obtained with the Kjeldahl method, while crude ash was evaluated following the incineration of samples in a muffle furnace for 12 hours at 525°C. Finally, Soxhlet extraction was utilized in the evaluation of crude fat values (AOAC, 2003).

Gastrointestinal Dissection

Fishes were anesthetized with 0.2 mL / L eugenol and in order to isolate the gastrointestinal region required for enzyme analyses, samples were taken from tilapia juveniles and the enzyme was isolated with the help of a scalpel in the gastrointestinal region from the pharynx to the rectum. After the removed gastrointestinal content was crushed in a ceramic mortar until it became completely homogeneous, pure water, glycerol (Isolab) and Tris HCl (Sigma-Aldrich) with a pH value of 7.5 were placed in falcon tubes and stored in a deep freezer at -20°C before analysis. Each enzyme was determined by its unique specific activity determination methods and enzyme activities were measured with the help of a spectrophotometer device (Jenway 6300-Visible Spectrophotometer).

Enzyme Analysis

Alkaline protease activity was determined by casein substrate analysis described by Alarcòn et al. (1998). The activity was calculated by measuring the value of the spectrophotometer device at a wavelength of 366 nm under the conditions specified at 37°C and pH 8.0. For determining the acid protease activity, bovine hemoglobin was used as a substrate, and the measurement was carried out at 280 nm in 30 minutes at 25°C (Anson, 1938). Enzymatic activities were expressed as specific activity (U/mg protein^{-1}).

Statistical Analysis

All data produced in the course of this study were analyzed with the help of IBM SPSS Statistics 21. Following the application of one-way analysis of variance (ANOVA), data were further evaluated with Tukey multiple comparison tests. The threshold for statistical significance in the differences among experimental groups was accepted as P<0.05 (Logan, 2010).

Results

Table 2 presents the experimental findings regarding average initial weight (IW), and final weight (FW), weight gain (WG), over the course of the experiment, feed conversion rate (FCR), specific growth rate (SGR), feed intake (FI) and survival Significant differences were not observed for the parameters of FCR, FI, or survival (P>0.05). However, the values of FW, WG, and SGR among the fish receiving probiotic-supplemented feed were seen to increase significantly (P<0.05) Our findings furthermore revealed that incorporating glycyrrhizic acid into fish feed at levels of 2-6 mL/kg had so significant impact on fish growth performance among juvenile Nile tilapia.

The highest level of alkaline protease activities were determined among the fish of the P30 group, to which probiotics were given, on the 60th day with a mean value of 3.87±0.01 U/mg protein. This group was followed by the fish of P20 (3.35±0.01 U/mg protein), P10 (2.78±0.01 U/mg protein), V6 (2.6±0.03 U/mg protein), V4 (2.45±0.04 U/mg protein) and V2 (2.27±0.05 U/mg protein), in descending order. For the control group, the average alkaline protease activity was calculated as1.92±0.04 U/mg protein at the conclusion of the experimental duration (Figure 1).

Table 1. Fish diet (pellet size: 1.5 mm) used for juvenile Nile tilapia (*Oreochromis niloticus*)

Chemical Analyses	(%)
Crude Protein	53
Crude Lipid	15
Crude Cellulose	1.5
Crude Ash	11
Macro Elements	(%)
Calcium	2.0
Phosphorus	1.5
Sodium	0.45
Vitamins	(per kg Feed)
A (IU)	24.000
D3 (IU)	5.000
E (mg)	500
C (mg)	400

Ingredients: Anchovy meal, krill meal, hydrolyzed fish meal, fish oil, soy protein concentrate, wheat gluten, pea protein, wheat flour, yeast and yeast by product, vitamin and mineral

Acid protease activity increased in all experimental groups from the beginning of the experimentation. The highest levels of acid protease activities were identified among the fish of the P30 group on the 60th day with a mean value of 2.73±0.12 U/mg proteinThis was followed by the fish of P20 (2.52±0.13 U/mg protein) and P10 (2.47±0.07 U/mg protein) in descending order. In contrast, the levels of acid protease activities were seen to be lower in the groups that received prebiotics compared to probiotics. At the conclusion of the experimental period, average acid protease activities among the fish of the V6, V4 and V2 groups were 1.86±0.07 U/mg protein, 1.56±0.06 U/mg proteinand 1.41±0.05 U/mg protein, respectively. For the control group, acid protease activity was calculated to be 1.27±0.07 U/mg protein at the conclusion of the experimentation. The alkaline and acid protease activities of the fish in the P30, P20, P10, V6, V4 and V2 groups reached statistically significantly high levels (P<0.05) in comparison to the fish of the control group(Figure 2).

Table 2. Growth performance and feed utilization of juvenile Nile tilapia (*Oreochromis niloticus*) at the end of 60-days the trial

	Dietary Viusid level (ml/kg^{-1})				Dietary Proakua level (ml/kg^{-1})		
	Control	V2	V4	V6	P10	P20	P30
Initial weight (g)	5.20±0.06	5.22±0.02	5.20±0.06	5.23±0.05	5.23±0.08	5.21±0.04	5.24±0.06
Final weight (g)	36.20±0.48^{b}	37.11±0.40^{b}	37.07±0.42^{b}	36.63±0.19^{ab}	39.04±1.88^{a}	38.97±0.19^{a}	39.13±0.60^{a}
Weight gain (g)	31.00±0.48^{b}	31.89±0.38^{b}	31.87±0.37^{b}	31.40±0.23^{b}	34.47±0.73^{a}	33.75±0.15^{a}	33.89±0.65^{a}
Feed conversion ratio (FCR)	1.85±0.01	1.82±0.09	1.84±0.02	1.83±0.01	1.76±0.09	1.71±0.04	1.74±0.02
Specific growth rate (SGR, %/d)	0.37±0.01^{c}	0.38±0.00^{cb}	0.38±0.00^{abc}	0.37±0.00^{c}	0.41±0.02^{ab}	0.41±0.00^{ab}	0.41±0.01^{a}
Feed intake (g/fish)	57.55±1.09	58.12±2.48	58.75±1.30	57.77±0.41	60.86±1.93	57.71±1.23	58.96±0.35
Survival (%)	100	100	100	100	100	100	100

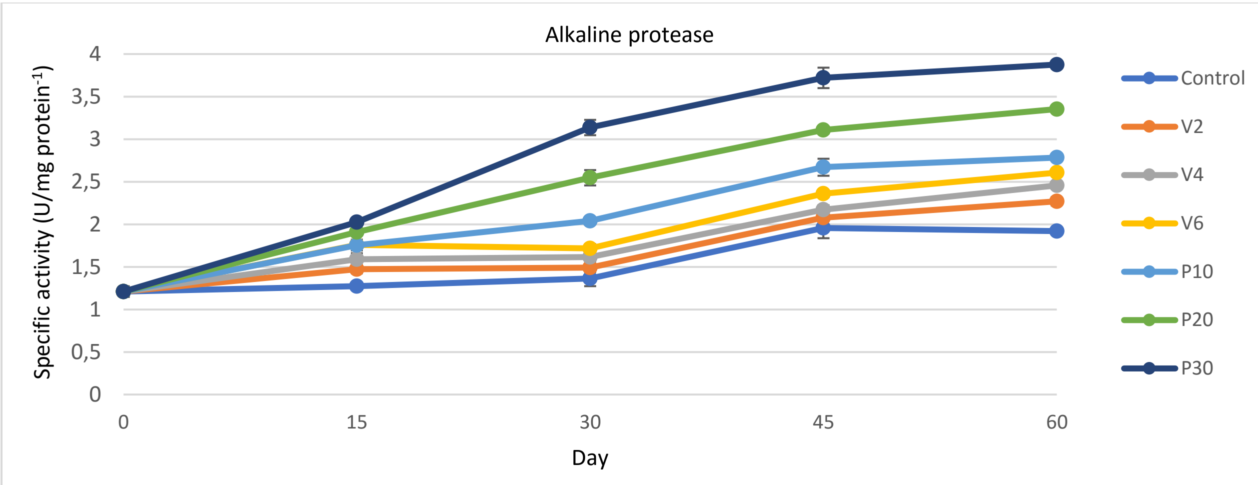

Figure 1. The changes observed in alkaline protease activities in tilapia juvenile fed experimental diets for 60 days.

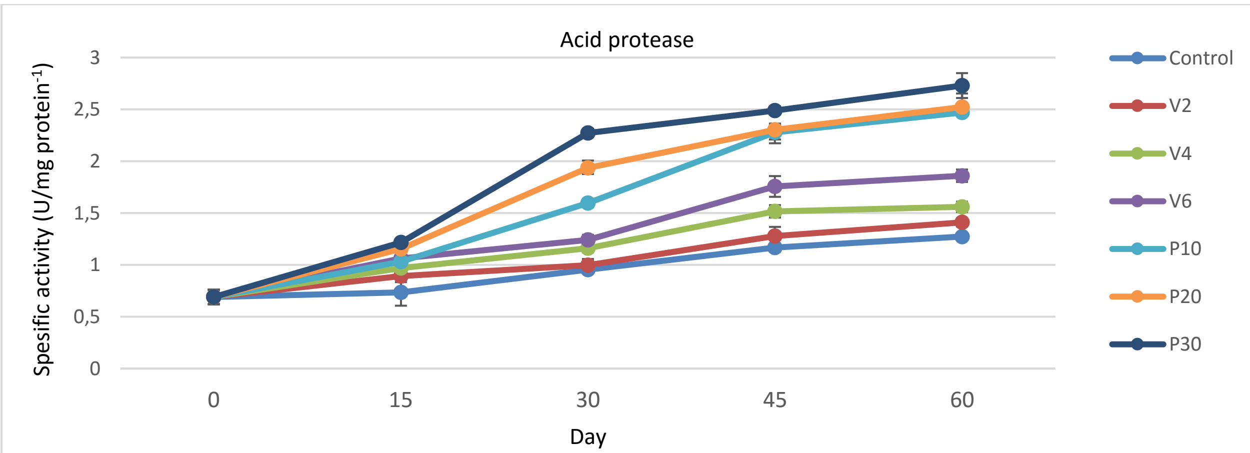

Figure 2. The changes observed in acid protease activities in tilapia juvenile fed experimental diets for 60 days.

Upon the conclusion of the experimental period, the nutritional compositions of the fish from the groups receiving *Lactobacillus* or glycyrrhizic acid were evaluated The relevant data are provided in Table 3. where it can be seen that the experimental diets had no statistically significant effects on the average values of fat, protein, ash or dry matter (P>0.05).

Discussion

The findings of the present study are in accordance with those presented by Byun et al. (1997) who found significantly higher final body weights and body weight gains among flounder (*Paralichthys olivaceus*) that were, fed *Lactobacillus* sp. in comparison to the fish of a control group. Other researchers investigated the effects of *Lactobacillus sp.* as a probiotic on changes in growth performance among common carp (*Cyprinus carpio*),, and, concluded thatFCR values were lowest and SGR values were highest when the experimental animals received supplementation of 1000 µL/1g bio active feed in their diets (Vignesh et al., 2011). Aderolu et al. (2013) demonstrated that supplementing the diet of African catfish (*Clarias gariepinus*) with *Lactobacillus* sp. produced better outcomes for both weight gain and SGR in conjunction with dose-dependent decreases in FCR values. It was also shown that the sole use of dietary *Lactobacillus* sp. (Amer & Tawil, 2011; Rahman et al., 2019), and a mixture of *Lactobacillus* sp. And *Saccharomyces cerevisiae* (Khunrang et al., 2021) both significantly improved tilapia growth. Andriani et al. (2019) incorporated a dietary of probiotic mixture containing *Lactobacillus sp*., *Bacillus sp*. and *Saccharomyces sp*., into feed for tilapia fry over the course of 40 days and indicated that 5 mL/kg Furthermore, Ramos et al. (2015) found higher growth rates among rainbow trout (*Oncorhynchus mykiss*) that received experimental, diets that included a mixture of bacterial genera (*Pediococcus, Bacillus,Enterococcus and Lactobacillus*) in comparison to a control group without special dietary treatment.. This particular probiotic mixture was also confirmed to have significantly increased the rates of SGR and weight gain among the experimental fish (Ramos et al., 2013). Another study of, juvenile Nile tilapia demonstrated the best increases in the weights of the fish and SGR when the animals received a diet that included a probiotic blend (species of the genera *Pediococcus*, *Enterococcus*, *Bacillus*, and *Lactobacillus*) at a dosage of 3 g/kg compared to the other experimental dietary groups (Ramos et al., 2017). Probiotics used as feed additives forgilthead sea bream (*Sparus aurata*) yielded similar findings, with increased growth performance among the of larvae (Suzer et al., 2008). In contrast, however, Ridha and Azad, (2012) observed the potential a growth-promoting influence of dairy yogurt species of *Lactobacillus* on tilapia (*O. niloticus*), and reported poor results for both growth and FCR. Similarly, the dietary inclusion of a probiotic supplement incorporating multiple species of bacteria from the genera *Enterococcus*, *Pediococcus, Bacillus*, and *Lactobacillus* at a level of 0.2% for 9 weeks was evaluated for rainbow trout (*Oncorhynchus mykiss*), but differences in growth performance were not observed (Ozório et al., 2015). The variations of the findings reported by different authors may be due to different times of feeding different types of fish, or differences in the levels of *Lactobacillus* in the diets of the experimental animals.

For example no statistical significance was observed for differences in growth among juvenile large yellow croakers that received feed that included glycyrrhizic acid (Xu et al., 2015). In another study, glycyrrhizic acid did not significantly affect growth performance (Harikrishnan et al., 2021). Jiang et al. (2012) similarly reported a lack of statisticallysignificant differences in the growth performance results achieved by juvenile channel catfish (*Ictalurus punctatus)* when glycyrrhizic acid was incorporated into their diets. It is considered that the reason why the data obtained in the studies are statistically insignificant depends on the species and the water temperature variation during the study. In an experiment designed to identify the impact of glycyrrhizic acid as a prebiotic on growth performance results of red swamp crayfish (*Procambarus clarkii*), the lowest values of FCR and highest SGR values were seen among animals that received diets containing glycyrrhizic acid at concentrations ranging from 75 to 100 mg·kg(Liu et al., 2021). Differences between species are believed to be the most important reason for this study to have differedfrom the present work. Supplementing the animals' diet with glycyrrhizic acid (Viusid-Vet® powder) was previously shown to improve growth parameters and immunity in pigs (Ocampo et al., 2017). In studies of humans, glycyrrhizic acid was found to improve fatty liver disease (Gomez et al., 2006; 2009). In line with the conclusions drawn at the end of our experiment, Jiang et al. (2012) did observed no changes of significance in protein, fat, ash and dry matter contents of juvenile channel catfish that received, glycyrrhetinic acid incorporation. While Amer and Tawil

Table 3. Biochemical composition of juvenile Nile tilapia (*Oreochromis niloticus*) at the end of 60-days the trial

		Dietary Viusid level (ml/kg^{-1})			Dietary Proakua level (ml/kg^{-1})		
	Control	V2	V4	V6	P10	P20	P30
Moisture (%)	27.54±1.98	26.81±2.85	27.17±1.51	27.49±1.01	28.38±2.36	31.38±3.59	28.04±1.97
Protein (%)	18.52±1.26	18.83±2.36	18.31±1.76	18.36±1.67	19.15±1.15	22.25±0.99	19.43±1.77
Fat (%)	4.74±0.52	4.08±0.44	4.21±0.76	4.07±0.28	4.02±0.63	4.51±0.69	4.22±0.06
Ash (%)	2.75±1.04	3.38±0.91	3.70±0.45	3.89±0.14	3.59±0.31	3.29±0.19	3.79±0.55

The percentages of protein, fat and ash results are expressed as % in dry matter.

(2011) found no differences of significance (P>0.05) in the, crude protein, crude fat, moisture, and ash contents of fish in a similar experiment.

Among the factors that affect the feed efficiency of fish, digestive enzymes have particular importance. Accordingly, analysis of these enzymes may yield important data on the capacities of different fish species to hydrolyze dietary carbohydrates, proteins and lipids (Lemieux et al., 1999). Heightened levels of protease activities among fish that received probiotic in their feed may have yielded better results in terms of growth and protein contents. Likewise, Xu et al. (2009) reported that the protease activity in the intestines and hepatopancreas of Prussian carp (*Carassius auratus gibelio*) receiving a diet XOS increased in a dose-dependent manner. According to those authors, these benefits observed with XOS administration for growth and heightened enzyme activities could be related to alterations in the gut microbiota. In another study Wen et al. (2009) concluded that pantothenic acid administered to juvenile Jian carp (*Cyprinus carpio var. Jian*) increased the alkaline phosphatase activity in the foregut and mid-gut. The activities of brush border enzymes in the intestines and digestive enzymes serve to keep intestinal epithelial cell membranes intact, which may be the reason why pantothenic acid increased fish growth. The results of the present study support those of previous work indicating that the administration of higher levels of a probiotic (that contained *L. lactis, B. subtilis,* and *S. cerevisiae*) increased the protease activity of Labeo rohita fingerlings (Mohapatraet al., 2012). In a different study, AQUA-PHOTO® containing [*Lactobacillus plantarum* and *Bacillus subtilis*] was added to the diets of various groups of Nile tilapia and alkaline protease activities were similar among all experimental groups when the trial ended (Guimarães et al., 2021).

Conclusions

The present study has successfully demonstrated that including a probiotic mixture at doses of 10 mL/kg, 20 mL/kgand 30 mL/kg in *O. niloticus* supports improvements in the growth parameters of these fish. Furthermore, it has been shown here that diets containing probiotics have positive effects on activities of the digestives enzyme of *O. niloticus* fingerlings. In the future, we recommend that more detailed research be conducted to explore the impacts of *Lactobacillus* sp. administration among fish subjected to environmental stressors or microbial challenges.

Ethical Statement

The experiments conducted in this study with fish were designed in full compliance with the guidelines of the Animal Ethics Committee of Aydin Adnan Menderes University for fish research (Protocol Number: 64583101/2021/122).

Funding Information

This study was funded Aydın Adnan Menderes University Research Fund (the project number ADÜ BAP ZRF-20013).

Author Contribution

E.Y.: Conceptualization, Software, Data curation, Writing – original draft; **D.Ç.:** Writing – review & editing, Validation, Investigation, **C.S.:** Supervision, Writing - review and editing, **M.E.:** Writing - review and editing, **O.A.:** Writing - review and editing, **İ.K.:** Writing - review and editing

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the Aydın Adnan Menderes University Research Fund for financial assistance (the project number ADÜ BAP ZRF-20013), and Aydın Adnan Menderes University Agricultural Biotechnology and Food Safety Application and Research Center- Tarımsal Biyoteknoloji ve Gıda Güvenliği Uygulama ve Araştırma Merkezi (ADÜ-TARBİYOMER) and Kılıç Company for providing research facilities.

References

Aderolu, A.Z., Ogun, A., Sanni, R.A., & Dguntoyinbo, F.A.P. (2013). Growth response of juvenile catfish (*Clarias gariepinus*) fed diets supplemented with *Lactobacillus sp* ınclusion into feeds and cultured water. *Nigerian Veterinary Journal*, 34(1). https://www.ajol.info/index.php/nvj/article/view/98883

Alarcón, F.J., Díaz, M., Moyano, F.J. & Abellán, E. (1998). Characterization and functional properties of digestive proteases in two sparids; gilthead seabream (*Sparus aurata*) and common dentex (*Dentex dentex*). *Fish Physiology and Biochemistry*, 19(3), 257-267. https://doi.org/10.1023/A:1007717708491

Amer, T.N., & El-Tawil, N.E. (2011). Effect of green seaweed (*Ulva* sp.) and probiotic (*Lactobacillus* sp.) as dietary supplements on growth performance and feed utilization of Red tilapia (♀ *O. mossambicus* × ♂ *O. niloticus*). *Abbassa International Journal for Aquaculture*, 4(1).

Anson, M.L. (1938). The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. *Journal of General Physiology*, 22(1),79-89. https://doi.org/10.1085/jgp.22.1.79

AOAC. (2003). Official Methods of Analysis (17th ed.). The Association of Official Analytical Chemists.

Azevedo, R.V.D., Fosse Filho, J.C., Cardoso, L.D., Mattos, D.D.C., Vidal Júnior, M.V., & Andrade, D.R.D. (2015).

Economic evaluation of prebiotics, probiotics and symbiotics in juvenile Nile tilapia. *Revista Ciência Agronômica*, 46, 72-79. https://doi.org/10.1590/S1806-66902015000100009

Bligh E.G., & Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8),911-917. https://doi.org/10.1139/o59-099

Byun, J.W., Park, S.C., Benno, Y., & Oh, T.K. (1997). Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). *The Journal of General and Applied Microbiology*, 43(5), 305-308. https://doi.org/10.2323/jgam.43.305

Bywater, R., McConville, M., Phillips, I., & Shryock, T. (2005). The susceptibility to growth-promoting antibiotics of *Enterococcus faecium* isolates from pigs and chickens in Europe. *Journal of Antimicrobial Chemotherapy*, 56(3), 538-543. https://doi.org/10.1093/jac/dki273

Cabello, F.C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8,1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Casewell, M., Friis, C., Marco, E., McMullin, P., & Phillips, I. (2003). The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of antimicrobial chemotherapy*, 52(2), 159-161. https://doi.org/10.1093/jac/dkg313

Chauhan, A., & Singh, R. (2019). Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis*, 77(2), 99-113. https://doi.org/10.1007/s13199-018-0580-1

Deng, M., Chen, J., Gou, J., Hou, J., Li, D., & He, X. (2018). The effect of different carbon sources on water quality, microbial community and structure of biofloc systems. *Aquaculture*, 482, 103-110. https://doi.org/10.1016/j.aquaculture.2017.09.030

El-Sayed, Abdel-Fattah M. (2019) Tilapia culture. Academic Press.

European Parliament and Council (2003). Regulation (EC) No 1831/2003 of the European parliament and of the council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Union. L268:29-43

Gobi, N., Vaseeharan, B., Chen, J.C., Rekha, R., Vijayakumar, S., Anjugam, M., & 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 immunology*, 74, 501-508. https://doi.org/10.1016/j.fsi.2017.12.066

Gomez, E., Rodriguez De Miranda, A., Gra Oramas, B., Arus Soler, E., Llanio Navarro, R., Calzadilla Bertot, L., & Del Rosario Abreu Vazquez, M. (2009). Clinical trial: a nutritional supplement Viusid, in combination with diet and exercise, in patients with nonalcoholic fatty liver disease. *Alimentary pharmacology & therapeutics*, 30(10), 999-1009. https://doi.org/10.1111/j.1365-2036.2009.04122.x

Gomez, E.V., Oramas, B.G., Soler, E., Navarro, R.L., & Domech, C.R. (2006). Viusid, a nutritional supplement, in combination with interferon a-2b and ribavirin in patients with chronic hepatitis C. *Liver International ISSN*, 1478-3223. https://doi.org/10.1111/j.1478-3231.2006.01411.x

Guimarães, M.C., da Silva Guimarães, A.I.C., Natori, M.M., Alarcon, M.F.F., Dias, D.D.C., Ishikawa, C.M., Tapia-Paniagua, S., Moriñigo, M.A., Moyano, F.J. & Tachibana, L. (2021). Oral administration of *Bacillus subtilis* and *Lactobacillus plantarum* modulates the gut microbiota and increases the amylase activity of Nile tilapia (*Oreochromis niloticus*). *Aquaculture International*, 29(1), 91-104. https://doi.org/10.1007/s10499-020-00612-2

Harikrishnan, R., Devi, G., Van Doan, H., Jawahar, S., Balasundaram, C., Saravanan, K., Arockiaraj, J., Soltani, M., & Jaturasitha, S. (2021). Study on antioxidant potential, immunological response, and inflammatory cytokines induction of glycyrrhizic acid (GA) in silver carp against vibriosis. *Fish & Shellfish Immunology*, 119, 193-208. https://doi.org/10.1016/j.fsi.2021.09.040

Hoseinifar, S.H., Esteban, M.Á., Cuesta, A., & Sun, Y.Z. (2015). Prebiotics and fish immune response: a review of current knowledge and future perspectives. *Reviews in Fisheries Science & Aquaculture,* 23(4), 315-328. https://doi.org/10.1080/23308249.2015.1052365

Hoseinifar, S.H., Ringø, E., Shenavar Masouleh, A., & Esteban, M.Á. (2016). Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. *Reviews in Aquaculture*, 8(1), 89-102. https://doi.org/10.1111/raq.12082

Jiang, G.Z., Liu, W.B., Li, G.F., Wang, M., & Li, X.F. (2012). Effects of different dietary glycyrrhetinic acid (GA) levels on growth, body composition and plasma biochemical index of juvenile channel catfish, *Ictalurus punctatus*. *Aquaculture*, 338, 167-171. https://doi.org/10.1016/j.aquaculture.2012.02.006

Khunrang, T., Pooljun, C., Wutisutimeethavee, S., & Direkbusarakom, S. (2021). Effects of mixed probiotic (*Lactobacillus sp*. and *Saccharomyces cerevisiae*) on the growth performance and immune gene expression of tilapia (*Oreochromis niloticus*) after *Streptococcus agalactiae* vaccination. *Aquaculture Research*, 52(8), 3882-3889. https://doi.org/10.1111/are.15232

Klemm, D.J., Stober, Q.J., & Lazorchak, J.M. (1993). Fish field and laboratory methods for evaluating the biological integrity of surface waters: Environmental Monitoring Systems Laboratory-Cincinnati Office of Modeling, Monitoring Systems, and Quality Assurance, Office of Research and Development, US Environmental Protection Agency (EPA/600/R-92/111), Cincinnati, OH.

Lemieux, H., Blier, P., & Dutil, J.D. (1999). Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*). *Fish Physiology and Biochemistry*, 20, 293-303. https://link.springer.com/content/pdf/10.1023/A:1007791019523.pdf

Li, S., Zou, J., Wang, X., Song, Z., Xu, Z., & Wang, Q. (2022). Effects of glycyrrhizic acid on hatchability, growth, and physiological responses of farmed dojo loach (*Misgurnus anguillicaudatus*) during early life stages. *Aquaculture*, 557, 738323. https://doi.org/10.1016/j.aquaculture.2022.738323

Liu, F., Shao, G.Y., Tian, Q.Q., Cheng, B.X., Shen, C., Wang, A.M., Zhang, J.H., Tian, H.Y., Yang, W.P., & Yu, Y.B. (2021). Enhanced growth performance, immune responses, immune-related gene expression and disease resistance of red swamp crayfish (*Procambarus clarkii*) fed dietary glycyrrhizic acid. *Aquaculture*, 533, 736202. https://doi.org/10.1016/j.aquaculture.2020.736202

Logan, M. (2010). Biostatistical design and analysis using r: a practical guide. Wiley Blackwell, London.

Meidong, R., Khotchanalekha, K., Doolgindachbaporn, S., Nagasawa, T., Nakao, M., Sakai, K., & Tongpim, S. (2018). Evaluation of probiotic *Bacillus aerius* B81e isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of Pla-mong *Pangasius bocourti*. *Fish & shellfish immunology*, 73, 1-10. https://doi.org/10.1016/j.fsi.2017.11.032

Mohapatra, S., Chakraborty, T., Prusty, A.K., Das, P., Paniprasad, K., & 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. *Aquaculture Nutrition*, 18(1), 1-11. https://doi.org/10.1111/j.1365-2095.2011.00866.x

Muaddama, F., & Putri, R.S. (2021). Application of fermented rice bran using *Lactobacillus sp*. in artificial feed for survival rate and fcr of tilapia (*Oreochromis niloticus*). In 3rd KOBI Congress, International and National Conferences (KOBICINC 2020) (pp. 529-534). Atlantis Press. https://doi.org/10.2991/absr.k.210621.088

Munoz-Atienzal, E., Gomez-Sala, B., Araujo, C., Campanerol, C., Del Campo, R., Hernandez, P.E., ... & Cintas, L.M. (2013). Antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology*, 13(1), 15.

Ocampo, L., Chavez, B., Tapia, G., Ibarra, C., & Sumano, H. (2014). Efficacy of a pharmaceutical preparation based on glycyrrhizic acid in a challenge study of white spot syndrome in white shrimp (*Litopenaeus vannamei*). *Aquaculture*, 428, 280-283. https://doi.org/10.1016/j.aquaculture.2014.03.005

Ocampo, L., Tapia, G., Gutiérrez, L., & Sumano, H. (2017). Effects of glycyrrhizic acid (Viusid-Vet® powder) on the reduction of influenza virus spread and on production parameters in pigs. *Veterinaria México*, 4(1), 1-13. https://doi.org/10.21753/vmoa.4.1.373

Ozorio, R.O., Kopecka-Pilarczyk, J., Peixoto, M.J., Lochmann, R., Santos, R.J., Santos, G., Weber, B., Calheiros, J., Ferraz-Arruda, L., Vaz-Pires, P., & Gonçalves, J.F. (2016). Dietary probiotic supplementation in juvenile rainbow trout (*Oncorhynchus mykiss*) reared under cage culture production: effects on growth, fish welfare, flesh quality and intestinal microbiota. *Aquaculture research*, 47(9), 2732-2747. https://doi.org/10.1111/are.12724

Paray, B.A., Hoseini, S.M., Hoseinifar, S.H., Van Doan, H. (2020). Effects of dietary oak (*Quercus castaneifolia*) leaf extract on growth, antioxidant, and immune characteristics and responses to crowding stress in common carp (*Cyprinus carpio*). *Aquaculture*, 524, 735276. https://doi.org/10.1016/j.aquaculture.2020.735276

Pohlenz, C., & Gatlin III, D.M. (2014). Interrelationships between fish nutrition and health. *Aquaculture*, 431, 111-117. https://doi.org/10.1016/j.aquaculture.2014.02.008

Rahman, Z. (2019). Influence of probiotics on the growth performance of sex reversed Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) fry. *Journal of Aquaculture Research & Development*. https://hal.archives-ouvertes.fr/hal-03639854/

Ramos, M.A., Batista, S., Pires, M.A., Silva, A.P., Pereira, L.F., Saavedra, M.J., Ozório, R.O.A. & Rema, P. (2017). Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. *Animal*, 11(8), 1259-1269. https://doi.org/10.1017/S1751731116002792

Ramos, M.A., Gonçalves, J.F.M., Batista, S., Costas, B., Pires, M.A., Rema, P., & Ozório, R.O.A. (2015). Growth, immune responses and intestinal morphology of rainbow trout (*Oncorhynchus mykiss*) supplemented with commercial probiotics. *Fish & shellfish immunology*, 45(1), 19-26. https://doi.org/10.1016/j.fsi.2015.04.001

Ramos, M.A., Weber, B., Gonçalves, J.F., Santos, G.A., Rema, P., & Ozório, R.O.A. (2013). Dietary probiotic supplementation modulated gut microbiota and improved growth of juvenile rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 166(2), 302-307. https://doi.org/10.1016/j.cbpa.2013.06.025

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433, 50-61. https://doi.org/10.1016/j.aquaculture.2014.05.048

Ridha, M.T., & Azad, I.S. (2012). Preliminary evaluation of growth performance and immune response of Nile tilapia *Oreochromis niloticus* supplemented with two putative probiotic bacteria. *Aquaculture Research*, 43(6), 843-852. https://doi.org/10.1111/j.1365-2109.2011.02899.x

Ringø, E., Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.I., & Bakke, A.M. (2010). Prebiotics in aquaculture: a review. *Aquaculture Nutrition*, 16(2), 117-136. https://doi.org/10.1111/j.1365-2095.2009.00731.x

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, 172(1-2), 63-92. https://doi.org/10.1016/S0044-8486(98)00436-0

Suzer, C., Çoban, D., Kamaci, H.O., 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

Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and molecular biology reviews*, 64(4), 655-671. https://doi.org/10.1128/MMBR.64.4.655-671.2000

Vignesh, V., Kanipandian, N., Parthiban, K., Vasuki, B., Nishanthini, A., & Thirumurugan, R. (2011). Evaluation of *Lactobacillus sp*. as a probiotic for the growth of freshwater fish *Cyprinus carpio*. *International Journal of Fisheries and Aquaculture Sciences*, 1, 89-98.

Wen, Z.P., Zhou, X.Q., Feng, L., Jiang, J., & Liu, Y. (2009). Effect of dietary pantothenic acid supplement on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture nutrition*, 15(5), 470-476. https://doi.org/10.1111/j.1365-2095.2008.00612.x

Xu, B., Wang, Y., Li, J., & Lin, Q. (2009). Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Fish physiology and* *biochemistry*, 35(3), 351-357. https://doi.org/10.1007/s10695-008-9248-8

Xu, H., Ai, Q., Mai, K., Xu, W., Wang, J., & Zuo, R. (2015). Effects of dietary supplementation of glycyrrhizic acid on growth performance, survival, innate immune response and parasite resistance in juvenile large yellow croaker, *L arimichthys* crocea (Richardson). *Aquaculture Research*, 46(1), 86-94. https://doi.org/10.1111/are.12164

Nowosad, J., Jasiński, S., Arciuch-Rutkowska, M., Abdel-Latif, H.M., Wróbel, M., Mikiewicz, M., ... & Kucharczyk, D. (2023). Effects of Bee Pollen on Growth Performance, Intestinal Microbiota and Histomorphometry in African Catfish. *Animals*, 13(1), 132. https://doi.org/10.3390/ani13010132