

Research Article

Assessing the Influence of Dietary *Pediococcus acidilactici* Probiotic Supplementation in the Feed of European Sea Bass (*Dicentrarchus labrax* L.) (Linnaeus, 1758) on Farm Water Quality, Growth, Feed Utilization, Survival Rate, Body Composition, Blood Biochemical Parameters, and Intestinal Histology

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The probiotics are being used as ecofriendly and bioremediation tools for developing sustainability to aquaculture. The present study was conducted to explore the practical capability of using dietary lactic acid bacteria (*Pediococcus acidilactici*) probiotics and see how its dose variation affected the water quality, growth performance, survival rate, body composition, blood biochemical parameters, and intestinal histology of European sea bass (*Dicentrarchus labrax* L.). A total of 120 fingerlings with an initial weight of 9 ± 0.2 g were divided into four groups, each with three replicates. The feeding experiment lasted for 60 days. In addition to the control (without probiotics) (T0), fish were fed diets containing (T1) 2.0, (T2) 2.5, and (T3) 3.0 g of probiotics per kg of diet twice a day. When compared to the control, sea bass fed probiotic-supplemented diets had significantly higher growth parameters, fish body "crude lipid," and villi height (p < 0.05, p < 0.01, and p < 0.001). The *P. acidilactici* probiotic treatments improved survival rate, feed conversion ratio, body composition, and blood biochemical markers, but not statistically significant (p > 0.05). Also, in regard to water quality, *P. acidilactici* drastically reduced ammonia and pH levels. In this experiment, fish fed with a dosage of 3.0 g of this commercial probiotic per kg of probiotics performed better. The study found that including probiotics in the diets of European sea bass improved growth, body composition, survival rate, blood biochemical markers, intestinal histology, and some water quality parameters.

1. Introduction

The aquaculture business, according to FAO, continues to be a significant source of food and income for millions of people worldwide [1]. It is an important food-producing sector around the world, according to the 2017 fishery yearbook, and around 89 percent of overall fishing produce was used for human use [2, 3]. Diseases and bad environmental conditions continually hinder productivity, resulting in serious financial losses for farmers. Unwise management practices like overfeeding, excessive stock densities, damaging fishing techniques, and water contamination also enhance the likelihood of disease signs in aquaculture industries [4-6]. Antibiotics are commonly used in fish farming industries to combat disease outbreaks; on the other hand, the longevity of antibiotics is not assured due to the potential of bacteria to develop antibiotic resistance. It is a problem not only for aquaculture but also for fish consumers and the environment [7-10]. As a result, probiotics are being used as ecofriendly and bioremediation tools for developing sustainability to aquaculture [11, 12], where probiotics have emerged as an excellent alternative to chemotherapeutics, particularly antibiotics, which are well known for their negative impact on living biota, particularly the natural beneficial bacterial flora [13-16].

The definition of probiotics has evolved throughout time. Probiotics, according to the WHO definitions, are living microorganisms that, when supplied in sufficient proportions, provide health benefits to the host [17]. Aquaculture probiotics are microbial cells; when introduced to water or feed, it improves the host organism's health by improving the microbial balance in the environment [18, 19]. They are biofriendly agents that can be administered in aquaculture environments to inhibit pathogenic microorganisms and improve feed utilization, survival, immunity, stress responses, disease resistance, growth rate, haematological parameters, plasma biochemical parameters, physiological conditions, and water quality, thus lowering production costs of farmed species [20-26]. The use of probiotics is critical for improving the habitat for aquatic animals and increasing their performance while having no negative consequences for customers [27, 28]. There is a lot of interest in using probiotics in fish as feed additives to improve feed values, nutrient absorption, gut microbial community [29-31], gut morphology, gut histology, production of lactic acid, and digestive enzymes and increase the available nutrients to the host [32–36]. Furthermore, they provide essential growth factors, vitamins, fatty acids, and amino acids [37]. Several studies have shown that probiotics can be useful in a variety of fish species such as snook, Rohu, red sea bream, Nile tilapia, rainbow trout, catfish, and sea bass [38-44].

The European seabass (*Dicentrarchus labrax*) is a commercially valuable fish that is frequently cultivated in various Mediterranean locations [45]. The top producers are Greece, Turkey, Italy, Spain, Croatia, and Egypt. Sea bass can tolerate a wide variety of temperatures and salinities and adapts to different rearing environments [46]. Farmed European sea bass is economically significant because aquaculture accounts for 96% of total production rather than fisheries

[47]. Despite rapid growth in global sea bass aquaculture, the farming business experienced a variety of harmful diseases, resulting in economic losses [48]. Outbreaks of infectious diseases continue to be a barrier to the expansion of the fish farming sector [49, 50]. Probiotics have been used successfully to prevent aquatic animal infections in several countries [51] because they are less expensive and safer than antibiotics [52], whereas in the commercial production of aquaculture, the most important goals are stimulating growth and maintaining health [53]. Manipulation of the intestinal microbiota with probiotic supplementation is thus an effective and safe method of improving host growth performance, survival rate, and disease resistance [54-57]. As a result, the general purpose of our research is to employ and test new methods for increasing disease resistance and productivity of cultured fish by modifying their water ecosystems or nutritional regimes; the current study was aimed at determining the effect of utilizing probiotics as feed supplements on water quality, growth performance, feed utilization, survival rate, body composition, blood biochemical parameters, and intestinal histology in European sea bass (Dicentrarchus labrax) (Linnaeus, 1758).

2. Materials and Methods

2.1. Experimental Design. The research was conducted at a private farm in Ismailia Governorate, Egypt, to test the effect of a commercial probiotic as a feed supplement on European sea bass. After two weeks of acclimatization, during which plain commercial sea bass food was administered, one hundred and twenty apparently healthy sea bass with a mean weight of 9 ± 0.2 g were randomly divided into four groups of triplicate tanks. The water salinity was around 37 ppt, temperature was adjusted to be $22 \pm 2^{\circ}C$ (Table 1), and a photoperiod regime of 12:12 (12h light and 12h dark). Each tank (120 L) was stocked with 10 fish and equipped with an air pump to ensure proper aeration. Every day, 25% of the entire volume of water was replaced. Fish were fed diets containing varying amounts of the tested probiotics (T1) 2.0, (T2) 2.5, and (T3) 3.0 g of probiotics per kg food, as well as a control (T0) diet devoid of them. The used probiotics is the commercial probiotics (Bactocell PA10, Lallemand SAS, Canada) which contains viable cells of a strain of the lactic acid bacteria (Pediococcus acidilactici) CNCM I-4622 with a concentration as declared not less than $1\times 10^{10}\,\text{CFU}$ per gram of the additive. Fish were fed experimental diets at a rate of 6% in the first month and 5% in the second month of their body weights, and feeding was done twice daily at 08:00 and 15:00 for 60 days. Every two weeks, the fish of each group were live-weighed to recalculate the amount of feed consumed during the experimental trial.

2.2. Experimental Diet Preparation. All the ingredients in each treatment (Table 2) were finely grounded and mixed, and carboxymethylcellulose was used as a binder. The mixture was then pelleted by passing it through a meat mincer of 2 mm die to produce 2 mm diameter pellets. The pellets were then packed in polythene bags and kept refrigerated during use.

 44.4^{**}

(Dicentrarchus labra	(X).				
Parameters	Treatment				
	T0	T1	T2	Т3	<i>F</i> -value
T (°C)	24.0 ± 1.84	24.2 ± 1.9	24.15 ± 1.89	23.95 ± 1.6	0.017
pН	8.6 ± 0.14	8.38 ± 0.09	8.23 ± 0.12	8.15 ± 0.23	6.19*
Salinity (ppt)	37.58 ± 0.29	37.53 ± 0.10	37.50 ± 0.20	37.48 ± 0.38	0.104
DO (mg/L)	5.50 ± 0.12	5.43 ± 0.09	5.50 ± 0.16	5.45 ± 0.10	0.380

 0.0042 ± 0.003

 0.0114 ± 0.001

TABLE 1: Effect of probiotic use on some water parameters (means \pm standard deviations) of rearing water of European sea bass (*Dicentrarchus labrax*).

 $p^* p \le 0.01$, and $p^* p \le 0.001$.

 NH_3 (mg/L)

TABLE 2: Composition and chemical analysis of experimental diets.

 0.0179 ± 0.0019

	Diets				
Components	T0	T1	T2	Т3	
Fish meal (65%)	310	310	310	310	
Soybean meal (45%)	370	370	370	370	
Wheat gluten	130	128	127.5	127	
Corn meal	60	60	60	60	
Rice bran	60	60	60	60	
Bactocell PA10 (probiotics)	0.0	2.0	2.5	3.0	
Corn oil	10	10	10	10	
Flax oil	10	10	10	10	
Fish oil	20	20	20	20	
Premix ¹	30	30	30	30	
	1000	1000	1000	1000	
Chemical analysis					
Dry mater	89.85	89.94	90.04	89.87	
Moisture	10.15	10.06	9.96	10.13	
Crude protein ($N \times 6.25$)	45.5	45.5	45.5	45.5	
Crude fat	14.48	14.27	14.24	14.32	
Crude fiber	2.16	2.11	2.17	2.15	
Ash	6.33	6.62	6.53	6.44	
Nitrogen free extract (NFE) ²	21.36	21.42	21.58	21.44	
Gross energy (kcal/100 g) ³	483.152	481.42	481.8	481.98	

¹Premix composition (Hymix, Egypt): each 1 kg contains Vit. A (400000 i.u.), Vit. D3 (100000 i.u.), Vit. E (230 mg), Vit. K3 (165 mg), Vit. B1 (300 mg), Vit. B2 (80 mg), Vit. B6 (200 mg), Vit. B12 (1 mg), Vit. C (650 mg), niacin (1000 mg), methionine (3000 mg), choline chloride (10000 mg), folic acid (100 mg), biotin (2 mg), pantothenic acid (220 mg), magnesium sulphate (1000 mg), copper sulphate (1000 mg), iron sulphate (330 mg), zinc sulphate (600 mg), cobalt sulphate (1000 mg), and calcium carbonate, up to 1000 mg. ²NFE =100 - (crude protein + lipid + ash +fiber content). ³Gross energy (G.E.) was calculated as 5.64, 9.44, and 4.11 kcal/ 100 g for protein, lipids, and NFE, respectively [58].

2.3. Ethical Issues. Following the ethical regulations and guidelines of the institute regarding the humane dealing with experimental fish, the minimal number of needed experimental fish was used for the present study. Ethical approval certificate (no. 19158) was issued by Institutional Animal Ethics Committee of National Research Centre, Dokki, Giza, Egypt, and was conducted in accordance with guidelines as per "Guide for the care and use of laboratory animal."

2.4. Water Quality Analysis. Water samples were collected twice monthly, and temperature and dissolved oxygen (D.O.) levels were determined using an oxygen meter equipped with an oxygen and temperature probes. A pH meter was used to record the pH values. The salinity of the water was assessed using a refractometer (Erma, Japan), and the unionized ammonia (NH_3) was calculated according to Boyd [59].

 0.0028 ± 0.002

2.5. Fish Growth Performance Measurements. Fish samples were collected and individually weighed in order to calculate the growth and feed utilization parameters using the formulae below:

Bodyweight gain (WG) = Final weight (g) – Initial weight (g),

Weight gain rate =
$$\frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$
,
Feed conversion ratio (FCR) = $\frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$,
Specific growth rate (SGR) = $100 \times \frac{(\ln W2 - \ln W1)}{T}$,
(1)

where ln is the natural logarithm, W1 is the initial weight, W2 is the final weight (g), and T is the number of days in the feeding period.

Survival rate (S.R.) =
$$\frac{Z}{X} \times 100$$
, (2)

where Z is the surviving fish number and X is the initial fish number.

2.6. Chemical Analysis. Diet and fish body composition analyses were performed using the standard methods [60] to determine moisture, crude protein, crude lipid, and ash levels. By oven-drying to constant weight at 105°C for feed and 70°C for fish, the moisture levels of fish and feed were determined. The protein content of fish and diets was determined using the Kjeldahl method. The ash levels of fish and diets were determined using a muffle furnace at 550°C for 8– 10 hours. Chloroform/methanol extraction was used to determine the lipid content of fish and diets. 4

Demonsterne	Treatment				
Parameters	TO	T1	T2	Т3	<i>F</i> -value
Initial weight (g)	9.1 ± 0.1	9.0 ± 0.0	9.1 ± 0.1	9.1 ± 0.1	1.000
Final weight (g)	28.2 ± 1.8	29.8 ± 1.2	32.1 ± 1.8	33.0 ± 1.04	6.287*
Weight gain rate (%)	209.8 ± 17.3	231.1 ± 13.5	252.64 ± 15.8	262.6 ± 8.49	8.269**
SGR (%/day)	1.88 ± 0.09	1.995 ± 0.07	2.099 ± 0.08	2.15 ± 0.04	7.976**
FCR	1.63 ± 0.15	1.53 ± 0.057	1.44 ± 0.12	1.39 ± 0.055	3.125
Survival (%)	93.33 ± 5.7	96.67 ± 5.8	96.67 ± 5.8	100.0 ± 0.0	0.889

TABLE 3: Effect of probiotic use on growth performance and survival rate (means ± standard deviations) of European sea bass (*Dicentrarchus labrax*).

 $p^* p \le 0.05$ and $p^* p \le 0.01$.

2.7. Blood Biochemical Analyses. At the end of the experiment, blood samples were collected from the caudal vein of fish (three for each replicate) in clean, dry centrifuge tubes, allowed to clot for 15 minutes, and spun at 3000 rpm for 10 minutes, and then, the collected serum was frozen at -20°C for biochemical analyses. The colorimetric method reported by Henry et al. [61] was used to assess serum total protein (g/dL), albumin (g/dL), and creatinine. Serum globulin (g/dL) levels were obtained by calculating the differences between total protein (g/dL) and albumin (g/dL), according to Sundeman [62]. Plasma glucose was determined according to Trinder [63]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured according to the method described by Reitman and Frankel [64].

2.8. Intestinal Histology. At the end of the experiment, the midintestines of European sea bass (n = 3) were sampled from each tank. They were fixed in 10% buffered formalde-hyde for 24 hours before being dehydrated in an ascending series of ethanol and then embedded in paraffin. Tissue blocks were sectioned $(5 \,\mu\text{m})$ and stained with hematoxylin and eosin (H&E). A light microscope (CX 43, Olympus, Japan) armed with a scientific digital camera for microscopy (E620, Olympus, Japan) was used to photograph and measure the villi height and muscle layer thickness. The images were analyzed using the ImageJ software version 1.36 (National Institute of Health, Bethesda, MD, USA) and converted into metric units.

2.9. Statistical Analysis. The results for each measured parameter were expressed as means \pm standard deviations. Statistical evaluation of results was carried out using the one-way analysis of variance (ANOVA), to detect the significance of differences of various parameters among the treatments according to Statistical Product and Service Solutions Software (SPSS version 20).

3. Results

3.1. Water Quality. The differences in water parameters between control and different probiotic treatments are shown in Table 1. The temperature differences were minor in T0, T1, T2, and T3 $(24.0 \pm 1.84, 24.2 \pm 1.9, 24.15 \pm 1.89,$

and $23.95 \pm 1.6^{\circ}$ C, respectively). The pH was significantly decreased (p < 0.01) in probiotic treatments (8.38 ± 0.09 , 8.23 ± 0.12 , and 8.15 ± 0.23 , respectively) less than control (8.6 ± 0.14). Salinity exhibited the same trend in its variations where there was an increase in T0 (37.58 ± 0.29 ppt) and decrease in T1, T2, and T3 (37.53 ± 0.10 , 37.50 ± 0.20 , and 37.48 ± 0.38 ppt, respectively). The dissolved oxygen of both control and probiotic diets was similar or less, while ammonia concentration was lower in different treatments (0.0114, 0.0042, and 0.0028 mg/L, respectively) than the control (0.0179 mg/L). There was a significant difference in ammonia measurements (p < 0.001).

3.2. The Growth Performance. The growth parameters, nutrient utilization, and survival rate values are presented in Table 3. The data indicated that European sea bass fed the diet supplemented with probiotics (T1, T2, and T3) had higher significant (p < 0.05, 0.01) final body weight, weight gain, weight gain rate, and specific growth rate (SGR) than fish fed the control diet (28.2 g, 19.1 g, 209.8 g, and 1.88%/day, respectively). The better FCR and survival rate were found in T3 (1.39 and 100%, respectively), where FCR was lower with 0.24 and survival rate was higher with 6.67% than the control, but the difference was not significant (p > 0.05).

3.3. Body Composition. From the findings of the present study, it was noted that dry matter, crude protein, and ash were not significantly different (p > 0.05) among different diets. On the other hand, the body composition analysis revealed higher lipid contents with high significance (p < 0.01); the values increased with the increase of probiotic inclusion in fish feed (T1, T2, and T3, respectively) compared with the control (T0) (Table 4).

3.4. Serum Biochemical Parameters. Serum biochemical parameters of sea bass fed probiotic and control diets are summarized in Table 5. According to the results, the average values of total protein $(5.83 \pm 0.47 \text{ g/dL})$, albumin $(2.72 \pm 0.08 \text{ g/dL})$, globulin $(3.11 \pm 0.53 \text{ g/dL})$, and glucose $(105.3 \pm 21.9 \text{ mg/dL})$ were lower in control (T0) than the values of these parameters in T1, T2, and T3. The serum creatinine levels of both control and probiotic diets were similar or less. In addition, AST and ALP concentrations decreased

Parameters	Treatment				
	Τ0	T1	T2	Т3	<i>F</i> -value
Dry matter	31.52 ± 1.92	32.42 ± 1.99	32.83 ± 1.68	32.92 ± 1.86	0.352
Crude protein	59.85 ± 4.54	60.10 ± 2.96	61.30 ± 4.37	61.50 ± 4.71	0.117
Crude lipid	22.05 ± 0.49	23.38 ± 0.83	24.26 ± 0.42	24.61 ± 0.85	8.502*
Ash	14.7 ± 1.19	14.83 ± 0.85	15.00 ± 1.11	15.05 ± 0.29	0.089

TABLE 4: Effect of probiotic use on body composition (means ± standard deviations) of European sea bass (Dicentrarchus labrax).

 $^{*}p\leq0.01.$

TABLE 5: Effect of probiotic use on some serum biochemical parameters (means ± standard deviations) of European sea bass (*Dicentrarchus labrax*).

D (Treatment				
Parameters	Τ0	T1	T2	Т3	<i>F</i> -value
Total protein (g/dL)	5.83 ± 0.47	5.87 ± 0.55	6.46 ± 0.72	6.56 ± 0.46	1.392
Albumin (g/dL)	2.72 ± 0.08	2.78 ± 0.18	2.8 ± 0.11	2.81 ± 0.09	0.349
Globulin (g/dL)	3.11 ± 0.53	3.12 ± 0.61	3.66 ± 0.72	3.75 ± 0.39	1.059
Glucose (mg/dL)	105.3 ± 21.9	105.7 ± 16.01	106.2 ± 7.91	107.0 ± 7.41	0.008
Creatinine (mg/dL)	0.25 ± 0.03	0.25 ± 0.04	0.24 ± 0.02	0.24 ± 0.01	0.306
AST (U/L)	59.53 ± 3.01	58.50 ± 8.26	59.27 ± 7.89	59.40 ± 16.5	0.020
ALT (U/L)	24.50 ± 6.18	23.30 ± 8.57	23.65 ± 5.32	23.80 ± 3.51	0.02

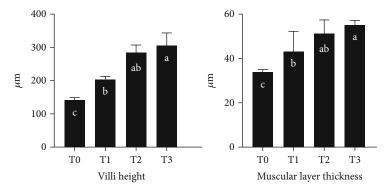


FIGURE 1: Effect of probiotic diets on intestinal histology (means ± standard deviations) of European sea bass (Dicentrarchus labrax).

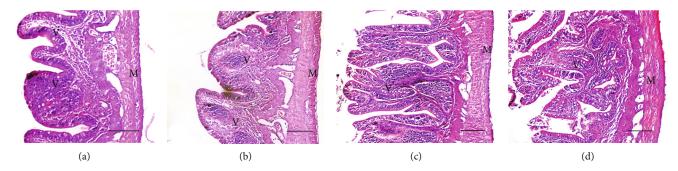


FIGURE 2: Histological sections of European sea bass (*Dicentrarchus labrax*) intestine fed with the experimental diets. (a) Control (T0), (b) T1, (c) T2, and (d) T3 (scale bar = 50μ m). M = muscular layer and V = villi.

in T1, T2, and T3 more than T0 (59.53 ± 3.01 and 24.50 ± 6.18 U/L, respectively). However, no significant differences were observed in serum biochemical parameters in different treatments (p > 0.05).

3.5. Intestinal Histology. The intestinal histology of sea bass fed experimental diets is described in Figures 1 and 2. No statistically significant differences (p > 0.05) were identified in the muscular layer thickness of sea bass in different

treatments, but it was thinner in control (33.81 μ m) than the probiotic diets. Correspondingly, the average villi height in T1, T2, and T3 (202.98, 284.3, and 304.7 μ m, respectively) significantly (*p* < 0.001) improved compared to the control (139.42 μ m).

4. Discussion

Good water quality is critical in aquaculture production. For optimal growth, survival, and production, a thorough understanding of the link between water quality and aquatic productivity is required [65, 66].

The findings revealed that the amplitude of change in water temperature was quite narrow, which is thought to be a feature of tropical waters [67]. In general, the variation of water temperature follows the pattern of fluctuation of air temperature [68]. The data demonstrated an increase in salinity in the control diet, while dissolved oxygen levels were comparable in the control and probiotic diets. There were no significant differences in the means of temperature, salinity, and dissolved oxygen between treatments, but they all lie within the optimal range. These findings are consistent with those of George et al. [29] and Kurdomanov et al. [69]. pH is a critical physicochemical parameter that influences fish development, metabolism, and other physiological functions. There was a significant difference in pH concentrations (p < 0.01), with control treatments continuously having higher pH than probiotic treatments. Mujeeb Rahiman et al. [70] had comparable results. Higher pH in all treatments implies more photosynthesis and water fertility. Ammonia, on the other hand, was considerably reduced in probiotic therapy (p < 0.001). The results are consistent with Sunitha and Krishna [71] research. In addition to the activities of nitrifying bacteria, the oxidation of various types of inorganic nitrogen in the well-oxygenated surface water may have resulted in a lower quantity of ammonia [72]. All of the water quality factors assessed in this study were within acceptable ranges for fish farming [73]. However, water parameters were more appropriate in probiotic treatments than in control water. These could be due to probiotic bacteria which were shed with fish excreta, which alter the bacterial community in favor of improved water quality [74]; as a conclusion, probiotics are essential for maintaining water quality and improving growth and survival rates [66].

Growth performance parameters are an important elements in aquaculture because they reflect production yield and are controlled by environmental conditions, genetic factors, and feeding quantity and quality [75]. As a result, they are often employed to assess the efficacy of various diets and supplements on fish [76]. The research showed that as probiotic levels increased and feed conversion decreased, growth parameters were dramatically optimized. These findings corroborate the findings of Sharibi et al. [77] and Adorian et al. [37]. The best growth performance can be attributed to improving nutrient digestibility and availability to fish [31, 41] by producing digestive enzymes (e.g., amylase, protease, and lipase), providing necessary growth factors (e.g., vitamins and amino acids), improving intestinal microbial flora, detoxifying potentially harmful compounds in feed, and stimulating the immune system [33, 41, 44]. The increased lifespan of fish fed probiotic-supplemented diets could indicate improved health conditions, which is consistent with the findings of Welker and Lim [78] and Abdel-Aziz et al. [50].

Fish administered with high dosages of probiotics had a significantly higher body lipid composition. Hassaan et al. [79] and Yones et al. [80] found similar results. Other body compositions, on the other hand, showed no significant variations but were improved by probiotic therapy. These findings confirm the findings of Morshedi et al. [81]. Increased nutritional deposition could have caused the rise in protein content. As a result, the higher body protein content seen in this study could be linked to more proteins released by the probiotics and efficient conversion of consumed food into structural protein, resulting in the development of more muscle [82, 83].

The health status of fish can be determined using plasma biochemistry measures [84, 85]. They are also used as markers for assessing the health of fish after they have been fed a diet supplemented with probiotics and have been exposed to stresses in the fish farming process [86].

The results showed that probiotic treatments had the highest levels of total protein, albumin, globulin, and glucose, indicating that probiotic-fed fish were healthier than the control group, but there was no statistical difference. These indicators also rose as the concentration of probiotics increased. The findings are similar to those of Kamgar and Ghane [87] and Nargesi et al. [42]. The protein profile could be attributable to the use of probiotics, which improve the intestinal environment, resulting in improved digestion and nutritional absorption [88]. A significant immunological response in fish is indicated by the increase in total protein might be an indication of a variety of disorders caused by liver disease, impaired protein absorption, or protein loss [90].

Furthermore, the data revealed that T1, T2, and T3 had lower creatinine, ALT, and AST levels than control, although the differences were not significant. Furthermore, the inclusion rate of probiotics in their diets had an effect on their activity. The liver enzymes ALT and AST are essential indicators of liver health and function. The key biochemical indicator that may be used to assess the influence of fish food supplements on metabolic activity and fish health is enzyme activity assessment [91, 92], as their levels increase in animal blood when liver cells are damaged [18]. Various research works revealed that their levels vary in relation to the used probiotics, as their levels were decreased significantly in Oreochromis niloticus plasma following the dietary usage of two strains of probiotic bacteria (Micrococcus luteus and Pseudomonas spp.) [93]. However, in another study, the ALT and AST levels significantly increased when the olive flounder (Paralichthys olivaceus) fish were fed with Lactobacillus plantarum-, Lactobacillus acidophilus-, or Saccharomyces cerevisiae-supplemented diets [94]. The results of the present study are following the study of Hassanien et al. [95] and Kurdomanov et al. [69], who found that probiotic-treated diets reduced ALT and AST levels when

compared to control diets, but disagree with those of Al-Hisnawi and Beiwi [96] who found that *Bacillus subtilis* supplemented diets, and Ghiasi et al. [97] who denoted that *P. acidilactici*-supplemented diets increased ALT and AST levels. The discrepancies across the studies could be due to the type of probiotics used, the concentration of probiotics utilized, the fish species studied, the time of administration, and/or environmental conditions.

The histology of the intestine has been determined in order to determine the health status and well-being of the gut [98]. According to histological observations, probiotic treatment improved significantly various aspects of intestinal histoarchitecture such as villus height and apparent muscular layer thickness, especially at higher doses, as compared to the control group. These findings are consistent with those of Zheng et al. [99], Won et al. [10], and Ashouri et al. [100] who detected also further improvement in villus width and apparent villus surface as well as crypt depth. The improvement in intestinal histology in probiotic treatments could be attributable to an increase in intestinal surface area, thus increasing absorption surface that is associated with improvement in integrity of brush borders which would promote nutrient absorption and, in turn, improve growth performance, feed consumption, and survival [100-103].

5. Conclusion

According to the findings, supplementing diets with probiotics increases growth, body composition, water quality measures, and intestinal histology. Its addition improves the survival and blood biochemistry of fish; especially, when supplementing fish feed with a dosage of 3.0 g of the experimental probiotic product per kg, the results were better. As a result, the authors recommend the usage of *P. acidilactici* probiotics in such dose to be included in the fish diet.

Data Availability

The authors declare that all data can be provided upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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