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Utilizing dietary probiotics can boost amberjack (*Seriola dumerili*) lysozyme activity, antioxidant capacity, and gut microbiota

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A R T I C L E I N F O

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A B S T R A C T

In aquaculture, natural and eco-friendly resources are prioritized over synthetic inputs (e.g., antibiotics), and optimizing sustainable production methods is necessary. Probiotics have been well known for various potential functions in animal performance and health when supplemented as a single or mix of strain (s) in the diet. Consuming the right probiotic blend (synergistic types) is more advantageous than using each kind individually due to combining each species' functions. The present trial aimed to examine the potential impacts of two probiotic mixtures on the performance and well-being of amberjack juveniles (5.63 ± 0.04 g; Number = 300) for a 60-day feed trial. Three experimental diets (43.5 ± 0.50% total protein and 12.55 ± 0.17% total lipid) were formulated with no probiotics supplement for the control (CD1) or with probiotics mixture supplement. Probiotic mixtures were supplemented at 2 g/kg diet for groups D2 [*Bacillus amyloliquefaciens* TOA5001 (BA) + *Streptococcus faecalis* T-110 (SF)] and D3 [*Bacillus amyloliquefaciens, Streptococcus faecalis* T-110, *Lactobacillus plantarum* TO-A, *Bacillus mesentericus* TO-A] respectively. The analysis revealed no difference in all means of growth parameters, feed utilization, survival rate, biometric indices, whole-body composition, and blood parameters between control and probiotic-supplemented diet groups. Liver lysozyme activity, superoxide dismutase, peroxidase activity, and biological antioxidant (BAP) were significantly improved in fish fed D2 compared to CD1 ($P < 0.05$). The higher ($P < 0.05$) content of intestinal lactic acid bacteria was noted in fish group fed diet D2, with an improved intestinal histological structure such as increased villi length, cryptal depth, and goblet cells compared to those provided with CD1 and D3 diets. In conclusion, incorporating a mix of probiotic bacteria [BA+SF] at a 2 g/kg diet has remarkable effects on intestinal health, immune responses, and oxidative status of amberjack, *S. dumerili*, suggesting a potential probiotic candidate for this species.

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Introduction

Sustainable development goals in Africa (Africa's Union's Agenda 2063) and globally (SDGs 2030) involve various common pillars, covering healthy and well-nourished citizens, modern agriculture, and the blue economy [1–3]. Aquaculture is one of the major sectors contributing to accomplishing several earlier sustainable development objectives [3]. Finfish aquaculture is a prominent part of food security. It has expanded influentially from extensive small-scale farming to large-scale intensive farming systems, progressively spreading across the globe [4–6]. It provides an alternative avenue to address food security and meet protein demand. Intensive aquaculture's rapid growth has brought many challenges, especially disease outbreaks, stressors, and contamination [7–12]. Chemotherapy, including antibiotics, has been applied for decades as growth and health promoters, but with rising apprehension about the outcomes of their application (e.g., generation of a resistant pathogen, suppression of the immune system, accumulation of toxins and causing environmental damage), it is discouraged in a lot of countries [13].

Functional feed additives, e.g., probiotics, prebiotics, synbiotics, medicinal herbs, and their extracts represent alternatives promising natural strategies to antibiotics [14–18]. Probiotics' effectiveness in aquaculture sustainability is well documented [19]. Integrating either life or killed probiotics forms in aqua feeds increases the success of aquatic organisms' production. Probiotic bacteria are a recommended additive in aquaculture due to their various functional benefits. They promote growth, modulate digestive enzyme activities, improve nutrient absorption, modify gut microbial community, immunostimulants, and improve tolerance to stress and unfavorable environmental conditions [20,21].

A mixture of probiotic strains may benefit the health condition of the host species or its surrounding environment [22]. Consuming the right probiotic blend (synergistic types) is more advantageous than using each kind individually due to combining each species' functions [23,24]. The supplementation of *Bacillus* strain has improved growth, immune responses, mRNA expression of immune and growth genes, and the digestibility of feed ingredients of Red Sea bream, *Pagrus major* [25]. The single-strain *Bacillus amyloliquefaciens* and mixed strains of *Streptococcus faecalis* (SF), *Lactobacillus plantarum* (LP), and *Bacillus amyloliquefaciens* (BA) supplementation in juvenile amberjack, *Seriola dumerili* revealed significant improvement in physiological condition [26]. Similar gains in Red Sea bream performance and immunity were reported with probiotic supplementations compared to the control, with the mixed strains outperforming the single strain [25]. However, the potential impacts on antioxidant activity, immune response, and intestinal health are yet to be provided.

Therefore, in this study, the effects of two probiotics mixtures [BA+SF: *Bacillus amyloliquefaciens* + *Streptococcus faecalis*] and [BA+SF+LP+BM: *Bacillus amyloliquefaciens, Streptococcus faecalis, Lactobacillus plantarum*, and *Bacillus mesentericus*] were investigated on *S. dumerili.*

Table 1
Trial diets ingredients and proximate analysis.

Ingredients, g	Experimental diets		
	CD1	D2 [BA+SF]	D3 [BA+SF+LP+BM]
Fish meal [1]	45	45	45
Soybean meal [2]	15	15	15
Wheat flour	15	15	15
Pollack liver oil [3]	4	4	4
Soybean lecithin [4]	2	2	2
n-3 LC-HUFA [5]	0.5	0.5	0.5
Methionine [6]	0.11	0.11	0.11
Lysine [7]	0.4	0.4	0.4
Taurine [8]	0.07	0.07	0.07
Vitamin mix [9]	4	4	4
Mineral mix [10]	4	4	4
Activated gluten [11]	5	5	5
CMC	1	1	1
Vitamin C stay [12]	0.3	0.3	0.3
Probiotic Mixture [BA+SF] [13]	0	0.2	0
Probiotic Mixture [BA+SF+LP+BM] 14	0	0	0.2
α – cellulose [15]	3.62	3.42	3.42
Total (g)	**100**	**100**	**100**
Chemical analysis [16]			
Total protein%	41.71 ± 0.33	42.55 ± 0.23	42.73 ± 0.24
Total lipid%	12.79 ± 0.71	12.05 ± 0.6	12.81 ± 0.17
Ash%	12.26 ± 0.02	12.20 ± 0.01	12.28 ± 0.01
Carbohydrate [17]	29.69 ± 1.20	29.86 ± 0.35	27.95 ± 0.86
Gross energy (KJ/g) [18]	20.0 ± 0.13	20.05 ± 0.04	20.30 ± 0.02

[1–12] and [15–18] Ingredient and sources are detailed by Shadrack et al. [26]. [13, 14] Toa Biopharma Co., Tokyo, Japan. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

Materials and methods

Probiotic supplements

The Toa pharmaceutical company (Japan) provides the bacterial strains. The cell contents in the dry products were *Bacillus amyloliquefaciens* 1×10^8 cfu/g, *Streptococcus faecalis* T-110 2×10^8 cfu/g, *Lactobacillus plantarum* TO-A 8×10^7 cfu/g, *Bacillus mesentericus* TO-A 2×10^4 cfu/g.

Test diets

Table 1 shows the experimental diets' ingredients and proximate composition as designed following Shadrack et al. [26]. Three experimental diets (43.5 ± 0.50% crude protein and 12.55 ± 0.17% crude lipid) were formulated with no probiotics supplement as the control group (CD1) or with probiotics mixture supplement at the level of 2 g/kg diet in groups D2 [*Bacillus amyloliquefaciens* TOA5001 (BA) and *Streptococcus faecalis* T-110 (SF)] and D3 [*Bacillus amyloliquefaciens* (BA), *Streptococcus faecalis* T-110 (SF), *Lactobacillus plantarum* TO-A (LP), *Bacillus mesentericus* TO-A (BM)]. The experimental feeds were manufactured by mixing the dry ingredients using a food mixing machine for 15 min, followed by adding lipid sources (Pollack liver oil and soybean lecithin). The probiotics were added onto the diets based on weight percent of the feed. Bacteria cells was weighed and mix with lipid source, and then mix with the rest of the feed ingredients. Further, 35% water was added to the mixture and blended for 10 min to produce a sticky mixture for pellet production. The final pellets were obtained with a grinder (1.90 mm), then dried in the oven (DK 400, Yamamoto Scientific, Tokyo, Japan) at 40 °C for about 9 h, and stored in a polyethylene plastic bag at −20 °C until use.

Experimental conditions

S. dumerili juveniles were purchased from commercial producer, Kagoshima, Japan. The aquatic laboratory of Kamoike Marine Production units, Faculty of Fisheries, Kagoshima University, Japan, was used for the rearing. The design and experimental conditions were maintained according to amberjack's requirements [26]. Water system used was a flow through system with a flow rate of 1.5 L min^{-1}. Fish were stock in 500 L polycarbonate tank at a density of 50 fish per tank and two tanks per treatment group. Fish were fed until satiation twice daily at every 09:00 hrs and 15:00 hrs. Water temperature was maintained at 23.32 ± 0.5 °C, pH 8 ± 0.5, salinity 33.3 ± 0.5 ppt, and dissolve oxygen 6.1 ± 0.5 mg L^{-1}. Thirty-five percent of water is exchanged on a daily basis.

Performance variables

The growth parameters, feed utilization, and survival parameters of amberjack fed the test diets were determined according to the formulas described per parameters by Dawood et al. [27]. The performance variable includes growth (Feed intake (FI), feed conversion ratio (FCR), Specific growth rate (SGR) protein efficiency ratio (PER), protein gain (PG), protein retention (PR), Hepatosomatic index (HSI), Viscerasomatic index (VSI), Survival)), blood health (Blood chemistry), gut health (Microbiota) and antioxidants.

Sampling and data collection

Fish were fasted for 24 h before sampling to slow the stress-related metabolic process. During sampling, all fish were anesthetized with 50 mg/L eugenol (Wako Pure Chemical Ind., Japan), then body weight and length were measured according to tank numbers. A subsample of 3 fish / tank was assembled and stored at −20 °C for further analysis. Blood gathered from 5 fish was used in the study of biochemical content. A fraction of the blood was analyzed for hematocrit. The heparinized blood sample was centrifuged at 3000 x g at 4 °C for 15 min using MX-160 microcentrifuge (Tomy Tech USA., Tokyo, Japan) for plasma and stored at −80 °C until used. The chemical parameters of blood plasma were measured spectrophotometrically using a dry chemistry analyzer (SPOTCHEM EZ model SP-4430, Arkray, Inc., Kyoto, Japan) [28]. Reactive oxygen metabolites (d-ROM) and Biological antioxidant potential (BAP) were determined using an automated analyzer (FRAS4, Diacron International s.r.l., Grosseto, Italy) according to the method of Morganti et al. [29] and Kader et al. [30].

Three fish / tank were dissected to evaluate the internal organs. The liver and viscera were removed and weighed for viscerasomatic index (VSI) and hepatosomatic index (HSI). The digestive tract from three dissected fish per tank was chopped to pieces, washed with pure water, filled into a polyethylene bag, and stored at −80 °C. The liver from three dissected fish per tank was pooled together and kept at −80 °C. The proximate feed and fish whole-body analyses were conducted using standard methods [31]. The viability of bacteria cells in the test diet and intestinal content was determined using total bacteria (TB) and lactic acid bacteria (LAB) kit (3 M foot safety, USA). The bacteria analysis was done according to Nikoskelainen et al. [32], and the count was made according to Ren et al. [33]. Bacteria analysis was carried out by spreading onto 3M™ Petrifilm aerobic count plates. Briefly, 1 g of a test diet was homogenized in 10 mL of 0.05 M PBS buffer (pH 7.4) and 1 ml was serially diluted in 10 ml PBS buffer to 4th dilution. Then, 1 ml of each dilution is spread over the Petrifilm plate and incubated at 26 °C for 3 days. The colony forming unit is determined using a colony counter (ACK-3 AS ONE, Japan).

Immune response and antioxidant activity

The lysozyme activity of plasma and liver was determined as described by Dawood et al. [34]. The neutrophils oxidative radical production was assessed from whole blood using nitro blue tetrazolium (NBT) [35]. The total peroxidase in plasma was measured according to Salinas et al. [36]. The catalase enzyme activity of plasma was determined according to the process of Goth [37]. The superoxide dismutase (SOD) activity of plasma was measured as described by Dawood et al. [38] using the SOD kit-WST (Dojindo, Lab., Kumamoto Japan). The lipid peroxidation of plasma was determined by measuring the malondialdehyde (MDA) as a biomarker with the TBARs microplate assay Kit (Oxford Biochemical Research, Inc., USA) following the manufacturer's instruction.

Intestine histological assessment

The intestine was cut and submerged in Bouin solution (5% acetic acid, 9% formaldehyde, and 1.5% picric acid) for 12 h. Fixing was rapidly done by rinsing in alcohol every 24 h until clear. The tissue was embedded in paraffin blocks, sectioned, deparaffinized, and rehydrated. A rotary microtome (RM 2135, Leica, Nussloch, Germany) was used to obtain sagittal sections (5 μm). The section was placed on a glass slide, rehydrated, and stained with hematoxylin and eosin. The slides were then permanently mounted and observed with BX41, Olympus light microscope, Tokyo, Japan.

Statistical analysis

The Paleontological statistical software package version 3.21 was used for data analysis. The data was checked and confirmed for normality and homogeneity by the Kolmogorov-Smirnov test and Levene's test, respectively, before performing the analysis of variances (ANOVA) test. Probabilities of $P < 0.05$ were considered significant, and further analysis of the Tukey-Kramer post hoc test was performed to evaluate the differences in means.

Results

Growth responses and proximate analysis

Table 2 shows the growth performance of fish fed over the 60 days of trial. No substantial differences were observed in means of all growth variables, feed utilization, survival rate, biometric indices, and whole-body composition between fish groups ($P > 0.05$). Table 3 shows whole body proximate and somatic index of fish fed over the 60 days of trial. No apparent significant difference was observed between each treatment groups and the control $P > 0.05$).

Blood chemical parameters and hematocrit

The results of the blood profile demonstrated in Table 4 show normal ranges with no significant differences among fish groups.

Immune response

Fig. 1 exhibits the immunological responses of amberjack after 60 days of feeding. Liver lysozyme activity (U/mg) was more significant ($P < 0.05$) in D2 and D3 fish groups and compared to control CD1. Plasma lysozyme activity and NBT activity showed no significant alteration among all fish groups (D2, D3CD1) ($P > 0.05$). High peroxidase activity was detected in the fish group fed D2 compared to D3 and CD1 groups ($P < 0.05$).

Table 2
Amberjack juvenile performance after 60-day feeding regime.

Items	Experimental diets		
	CD1	D2 [BA+SF]	D3 [BA+SF+LP+BM]
Initial weight, g/fish	5.61 ± 0.15	5.59 ± 0.00	5.69 ± 0.01
Final weight, g/fish	45.88 ± 1.23	47.46 ± 0.11	48.5 ± 1.39
Weight gain,%	718.03 ± 8.05	748.33 ± 1.06	752.39 ± 26.5
Specific growth rate,%/day	3.5 ± 0.02	3.55 ± 0.02	3.57 ± 0.05
FI, g/fish /60 days	43.40 ± 0.71	45.0 ± 2.83	44.5 ± 0.71
Feed conversion ratio (FCR)	1.08 ± 0.01	1.07 ± 0.08	1.07 ± 0.01
Protein efficiency ratio (PER)	2.18 ± 0.06	2.17 ± 0.17	2.17 ± 0.13
Protein gain (PG)	807.83 ± 24.16	827.34 ± 25.49	847.08 ± 75.26
Protein retention (PR)	43.53 ± 1.5	43.36 ± 4.38	44.51 ± 2.89
Survival rate,%	100 ± 0.00	98 ± 2.83	100 ± 0.00

CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM). Values are the means of triplicate groups ± SEM of the mean. The absence of superior letters implies no difference.

Table 3
Amberjack juvenile whole-body proximate analysis and somatic index after 60-day feeding regime.

Items	Experimental Diets CD1	D2 [BA+SF]	D3 [BA+SF+LP+BM]
Moisture	74.36 ± 0.42	73.44 ± 0.34	73.73 ± 0.47
Total protein	17.55 ± 0.06	17.56± 0.29	17.99 ± 0.21
Total lipid	2.00 ± 0.01	2.09 ± 0.05	2.11 ± 0.08
Ash	3.82 ± 0.25	3.83± 0.37	3.83 ± 0.20
Hepatosomatic index,% (HSI)	0.70 ± 0.40	0.60 ± 0.10	0.60 ± 0.20
Viscerasomatic index,% (VSI)	4.80 ± 0.80	4.20 ± 0.00	4.10 ± 0.20

Whole-body proximate analyses are expressed on a wet weight basis. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM). Values are the means of triplicate groups ± SEM of the mean. The absence of superior letters implies no difference.

Table 4
Blood analysis of amberjack juvenile fed experimental diets for 60 days.

Items	Experimental diets CD1	D2 [BA+SF]	D3 [BA+SF+LP+BM]
Hematocrit (%)	40.2 ± 0.85	44.0 ± 1.13	43.2 ± 1.13
Glucose (mg/dl)	128.5 ± 9.19	134.5 ± 24.75	137.0 ± 24.04
Total cholesterol (T-Cho, mg/dl)	186.5 ± 20.51	201 ± 9.90	222.50 ± 38.89
Blood urea nitrogen (Bun, mg/dl)	13.5 ± 2.12	13.0 ± 1.41	14.5 ± 3.54
Total bilirubin (T-Bill, mg/dl)	1.10 ± 0.28	1.65 ± 0.35	1.40 ± 0.71
Total protein (TP, g/dl)	3.30 ± 0.28	4.20± 0.85	3.95 ± 0.78

Values are means of 3 pooled samples (means ± S.E.M). The absence of superior letters implies no difference. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

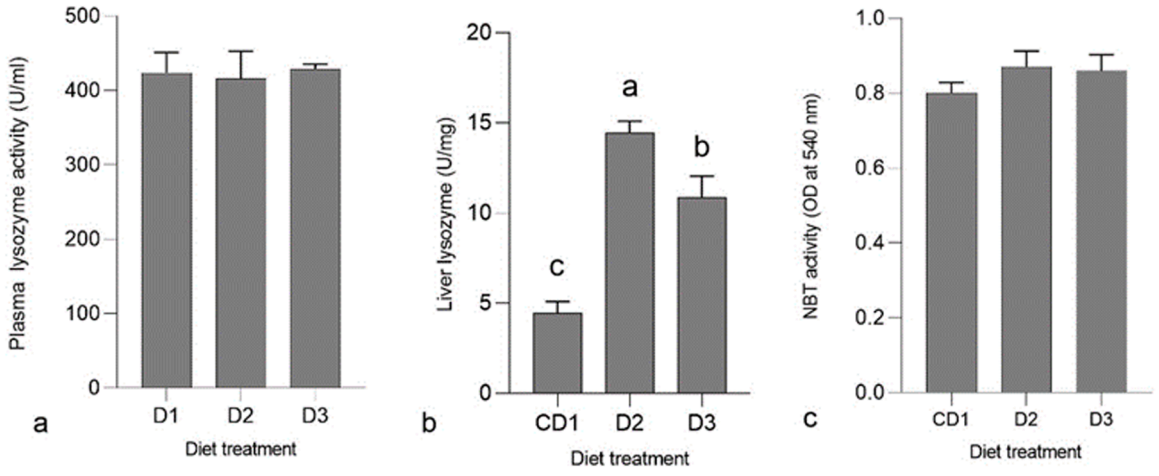

Fig. 1. Amberjack immunological responses after 60 days of the experimental period. (a) Plasma lysozyme activity (unit/ml); (b) Liver lysozyme activity (U/mg); (c) NBT activity (OD_{540} nm). Values are means ± SEM ($n = 3$). Different superscripts imply a significant difference ($P < 0.05$). The absence of letters shows no difference. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

Antioxidant potential

Table 5 and Figs. 2 & 3 show antioxidant activity over the 60 days of the feeding regime. High SOD and antioxidant activity were noted in fish fed D2 against D3 and CD1 groups (Table 5) ($P < 0.05$). The MDA and d-ROM results were not significantly altered among treatment groups (Table 4), while the D2 fish group showed significant peroxidase activity compared to the control CD1 ($P < 0.05$: Fig. 2, a & b). No considerable alteration was noted in catalase activities between fish groups fed the test diets ($P > 0.05$; Fig. 2, c). The combined effect of d-ROM and BAP showed D2 fish group was in Zone A (good condition), indicating a high ability to tolerate oxidative stress, diet CD1 (control diet) is Zone C (acceptable condition), showing lower d-ROM and lower BAP values, while diet D3 is in Zone D (poor condition) reflexing higher tolerance of d-ROM and low BAP values (Fig. 3).

Table 5
Antioxidant potential of amberjack juvenile fed test diets for 60 days.

Items	Experimental diets		
	CD1	D2 [BA+SF]	D3 [BA+SF+LP+BM]
MDA (nmol/ml)	1.78±1.36	3.01±1.62	1.83±0.61
SOD (50% inhibition)	31.72±12.83^{b}	82.41±17.47^{a}	59.22±18.20^{ab}
d-ROMs (μMol/L)	176.50±13.2^{b}	200.0 ± 15.9^{ab}	285.5 ± 20.5^{a}
BAP (U. Carr)	376.50±25.0^{c}	1578.0 ± 14.10 ^{a}	587.5 ± 42.50^{b}
MDA (nmol/ml)	1.78±1.36	3.01±1.62	1.83±0.61

CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM). Values are means of 3 pooled samples (means ± S.E.M). Different superiors imply a significant difference ($P < 0.05$). The absence of letters shows no difference. MDA – malondialdehyde, SOD - superoxide dismutase, NBT - nitro blue tetrazolium, d-ROM - Reactive oxygen metabolites, BAP - antioxidant potential.

Intestinal microflora and histology

The intestinal bacteria count of amberjack juveniles that consumed the test diets for 60 days is demonstrated in Fig. 4. LAB in diet and intestinal content was elevated ($P < 0.05$) in diet D2 compared to diets D3 and CD1 groups. Fig. 5 presents the cross-section in the small intestine of fish fed on the experimental diets for 60 days. The histological structure of the Amberjack juvenile's intestine in all experimental groups displayed a normal and intact structure in terms of serosa, tunica muscularis, lamina propria, and simple columnar epithelium villi lining around the connective tissue core. The cross-section in the small intestine of fish groups fed on D2 and D3 diets showed an increase in villi length, cryptal depth, and the number of goblet cells compared to the control group.

Discussion

Aquaculture of finfish is currently a growing sector in food protein production. It provides an avenue to meet the consumer demand for a protein source. Despite the existing challenges with production and quality, intensive farming systems to boost production have been the current focus [39]. Several approaches were investigated, including the use of functional feed, such as nucleotide supplementation [40], fermented feed [41–43], and the inclusion of bacteria as probiotics in the feed [44]. From this perspective, feed additives and immunostimulants help enhance growth and improve the disease resistance of cultured species [45]. This study presents the effects of probiotic bacteria as feed additives for *S. dumerili* juveniles.

Probiotic bacteria are known to be promising feed additives in aquaculture, regardless of limited, contradictory reports [21]. The results of some previous studies indicate the improvement of various growth and immune parameters with probiotic supplements [46–51]. The growth and feed efficacy were not altered in the current trial, but a numerical improvement was observed in SGR and FI for the D2 group with slightly low FCR. This may link to the probiotic roles in the gastrointestinal tract of fish, such as producing pathogens growth inhibition substances (bacteriocins, hydrogen peroxides, diacyl, etc.) [52]. A prolonged rearing period could achieve significant growth differences as observed in Shadrack et al., 2020, where growth genes were significantly improved in Red sea bream, *Pagros Major*, reared with mix probiotic bacteria strains though growth parameters were not different from the control. Thus, acknowledging the dietary supplementation ratio and rearing period at which favorable effects on growth can be identified but may need further examination.

Blood condition is a good indicator of fish welfare. It is also a direct reflector of stressors and external stimuli [53]. The blood results demonstrated normal values with no significant difference among fish groups, implying the safety of the probiotic supplements. A numerically high Haematocrit and TP for D2 and D3 dietary supplement groups suggest an improvement in the health condition of fish. Previous studies reported high hematocrit in *S. dumerili* and rainbow trout fed with heat kill-lactobacillus [54] and *Enterococcus feacalis* supplement diets [55], respectively, indicating an evenly distributed ions without any decrease in the synthesis of hemoglobin [54]. The reactive oxygen metabolites, d-ROM, and antioxidant potential (BAP) tests were used as a measure of oxidative stress [27] due to their reliability reported in various studies [56]. The results presented here concluded that the D2 group was less stressed than the other treatment groups. The antioxidant enzymes such as SOD results showed significantly high values in the fish group fed D2 compared to CD1 (control) and D3 group, suggesting an improved antioxidant status of fish [57].

The first defensive mechanism in fish includes phagocytosis, executed by lysozyme and respiratory burst activity (Nitroblue Tetrazolium, NBT) [58,59]. Lysozyme activity is measured as a non-specific immune defense in fish, while nitro blue tetrazolium (NBT) is monitored as an indicator of innate immunity [59,60]. High lysozyme activity was observed in fish fed D2 compared to other fish groups, suggesting enhanced phagocytosis cells stimulated by LAB supplementation. The fish group provided D2 and D3 showed high NBT activity compared to the control ($P > 0.5$), suggesting improved immune response of fish consistent with Abmughaid et al. [61], where supplementation of probiotic bacteria in diets of tilapia (*Oreochromis niloticus*) significantly improve NBT activity. The fish group fed D2 and D3 showed an exceptionally high level of peroxidase (GPx) and improved catalase (CAT) consistent with Salinas et al. [36] and Dawood et al. [27], where heat-killed bacteria supplement in diets of Red Sea bream, and amberjack causes a high level of these parameters. These results concluded that the non-specific immune response for amberjack was enhanced by probiotic bacteria supplementation.

Probiotic bacteria are favorable microorganisms because of their various function, including GI development, digestive function, mucosal tolerance, stimulating immune responses, and improved resistance [21,54]. The analysis of LAB in feed and intestinal content

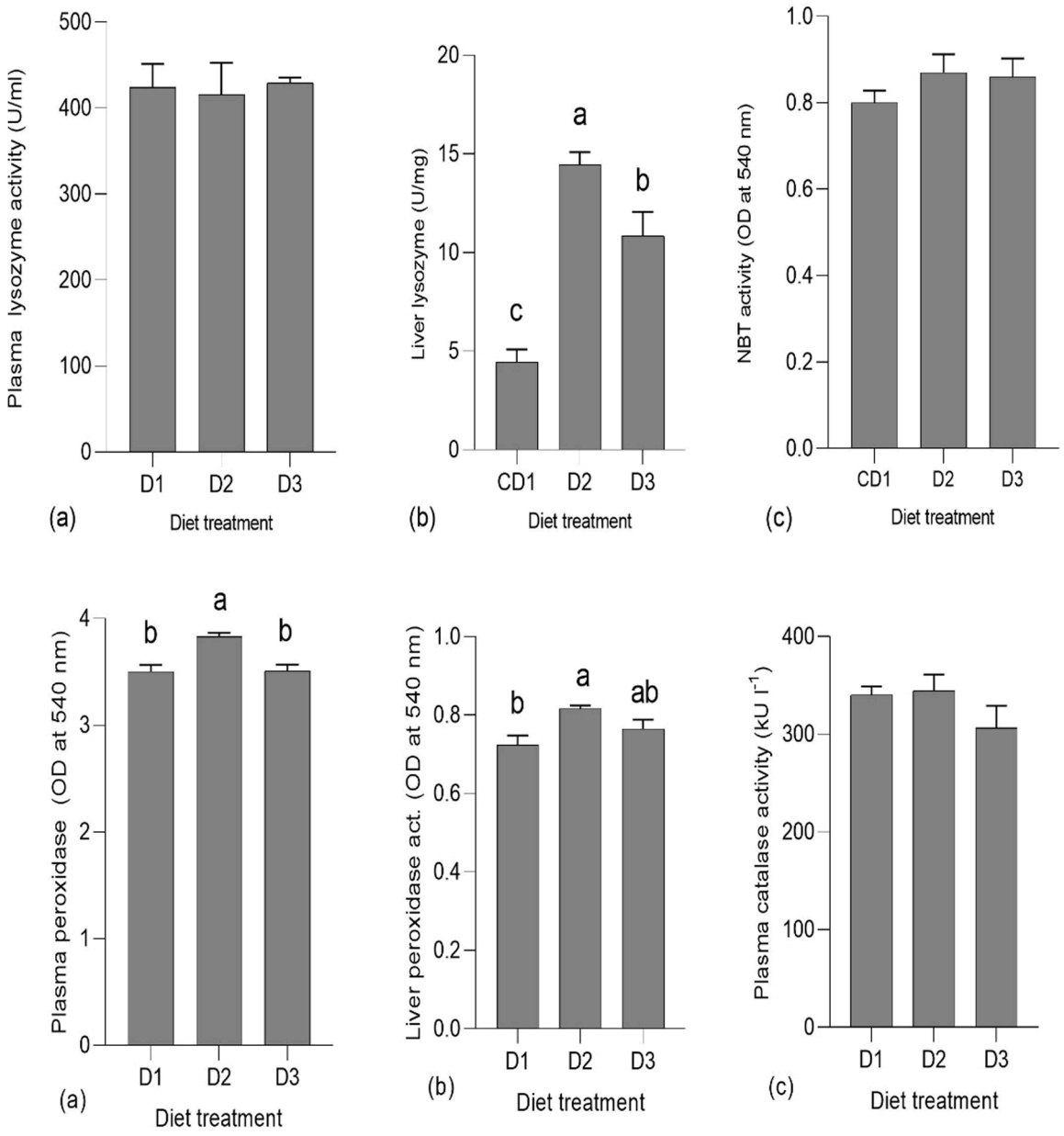

Fig. 2. Peroxidase and catalase activities of amberjack juvenile fed test diets for 60 days. Values are means of 3 pooled samples (means ± S.E.M). Different superiors imply a significant difference ($P < 0.05$). The absence of letters shows no difference. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

of fish revealed less LAB in the control and D3 groups compared to a significantly high LAB count for the D2 group, hence the improved immune and antioxidant activity. The findings of this study suggest D2 probiotic strain has regulated the microflora in favor of the beneficial LAB strain, following the report of Kuebutornye et al. [62]. The histological features are valuable indicators of inflammatory and pre-inflammatory alteration induced by biotic and abiotic factors in the aquatic environment [63]. The supplementation of probiotic bacteria improves intestinal features such as increased villi length, goblet cells, and crypt depth, which aligns with the findings of Ringø et al. [21]. Similarly, the micrograph of the intestine observed in this study revealed an increase in villi length, cryptal depth, and increased number of goblet cells supplemented group (D2-D3) compared to the control group (CD1).

The current investigation results indicate the advantages of using probiotic mixtures on gut health, immunity, and the oxidation system in fish, compared with the control group. The outcomes of using the first mixture [BA+SF] were superior to the second mixture [BA+SF+LP+BM] in lysozyme activity, LAB count, and BAP value. These findings may be attributed to an incongruousness between the types used in the second mixture. Therefore, choosing the compatible strain in a mixture is vital [23,24].

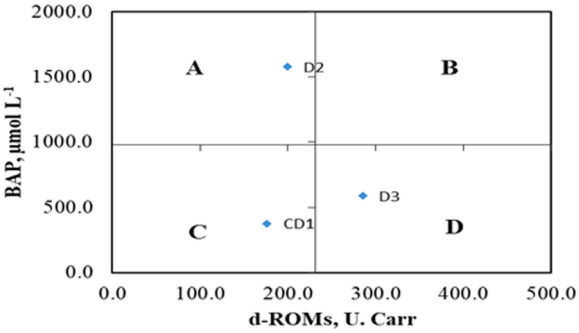

Fig. 3. Peroxidase and catalase activities of amberjack juvenile fed test diets for 60 days. Values are means of 3 pooled samples (means ± S.E.M). Different superiors imply a significant difference ($P < 0.05$). The absence of letters shows no difference. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

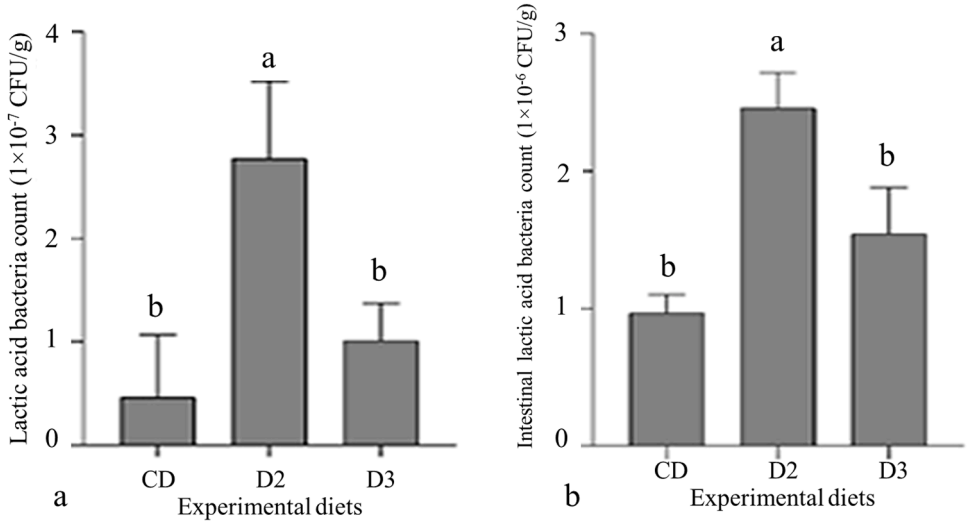

Fig. 4. The bacterial count in experimental diets and intestines of amberjack juveniles fed test diets for 60 days. (a) Lactic acid bacteria in experimental diets. (b) Lactic acid bacteria in amberjack intestine. Values are means ± SEM ($n = 3$). CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

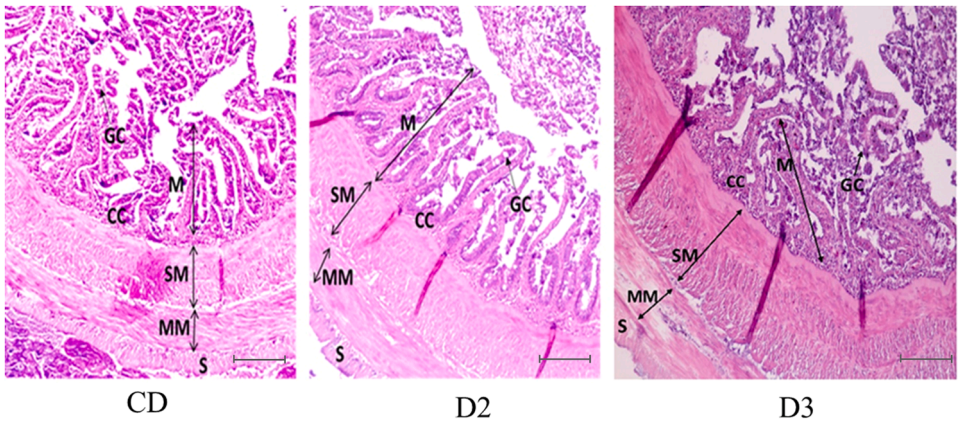

Fig. 5. The cross-section in the small intestine of amberjack juveniles fed on the experimental diets for 60 days (H & E stain, x100). Where, *M*= Mucosa; SM= Submucosa; MM= Muscularis mucosa; *S*= Serosa; GC= Goblet cell; CC= Crypts cell. CD1= the basal diet; D2= the basal diet + a probiotic mix (BA and SF); D3= the basal diet + a probiotic mixture (BA, SF, LP, and BM).

Conclusions

In conclusion, the current investigation reveals that the probiotic bacteria BA+SF (D2) supplement in the diet of amberjack has improved performance, immune and blood condition, oxidative status, and intestinal condition of fish. The D2 fish group showed higher liver SOD, lysozyme activity, Peroxidase activity, and BAP activity than other fish groups, consistent with high LAB in diet and intestinal content, respectively. These results suggest that D2 dietary supplements containing *Bacillus amyloliquefaciens* strain have the potential to improve immune and antioxidant activity in *S. dumerili*.

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Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data availability statement

The data are available from the first author upon reasonable request.

CREDIT author statement

Statement
All authors have seen and approved the manuscript and have contributed significantly to the paper.
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All authors contributed equally to this manuscript.

Declaration of Competing 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.

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