Probiotics and Antimicrobial Proteins (2020) 12:389–399
https://doi.org/10.1007/s12602-019-09552-7

Synergetic Effects of *Lactobacillus plantarum* and β-Glucan on Digestive Enzyme Activity, Intestinal Morphology, Growth, Fatty Acid, and Glucose-Related Gene Expression of Genetically Improved Farmed Tilapia

Mahmoud A. O. Dawood[1] · Fawzy I. Magouz[1] · Mahmoud F. I. Salem[2] · Zizy I. Elbialy[3] · Hanaa A. Abdel-Daim[1]

Published online: 11 May 2019

Abstract

The current study was conducted to evaluate the synergetic effects of heat-killed *Lactobacillus plantarum* (HK L-137) and β-glucan (BG) on digestive enzyme activity and intestinal morphology of genetically improved farmed tilapia (GIFT) with focus on insulin-like growth factor I (*IGF-I*), fatty acid synthase (*FAS*), and glucose-6-phosphate dehydrogenase (*G6PD*). For 12 weeks, fish fed the control, or three diets incorporated with 100 HK L-137, 100 BG, or 50 HK L-137 + 50 BG mg/kg (HK L-137, BG, and HK L-137/BG diets). After final sampling, fish fed HK L-137 or HK L-137/BG diets exhibited significantly ($P < 0.05$) increased final body weight and weight gain while the specific growth rate and feed efficiency ratio enhanced only in HK L-137/BG group. Mucosal and villi lengths and muscle thickness significantly ($P < 0.05$) increased by HK L-137 or/and BG for the middle intestine. Lipase and protease improved significantly ($P < 0.05$) in fish fed both HK L-137 and BG when compared to the control group. Interestingly, qRT-PCR revealed a significant ($P < 0.05$) upregulation in the *IGF-1* gene expression in fish fed HK L-137 or/and BG additives compared to the control. Muscle and liver *G6PD* gene expression were upregulated significantly ($P < 0.05$) in fish fed HK L-137/BG diet as compared to the control group. In addition, feeding HK L-137 or both additives effectively elevated the hematocrit, hemoglobin, and WBCs and decreased triglyceride and glucose levels. Accordingly, the use of both HK L-137 and BG is an efficient scheme to reach economically feasible and sustainable tilapia production.

Keywords GIFT tilapia · HK L-137 · β-Glucan · Insulin-like growth factor 1 · Fatty acid synthase · Glucose-6-phosphate dehydrogenase · Digestive enzyme activity · Intestinal morphometry

Introduction

Genetically improved farmed tilapia (GIFT), an enhanced strain of Nile tilapia (*Oreochromis niloticus*), exhibits better performance rates than routinely available strains of tilapia [1]. Currently, there is increased interest in Egypt in the culture of the GIFT tilapia mainly because of its rapid growth rate, high fillet yield, and high disease resistance [2]. GIFT also can be farmed in different aquaculture systems. The intensive culture systems are commonly used for tilapia culture which causes stressful conditions that reduce fish growth and wellbeing. In addition, there are increasing concerns about the use of antibiotics in tilapia culture to overcome infectious diseases [3–5]. Key driving forces include restrictions or bans on the use of prophylactic antibiotics in various countries. The use of functional feed additives as bio-friendly agents is a sustainable way to improve cultured fish performance. The use of probiotics and/or functional ingredients with immune modulatory properties has become more prominent across the animal feed industry [6–8]. In a wide range of species, both terrestrial and aquatic, a substantial body of literature exists demonstrating the advantageous effect of feeding functional immune modulatory substances [9–11].

The impetus of using functional feed additives in aquafeeds is to promote intestinal health and improve fish performance

✉ Mahmoud A. O. Dawood
mahmouddawood55@gmail.com

[1] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

[2] Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Sakha Aquaculture Research Unit, Kafrelsheikh, Egypt

[3] Fish Processing and Biotechnology Department, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh, Egypt

[12, 13]. According to Ouwehand and Salminen [14], probiotics can be existed in both live and killed bacterial forms. The application of probiotics "live bacterial cells" might affect the aquaculture environment negatively by the interaction with the ecosystem. Dead beneficial cells remarkably help in increasing feed efficiency along with disease resistance by immune modulation and/or competitive exclusion for adhesion sites [15]. Many reports have concluded that the dietary inclusion of heat-killed *Lactobacillus plantarum* (HK strain L-137) can positively impact the performance and wellbeing of fish, shrimp, and sea cucumber [16–21].

It is well documented that the supplementation of β-glucan (BG) in aquafeed resulted in improved performance, physiological and oxidative status as well as the enhancement in the immune response by increasing the lysozyme and complement system levels which increase macrophages phagocytic activity [22–24]. Further, BG can be used as a decent preventive and restorative choice against infectious diseases in cultured fish [9, 24].

Using synergistically related functional supplements as biofriendly agents is a sustainable way for improving the performance of aquatic species [25–27]. As far as we know, no published data about the detailed supplemental effects of HK L-137 or/and BG on tilapia's growth-related gene expression, digestive enzymes activity, intestinal morphometry, hemato-immune response, and antioxidative status. Thus, the present study is done to examine the probability of feeding a novel HK L-137 or/and BG on several performances of GIFT tilapia.

Materials and Methods

Fish, Diet, and Experimental Protocol

GIFT tilapia fingerlings were obtained from a private farm located in Kafrelsheikh, Egypt, and transported to Sakha Aquaculture Research Unit, Kafrelsheikh, Egypt. After 2-week acclimation, 180 fish (15.94 ± 0.02 g) were put into 12 glass aquaria (70 L) (15 fish/tank) for 12 weeks. Feeding rate was fixed at 3% of body weight per day with two feeding times 08:00 and 15:30 and batch-weighed every 2 weeks to adjust daily feed input. The leftover feed was siphoned out after 3 h, and 50% of water was replaced daily. During the trial, the rearing conditions were 12:12 h light/dark photoperiod, water temperature 23.1 ± 0.8 °C, pH 6.8–7.5, DO 7.5–8 mg/l, and ammonium 0.04–0.08 mg/l.

Four experimental diets were tested in triplicate: the basal (control) diet and the basal diet supplemented with HK L-137 "House Wellness Foods Corp., Itami, Japan [28]" at 100 mg/kg, β-glucan "BG, Daigon do, Tokyo, Japan" at 100 mg/kg or 50 HK L-137 + 50 BG mg/kg (control, HK L-137, BG, and HK L-137/BG diets) (Table 1). The doses of HK L-137 and BG were selected by following Dawood et al. [2]. HK L-137, BG or HK L-137/BG additives were thoroughly mixed with lipid source before adding other ingredients. Fish oil was well mixed the additives, added to the dry ingredients and mixed for 15 min. All ingredients were combined well into a homogenous mixture to produce the test diets. Water was then added to achieve a uniform texture appropriate for pelleting (1 to 2 mm pellets) using an extruder machine, followed by air drying at room temperature. The nutritional profile for each diet was fixed by following AOAC [29] certified procedures (Table 1).

Sampling Schedule

Three fish per group (one fish per aquarium) were randomly caught and euthanized by "diluted tricaine methanesulfonate (MS-222; 1:2,500 ratio; Sigma-Aldrich, Egypt)." Fish were individually measured for final body-weight, length and the intestine sampled for morphometrical and digestive enzymes analysis. Liver and viscera were removed then weighed to get hepatosomatic index "HSI = weight of liver/weight of fish × 100" and viscerasomatic index "VSI = weight of viscera/weight of fish × 100," respectively.

For digestive enzyme analysis, intestine from the control and treated fish were aseptically taken, washed with PBS (pH 7.5; 1 g per 10 ml), homogenized and centrifuged for 5 min at 8000 rpm. The supernatant then kept at 4 °C. Protease, lipase, and amylase enzyme activities performed according to Lowry et al. [30], Borlongan [31], Jin [32], and Worthington [33], respectively. Protease, lipase, and amylase activities were stated as "specific activity" (units per mg of protein) of intestine content [34, 35].

Samples from the different intestinal portions (anterior, middle, and posterior) were collected from the tested fish, then fixed in 10% formalin. After dehydration and clearance, the tissues were embedded in paraffin and sectioned in 5-μm thickness. The serial sections were subjected to H/E staining [36]. The villus height was performed using "Image J analysis software (National Institutes of Health, MD, USA)."

Tissue samples from different organs (liver and muscle) were collected from three fish per group (one fish per aquarium), immediately in 2-ml Eppendorf tubes and shocked in liquid nitrogen then stored at − 80 °C for RNA extraction.

Blood Assays

Blood was collected from the caudal vein of nine anesthetized fish per group (three fish per aquarium) to collect enough amount of blood. The collected blood was then put into either heparinized (with EDTA) or non-heparinized (for serum analysis) separate tubes. Blood hematology markers "hemoglobin, red blood cells (RBCs), white blood cells (WBCs), total and differential count of heterophil and lymphocyte" were analyzed according to Brown [37]. Hematocrit was determined

Table 1 Basal diet and proximate chemical composition (on dry matter basis)

Ingredient	%	Chemical composition	%
Fish meal	10	Dry matter	92.8
Soybean meal	44.4	Crude protein	30.9
Wheat bran	10	Ether extract	7.1
Yellow corn	18.6	Total ash	7.2
Rice bran	10	Gross energy (kcal/100 g)*	446
Fish oil	5		
Dicalcium phosphate	1		
Vitamins and minerals mixture	1		
Total	100		

*Gross energy was calculated as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively

using "the microhematocrit technique." Blood smears were prepared for the determination of differential leukocyte counts [38]. The remaining samples (1 ml blood) were left for 30 min till blood clotting then serum separation by centrifugation at 3000 rpm for 10 min. Serum samples were stored at − 20 °C until further analysis. Blood biochemical investigations to all studied subjects "cholesterol, triglycerides, glutamyl oxaloacetic transaminase (GOT), and glutamic-pyruvate transaminase (GPT)" were carried out by RA-50 chemistry analyzer (Bayer) using readymade chemicals (kits) supplied by Spinreact Co. Spain, following manufacturer's guidelines.

Total RNA Extraction and cDNA Synthesis

A total of 100 mg tissue was homogenized in liquid nitrogen, and total RNA from the tested samples (collected on liquid nitrogen) were extracted using easy RED total RNA extraction kits (iNtRON Biotechnology, Inc., Korea) according to the manufacturer's instructions. The RNA concentration (OD 260 nm) and purity (OD 260 nm/OD 280 nm ratio, range 28.15–103.1) of each sample. The RNA integrity was verified by agarose gel electrophoresis while the concentration and purity of the samples were examined by NanoDrop spectrophotometer. The first-strand cDNA was synthesized using HiSenScript cDNA synthesis kit (iNtRON Biotechnology, Inc., Korea).

qRT-PCR Assay

The specific primers were used to amplify the selected genes of the Nile tilapia (*O. niloticus*) with *β-actin* as a housekeeping (internal standard) gene-primer sequence and references are shown in Table 2. Real-time quantitative (qRT-PCR) assay was carried out using Stratagene MX300P real-time PCR system (Agilent Technologies, USA), using TOP real™ preMIX SYBR Green qPCR master mix (Enzynomics, cat. RT 500) following the manufacturer's recommendations. The thermocycling conditions for the reaction were as follows: 95 °C for 30 s, followed by 45 cycles of denaturation at 63 °C for 60 s and annealing at 72 °C for 60 s. MxPro QPCR Software was used for data collection. The relative gene expression levels were evaluated using the $2^{-\Delta\Delta ct}$ method as described by Pfaffl [41]. All samples were analyzed in triplicate and along with non-template control and negative RT controls in each plate.

Growth Performance Calculations

During the final sampling, 15 fish per tank were weighed separately. Growth and feed efficiency were evaluated using weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER), and condition factor (CF). Calculations were made using the following formulae: WG (%) = (FBW − IBW) × 100/IBW; SGR (%BW/day) = 100((lnFBW − lnIBW)/T); FER = WG /FI; CF = BW/FL^3, where FBW = body weight final (g), IBW = body weight initial (g), T = duration of the trial in days, WG = wet weight gain (g), FI = estimated feed intake (g), and FL = standard fork length (cm).

Statistical Analysis

Shapiro–Wilk and Levene tests confirmed normal distribution and variance homogeneity. All statistical differences (growth performance, digestive enzymes, intestinal morphometry, blood indices, immune, oxidative responses, and qRT-PCR data) were assessed by one-way ANOVA tests (SPSS version 22, SPSS Inc., IL, USA) with Duncan's as post hoc test where differences in experimental groups occurred. The level of significance was accepted at $P < 0.05$. All data are presented as means ± standard error (SE).

Results

Growth Parameters Analysis

The different growth and feed utilization parameters are represented in Table 3. A significant ($P < 0.05$) enhancement of

Table 2 Primers used for qRT-PCR analysis

Gene name	Sequence	Reference
IGF-1	For. 5′-TCCTGTAGCCACACCCTCTC-3′	[39]
	Rev. 5′-ACAGCTTTGGAAGCAGCACT-3′	
FAS	For. 5′-TGAAACTGAAGCCTTGTGTGCC-5′	[40]
	Rev. 5′-TCCCTGTGAGCGGAGGTGATTA-3′	
G6PD	For. 5′-ACAGGAACTGTCAGCCCACCTT-3′	[1]
	Rev. 5′-AGCACCATGAGGTTCTGGACCA-3′	
β-actin	For. 5′-CCACACAGTGCCCATCTACGA-3′	[1]
	Rev. 5′-CCACGCTCTGTCAGGATCTTCA-3′	

IGF-1 insulin-like growth factor 1, *FAS* fatty acid synthase, *G6PD* glucose-6-phosphate dehydrogenase

FBW and WG was observed in fish fed only HK L-137 and both (HK L-137/BG) over the control group. Further, specific growth rate (SGR) and feed efficiency ratio (FER) were significantly ($P < 0.05$) higher in fish fed both HK L-137 and BG (HK L-137/BG) than the control. No significant ($P > 0.05$) differences on the survival rate and somatic indices (CF, HSI, and VSI) were observed among all groups.

Digestive Enzyme Analysis and Intestinal Morphometry

Lipase and protease activities increased significantly ($P < 0.05$) in HK L-137/BG group over the control regime without no differences among the other group (Table 4). Amylase activity showed no significant differences among the groups (Table 4).

The results of the intestinal morphological analysis of GIFT fish fed test diets for 12 weeks are summarized in Table 5. The anterior intestine, mucosal, and villi lengths showed no significant differences ($P > 0.05$) among the groups. For the middle intestine, mucosal and villi lengths as well as the muscle thickness were increased significantly ($P < 0.05$) in HK L-137 or/and BG over the control. For the last section of the intestine (posterior), no changes were observed among all the experimental groups.

Fish fed the basal diet showed normal thick and blunt ended anterior villi, while fish fed HK L-137 or/and BG diets showed mild increase of intestinal villi length (Fig. 1A). Middle intestine of fish fed with basal diet showed normal thick and blunt ended villi, while fish fed HK L-137 or/and BG diets showed clear increase of intestinal villi length and branches (Fig. 1B). Fish fed with basal diet showed normal thick and blunt ended villi, while fish fed HK L-137 or/and BG diets showed normal villi number and length (Fig. 1C).

Gene Expression

Gene expression analysis revealed a significant upregulation ($P < 0.05$) in *IGF-1* expression in fish fed HK L-137 or/and BG compared to the control with no differences among the supplemented groups. The expression of *G6PD* in the muscle and liver tissues were also upregulated in fish fed both HK L-137 and BG over the control with no differences between the other groups (Fig. 2). In contrast, fish fed HK L-137 or both HK L-137 and BG exhibited relatively down regulation in *FAS* gene expression compared to fish fed the control diet.

Table 3 Growth performance, nutrient utilization, survival and somatic indices of fish fed test diets for 12 weeks

Item	Test diet Control	HK L-137	BG	HK L-137/BG
IBW (g)	15.9 ± 0.1	15.92 ± 0.03	15.95 ± 0.02	15.98 ± 0.01
FBW (g)	36.1 ± 1.8a	45.2 ± 2.4b	41.4 ± 3.1ab	50.7 ± 3.2b
WG (%)	126.2 ± 9.2a	183.4 ± 13.4b	159.2 ± 14.6ab	216.2 ± 21.6b
SGR (%/day)	1.81 ± 0.3a	2.31 ± 0.5ab	2.11 ± 0.6ab	2.55 ± 0.5b
FER	0.53 ± 0.1a	0.73 ± 0.1ab	0.59 ± 0.04a	0.85 ± 0.14b
Survival	91.1 ± 2.2	100 ± 0	93.3 ± 3.8	93.3 ± 3.8
CF	1.82 ± 0.2	1.84 ± 0.3	1.71 ± 0.2	1.73 ± 0.4
HSI	2.3 ± 0.2	2.1 ± 0.3	2.4 ± 0.3	1.54 ± 0.1
VSI	2.9 ± 0.3	2.6 ± 0.4	2.6 ± 0.5	2.24 ± 0.2

Values expressed as means ± SE ($n = 3$). Different letters indicate significant differences for each pairwise comparison between treatments

Table 4 Digestive enzymes activity in fish fed test diets for 12 weeks

Item	Test diet Control	HK L-137	BG	HK L-137/BG
Amylase (U mg^{-1})	22 ± 2.1	31. ± 3.2	27.5 ± 2.8	44.5 ± 4.4
Lipase (U/mg)	25.5 ± 2.6a	38.5 ± 6.1ab	49 ± 10.2ab	58.5 ± 12.6b
Protease (U/mg)	18.5 ± 1.6a	23 ± 2.1ab	20 ± 2.6ab	24.5 ± 3.9b

Values expressed as means ± SE ($n = 9$). Different letters indicate significant differences for each pairwise comparison between treatments

Blood Markers

Hematocrit increased significantly ($P < 0.05$) in BG and HK L-137/BG groups over the control group (Table 6), while no differences between BG and HK L-137/BG or HK L-137. Further, the hemoglobin increased significantly ($P < 0.05$) in HK L-137/BG group over the control group without no differences with the other groups (Table 6). WBCs increased significantly ($P < 0.05$) in HK L-137 and HK L-137/BG groups over the control group without no differences with BG group (Table 6). Blood glucose level decreased significantly ($P < 0.05$) in HK L-137/BG in comparison with the control without no differences with the other groups (Table 6). Blood triglyceride decreased significantly ($P < 0.05$) in HK L-137/BG group, while no alterations were detected between the control, HK L-137, and BG groups (Table 6). Blood GPT also decreased significantly ($P < 0.05$) in fish fed HK L-137 or/and BG. No significant changes ($P > 0.05$) were observed in the remaining blood variables of tilapia in the current study (Table 6).

Discussion

Functional feed additives have got superior consideration in aquaculture recently due to its beneficial activity not only in growth promotion but also in disease resistance and immune modulation [18, 42, 43]. Among them, dead *Lactobacillus* sp. and BG which got greater attention because of their immunomodulation prosperities to overcome unfavorable conditions [16, 17, 24, 27]. In this study, we revealed detailed mechanistical effects of HK L-137 or/and BG on GIFT's growth performance, digestive enzymes activity, intestinal morphometry, hemato-immune response, and antioxidative status.

Inclusion of HK L-137 or/and BG in GIFT feed were shown to increase FBW, WG, SGR, and FER significantly compared to control. It may be suggested that the beneficial bacterial cells modulated growth and caused total physiological changes [9]. The probiotic bacterium was analyzed for its growth-promoting effects by means of growth rate, feed utilization, modulation of digestive enzyme activity, antioxidant status, and upregulation of immune response in fish [16, 17, 24, 27]. It is well known that bacterial cells can colonize in the host intestine and then acting its effects by improving the digestion and absorption process as well as the supportive role to the intestinal digestive enzymes [14]. Similarly, tilapia fed probiotic showed improved growth rates and feed efficiency [20, 44]. Also, the results suggested that tilapia utilized test diets efficiently by HK L-137 resulting in improved FER, which would be one reason for the quicker growth in fish

Table 5 Intestinal morphometry in fish fed test diets for 12 weeks

Item		Test diet Control	HK L-137	BG	HK L-137/BG
Anterior	Mucosal length	155.5 ± 20.6	271.6 ± 41.5	246.5 ± 25.8	382 ± 62.6
	Villi length	102.3 ± 15.6	203.1 ± 19.8	170.7 ± 25.2	268.9 ± 51.6
	Muscle thickness	23.4 ± 4.6	41.9 ± 8.2	48.1 ± 5.9	45.7 ± 11.3
Middle	Mucosal length	162.4 ± 32.1a	408.4 ± 65.5b	399.8 ± 56.3b	442.7 ± 71.8b
	Villi length	124.5 ± 32.5a	349.1 ± 54.7b	384.1 ± 65.5b	328.3 ± 36.5b
	Muscle thickness	18.1 ± 3.1a	56.5 ± 10.2b	77 ± 17.8b	67.3 ± 16.5b
Posterior	Mucosal length	136.9 ± 15.3	170.3 ± 20.8	186.9 ± 46.2	132.8 ± 14.2
	Villi length	100.6 ± 11.2	106.4 ± 13.26	117.9 ± 20.5	111.3 ± 12.5
	Muscle thickness	37.9 ± 6.1	38.4 ± 5.3	42.7 ± 7.5	28.5 ± 5.4

Values expressed as means ± SE ($n = 9$). Different letters indicate significant differences for each pairwise comparison between treatments

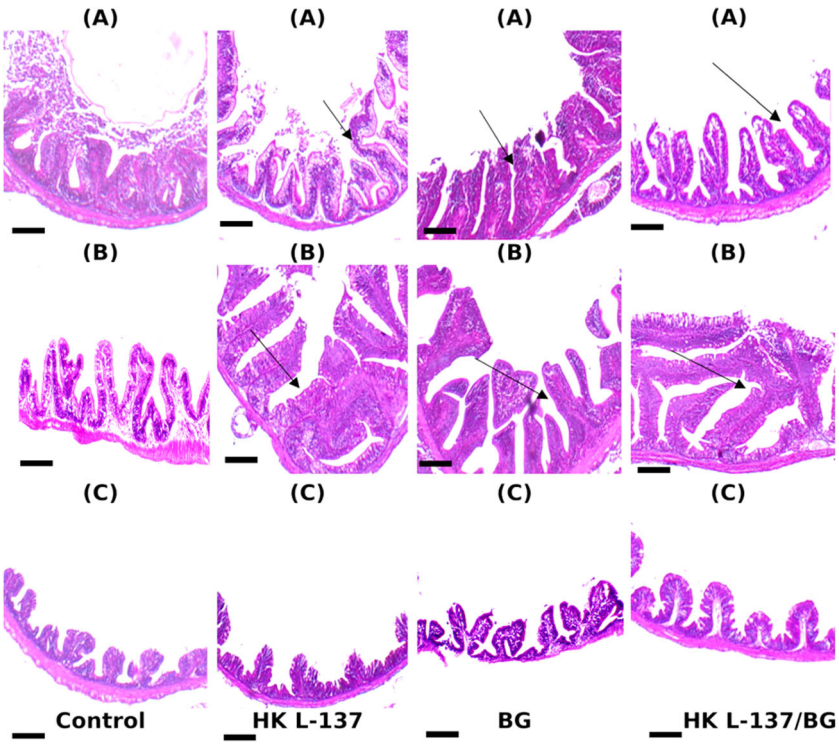

Fig. 1 Intestinal morphology ((A) anterior intestine, (B) middle intestine, and (C) posterior intestine) of fish fed control, HK L-137, BG, and HK L-137/BG diets (H&E, × 100) (scale bar = 50 μm). Fish fed the control diet showed normal thick and blunt ended anterior villi, while fish fed HK L-137 or/and BG diets showed mild increase of intestinal villi length (A). Middle intestine of fish fed with basal diet showed normal thick and blunt ended villi, while fish fed HK L-137 or/and BG diets showed clear increase of intestinal villi length and branches (B). Fish fed with basal diet showed normal thick and blunt ended villi, while fish fed HK L-137 or/and BG diets showed normal villi number and length (C)

fed HK L-137. The improved feed utilization may affect feed protein and energy to be more offered for helping in the fish growth [45]. An improvement of growth has been detected in red sea bream [17], mirror carp [24], cyprinid rohu [46], and Nile tilapia [47] fed with BG. BG can be absorbed by fish to produce energy and proteins required for the growth and development [17, 23]. Nile tilapia fed BG exhibited high feed intake and accordingly quick growth rate [47]. Probiotics could enhance feed utilization and weight gain of aquatic animals through affecting the host's appetite and feed digestion by breaking down indigestible components, increase production of vitamins, and detoxify compounds in the diet [3, 48]. Fermented prebiotics including BG can play an important role in improving the activity of intestinal microbiota [46, 49]. Further, BG has beneficial effects focused on growth performance and health, particularly prevention of the attachment

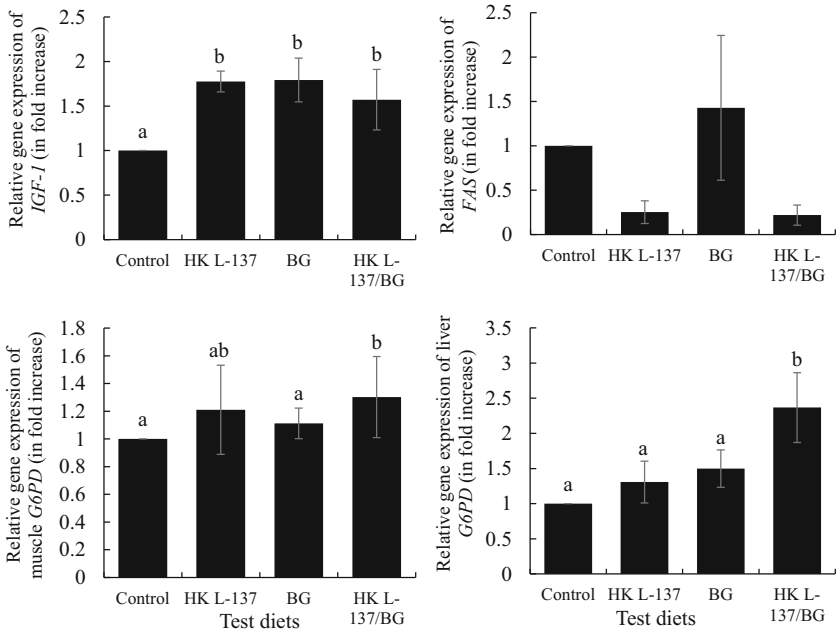

Fig. 2 Relative expression of growth-related genes in the muscle and liver tissues of fish fed test diets for a period of 12 weeks. Values are expressed as mean ± SE from triplicate groups ($n = 9$). Bars with an asterisk are significantly different from those of control group ($P < 0.05$)

Table 6 Blood hematological and biochemical parameters of fish fed test diets for 12 weeks

Item	Test diet Control	HK L-137	BG	HK L-137/BG
Hematocrit (%)	27.3 ± 2.5a	29.6 ± 2.8ab	30.1 ± 1.4b	30.7 ± 3.6b
Hemoglobin (g/dl)	7.8 ± 0.2a	9.1 ± 0.1ab	9.5 ± 0.1ab	9.9 ± 0.02b
RBCs (10^6/μl)	2.2 ± 0.2	2.6 ± 0.3	2.5 ± 0.5	2.7 ± 0.3
WBCs (10^3/μl)	64.3 ± 7.2a	91.1 ± 4.1b	77.6 ± 6.2ab	94.6 ± 4.2b
Heterotrophil (%)	24 ± 1.7	21.5 ± 2.1	20 ± 2.3	21 ± 2.5
Lymphocyte (%)	62.5 ± 4.5	63 ± 6.2	66 ± 3.8	65.5 ± 4.8
Esinophil	8 ± 1.6	8 ± 0.6	8 ± 1.2	8 ± 1.8
Basophil	5.5 ± 0.8	5.5 ± 1.3	6 ± 1.1	7 ± 1.4
Glucose (mg/dl)	186 ± 13.2b	158.4 ± 17ab	139.3 ± 21.1ab	74.5 ± 5.6a
Total cholesterol (mg/dl)	82.3 ± 2.3	93.1 ± 4.1	98.6 ± 10.3	77.7 ± 8.5
Triglyceride (mg/dl)	88.8 ± 5.4b	81.3 ± 5.5ab	81 ± 0.1ab	72.1 ± 1.4a
GOT (U/l)	55.9 ± 4	56.7 ± 1.5	48.9 ± 2	48.02 ± 1.5
GPT (U/l)	30.6 ± 0.6b	17.6 ± 0.8a	20.9 ± 1.3a	21.8 ± 2.4a

Values expressed as means ± SE ($n = 9$). Different letters indicate significant differences for each pairwise comparison between treatments

and colonization of pathogenic bacteria in the gastrointestinal tract (GIT) as well as the modulation of intestinal microbiota and the promotion of intestinal integrity in fish [9].

The effects of probiotics have been linked to modulation of gut microbiota and total digestive enzyme activity in the brush-border membrane of GIT, which increases nutrient digestibility and feed utilization and ultimately improves growth performance [50, 51]. The major digestive enzymes produced by fish are protease, lipase, and amylase, which play roles in feed digestion and assimilation [52]. If the activity of these enzymes increases, overall body metabolism may increase [24, 27]. In this study, the obtained data showed enhanced activity of lipase and protease digestive enzymes in case of feeding HK L-137 or/and BG over the control. Similarly, use of probiotics caused the increase in the protease and lipase activity regardless of its concentration in sea bream [45]. Digestive enzymes exhibited elevated activity by HK L-137 or/and BG supplementation resulting in high feed efficiency. Synbiotics mode of action depends essentially on its content, dose, and period of supplementation. However, more studies are required to find the mechanistic role in improving the feed efficiency and growth (e.g., microbiome, proteome, and transcriptome studies).

Morphometric analysis of the intestine was conducted to enable thorough evaluation of the effects of HK L-137 or/and BG at the digestive organs level which provide information about the histological condition of the intestine and consequently the growth of the fish [24, 27, 53]. Supplementation of HK L-137 or/and BG resulted in improved length of villi in the current study especially in the middle portion. Increased villi length could cause an improvement in the absorptive surface area resulting in better nutrient utilization followed by improved growth performance [24, 27]. The inclusion of probiotics and prebiotics in aquafeeds has improved feed absorbance efficiency in fish due to increasing the absorptive area, microvilli density, and height. The authors concluded that the beneficial role of HK L-137 or/and BG possibly depends on the duration of feeding, the composition, and the dose. In this study, improved growth and feed efficiency is related to increased villi length and enhanced secretion and activity of digestive enzymes.

Although there are several assumptions regarding the growth benefits triggered by beneficial bacteria, the actual molecular mechanism behind the elevated growth is unclear [2]. In addition, the relationship between the "central nervous system (CNS)" and the bacteria inhabiting the gut remains a mystery. If bacteria can control the expression of local growth factors (*IGF-1*) and the receptor (*GHR-I*) responsible for binding growth factors/hormones (growth hormones and steroid hormones), mediation of such signaling may lead to better growth [1, 39, 40]. The expression of *IGF-1* varies according to the type of tissue, feed intake, and environmental stress [40]. In this study, the results showed that *IGF-1* expression was significantly upregulated over the control in fish fed HK L-137 or/and BG, which agrees with previous studies on European sea bass or Yellow perch [54, 55], respectively. In response to growth hormone, cellular level biological responses of *IGF* signaling such as growth, proliferation, survival, immune response, and cell migration have been reported to vary. The high level of glycolysis, as indicated by increased glucose-6-phosphate dehydrogenase (*G6PD*) expression, is responsible for maintaining energy requirements needed for fish growth [1, 39, 40]. The current study revealed that *G6PD* in liver and muscle tissues of fish fed HK L-137 and BG was

significantly upregulated over the control regime. The increased expression of fatty acid synthase (*FAS*) is one of the reasons of elevated fat deposition such as triglyceride in the body [40]. In fish, expression of mRNA genes involved in triglyceride metabolism was downregulated by dietary probiotics [56]. In our study, a relatively reduction in *FAS* was observed in fish fed HK L-137 and BG. The downregulation of *FAS* might be the reason of low blood triglyceride levels in GIFT tilapia. The results also suggested that *FAS* have a positive feedback regulator on lipase activity by decreasing lipid droplet size and increasing fatty acid levels in the intestinal epithelium.

Blood hematological and biochemical markers could be utilized (as physiological biomarkers) to recognize probable enhancements in fish health condition up on supplementation of functional feed additives [57–61]. In this study, not only the growth rate was ameliorated by HK L-137 or/and BG feeding but also hematocrit, hemoglobin, and WBCs upregulated. It has been assumed that dietary probiotic can induce the blood biomarkers in fish as an indicator of improved health status. In addition, it is assumed that hemato-immunostimulating effects of HK L-137 or/and BG associated with its digested products that promote the innate immune reaction. Similarly, enhanced hematocrit by feeding HK L-137 or/and BG in red sea bream was observed [16, 17]. Supplementations of HK L-137 also improved hemoglobin as a general sign of healthy fish. Similarly, hemoglobin was increased in tilapia fed beneficial bacterial cells [62, 63]. In this study, a significant decreased glucose, triglycerides, and GPT by HK L-137 or/and BG, hypothetically signifying the influence of HK L-137 and BG in maintaining the wellbeing of GIFT tilapia. Low triglycerides in fish potentially indicate the role of HK L-137 in regulating the normal range of blood lipid derivatives of tilapia [56]. Similarly, blood lipids were influenced by probiotic supplementation in rainbow trout [64]. Blood GPT content is regularly used to assess the capacity of liver, while GPT is emitted into blood and the qualities expanded or diminished relying upon the injury of the hepatic tissue [45]. Low GPT in fish fed HK L-137 or/and BG showed that the secretion of GPT into blood was at normal level, representing enhanced health status of fish.

The reason of using functional feed additives in aquafeeds is to promote intestinal health and improve fish performance [65, 66]. The findings of this trial are in the line of previous studies that recommended the supplementation of probiotics, prebiotics, or both to improve the growth performance, feed utilization, and health status [49, 51, 67–69].

Conclusion

In conclusion, the findings of this trial have demonstrated that dietary inclusion of HK L-137 or/and BG can increase the performance of GIFT tilapia by promoting FBW, WG, SGR, FER, digestive enzyme activity, villi length, and increasing the intestinal surface area. For the first time, our results revealed an elevation in *IGF-1*, *G6PD*, and decreased *FAS* gene expression profiles. Dietary HK L-137 or/and BG also increased the hematocrit, hemoglobin, and WBCs and decreased triglyceride and glucose levels and could have significant effect on preserving a healthy fish in turn potentially enhance defense against infectious pathogens. In most of the studied parameters, fish fed both HK L-137 and BG exhibited the highest performances among the other groups which confirm the synergistic relation between both additives. This result merits further attention using mechanistic and disease challenge studies.

Acknowledgements The authors wish to thank Mr. Satoru Onoda (House Wellness Foods Corporation, Japan) for providing HK L-137 and for his input on the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Qiang J, He J, Yang H, Wang H, Kpundeh MD, Xu P, Zhu ZX (2014) Temperature modulates hepatic carbohydrate metabolic enzyme activity and gene expression in juvenile GIFT tilapia (*Oreochromis niloticus*) fed a carbohydrate-enriched diet. J Therm Biol 40:25–31. https://doi.org/10.1016/j.jtherbio.2013.12.003
2. Dawood MAO, Magouz FI, Salem MFI, Abdel-Daim HA (2019) Modulation of digestive enzyme activity, blood health, oxidative responses and growth-related gene expression in GIFT by heat-killed *Lactobacillus plantarum* (L-137). Aquaculture 505:127–136. https://doi.org/10.1016/j.aquaculture.2019.02.053
3. Dawood MAO, Koshio S, Ishikawa M, El-Sabagh M, Esteban MA, Zaineldin AI (2016) Probiotics as an environment-friendly approach to enhance red sea bream, *Pagrus major* growth, immune response and oxidative status. Fish Shellfish Immunol 57:170–178. https://doi.org/10.1016/j.fsi.2016.08.038
4. Elumalai P, Prakash P, Musthafa MS, Van Doan H, Hoseinifar SH, Faggio C (2019) Effect of alkoxy glycerol on growth performance, immune response and disease resistance in Nile Tilapia (*Oreochromis niloticus*). Res Vet Sci 123:298–304. https://doi.org/10.1016/j.rvsc.2019.01.006
5. Dawood MAO, Koshio S, Ishikawa M, Yokoyama S (2016) Effects of dietary inactivated *Pediococcus pentosaceus* on growth performance, feed utilization and blood characteristics of red sea bream, *Pagrus major* juvenile. Aquac Nutr 22(4):923–932. https://doi.org/10.1111/anu.12314
6. Adel M, Yeganeh S, Dawood MAO, Safari R, Radhakrishnan S (2017) Effects of *Pediococcus pentosaceus* supplementation on growth performance, intestinal microflora and disease resistance of white shrimp, *Litopenaeus vannamei*. Aquac Nutr 23(6):1401–1409. https://doi.org/10.1111/anu.12515
7. Hoseinifar SH, Sun Y, Wang A, Zhou Z (2018) Probiotics as means of diseases control in aquaculture, a review of current knowledge

and future perspectives. Front Microbiol 9:2429. https://doi.org/10.3389/fmicb.2018.02429

8. Hosseini M, Miandare HK, Hoseinifar SH, Yarahmadi P (2016) Dietary *Lactobacillus acidophilus* modulated skin mucus protein profile, immune and appetite genes expression in gold fish (*Carassius auratus* gibelio). Fish Shellfish Immunol 59:149–154. https://doi.org/10.1016/j.fsi.2016.10.026
9. Dawood MAO, Koshio S (2016) Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture 454:243–251. https://doi.org/10.1016/j.aquaculture.2015.12.033
10. Faggio C, Fazio F, Marafioti S, Arfuso F, Piccione G (2015) Oral administration of gum Arabic: effects on haematological parameters and oxidative stress markers in *Mugil cephalus*. Iran J Fish Sci 14(1):60–72 http://jifro.ir/article-1-1821-en.html
11. Hoseinifar SH, Yousefi S, Capillo G, Paknejad H, Khalili M, Tabarraei A, Van Doan H, Spanò N, Faggio C (2018) Mucosal immune parameters, immune and antioxidant defence related genes expression and growth performance of zebrafish (*Danio rerio*) fed on *Gracilaria gracilis* powder. Fish Shellfish Immunol 83:232–237. https://doi.org/10.1016/j.fsi.2018.09.046
12. Dawood MAO (2016) Effect of various feed additives on the performance of aquatic animals. Dissertation, Kagoshima University. Accessed December 6, 2016. http://hdl.handle.net/10232/00029468
13. Yan J, Guo C, Dawood MAO, Gao J (2017) Effects of dietary chitosan on growth, lipid metabolism, immune response and antioxidant-related gene expression in *Misgurnus anguillicaudatus*. Benefic Microbes 8(3):439–449. https://doi.org/10.3920/BM2016.0177
14. Ouwehand AC, Salminen SJ (1998) The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 8:749–756. https://doi.org/10.1016/S0958-6946(98)00114-9
15. Biswas G, Korenaga H, Nagamine R, Takayama H, Kawahara S, Takeda S, Kikuchi Y, Dashnyam B, Kono T, Sakai M et al (2013) Cytokine responses in the Japanese pufferfish (*Takifugu rubripes*) head kidney cells induced with heat-killed probiotics isolated from the Mongolian dairy products. Fish Shellfish Immunol 34:1170–1177. https://doi.org/10.1016/j.fsi.2013.01.024
16. Dawood MAO, Koshio S, Ishikawa M, Yokoyama S (2015) Effects of heat killed *Lactobacillus plantarum* (LP20) supplemental diets on growth performance, stress resistance and immune response of red sea bream, *Pagrus major*. Aquaculture 442:29–36. https://doi.org/10.1016/j.aquaculture.2015.02.005
17. Dawood MAO, Koshio S, Ishikawa M, Yokoyama S (2015) Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. Fish Shellfish Immunol 45(1):33–42. https://doi.org/10.1016/j.fsi.2015.01.033
18. Dawood MAO, Eweedah NM, Moustafa EM, Shahin MG (2019, 2019) Synbiotic effects of *Aspergillus oryzae* and β-glucan on growth and oxidative and immune responses of Nile tilapia, *Oreochromis niloticus*. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12602-018-9513-9
19. Thanh Tung H, Koshio S, Ferdinand Traifalgar R, Ishikawa M, Yokoyama S (2010) Effects of dietary heat-killed *Lactobacillus plantarum* on larval and post-larval kuruma shrimp, *Marsupenaeus japonicus* bate. J World Aquacult Soc 41:16–27. https://doi.org/10.1111/j.1749-7345.2009.00329.x
20. Van Nguyen N, Onoda S, Van Khanh T, Duy Hai P, Trung NT, Hoang L, Koshio S (2019) Evaluation of dietary heat-killed *Lactobacillus plantarum* strain L-137 supplementation on growth performance, immunity and stress resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 498:371–379. https://doi.org/10.1016/j.aquaculture.2018.08.081
21. Yang H, Han Y, Ren T, Jiang Z, Wang F, Zhang Y (2016) Effects of dietary heat-killed *Lactobacillus plantarum* L-137 (HK L-137) on the growth performance, digestive enzymes and selected non-specific immune responses in sea cucumber, *Apostichopus japonicus* Selenka. Aquac Res 47(9):2814–2824. https://doi.org/10.1111/are.12731
22. Carbone D, Faggio C (2016) Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish Shellfish Immunol 54:172–178. https://doi.org/10.1016/j.fsi.2016.04.011
23. Dawood MAO, Koshio S, Ishikawa M, Yokoyama S, El Basuini MF, Hossain MS, Nhu TH, Moss AS, Dossou S, Wei H (2017) Dietary supplementation of β-glucan improves growth performance, the innate immune response and stress resistance of red sea bream, *Pagrus major*. Aquac Nutr 23(1):148–159. https://doi.org/10.1111/anu.12376
24. Kühlwein H, Merrifield DL, Rawling MD, Foey AD, Davies SJ (2014) Effects of dietary β-(1, 3) (1, 6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L.). J Anim Physiol Anim Nutr 98:279–289. https://doi.org/10.1111/jpn.12078
25. Dawood MAO, Koshio S, Abdel-Daim MM, Van Doan H (2018) Probiotic application for sustainable aquaculture. Rev Aquac. https://onlinelibrary.wiley.com/doi/abs/10.1111/raq.12272. Accessed July 9, 2018
26. Dawood MAO, Koshio S, Esteban MÁ (2018) Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev Aquac 10(4):950–974. https://doi.org/10.1111/raq.12209
27. Sewaka M, Trullas C, Chotiko A, Rodkhum C, Chansue N, Boonanuntanasarne S, Pirarat N (2019) Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis spp*.). Fish Shellfish Immunol 86:260–268. https://doi.org/10.1016/j.fsi.2018.11.026
28. Murosaki S, Yamamoto Y, Ito K, Inokuchi T, Kusaka H, Ikeda H, Yoshikai Y (1998) Heat killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice. J Allergy Clin Immunol 102(1):57–64. https://doi.org/10.1016/S0091-6749(98)70055-7
29. AOAC (2012) Official Methods of Analysis of AOAC international, 19th edn. AOAC International, Gaithersburg, Maryland www.eoma.aoac.org
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193:265–275 http://www.jbc.org/content/193/1/265.long
31. Borlongan IG (1990) Studies on the digestive lipases of milkfish, *Chanos chanos*. Aquaculture 89:315–325. https://doi.org/10.1016/0044-8486(90)90135-A
32. Jin ZL (1995) The evaluation principle and method of functional food. In: Jin ZL (ed) Beijing publishers. Beijing University Press, Beijing, pp 100–120
33. Worthington V (1993) Alpha amylase. Diabetes. In: Worthington enzyme manual: Worthington Biochemical Corporation. Freehold, New Jersey, pp 36–41
34. Gupta A, Verma G, Gupta P (2016) Growth performance, feed utilization, digestive enzyme activity, innate immunity and protection against *Vibrio harveyi* of freshwater prawn, *Macrobrachium rosenbergii* fed diets supplemented with *Bacillus coagulans*. Aquacult Int 24(5):1379–1392. https://doi.org/10.1007/s10499-016-9996-x
35. Yuan L, Wang M, Zhang X, Wang Z (2017) Effects of protease and non-starch polysaccharide enzyme on performance, digestive function, activity and gene expression of endogenous enzyme of

broilers. PLoS One 12(3):0173941. https://doi.org/10.1371/journal.pone.0173941
36. Suvarna SK, Layton C, Bancroft JD (2012) The hematoxylins and eosin. Bancroft's theory and practice of histological techniques. E-book 7th edition. Accessed October 1, 2012:173–186. https://www.elsevier.com/books/bancrofts-theory-and-practice-of-histological-techniques-e-book/suvarna/978-0-7020-5032-9
37. Brown BA (1988) Routine hematology procedures. In: Brown BA (ed) Hematology, principles and procedures. Leo and Febiger, Philadelphia, PA, pp 7–122
38. Blaxhall PC, Daisley KW (1973) Routine haematological methods for use with fish blood. J Fish Biol 5(6):771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x
39. Costa LS, Rosa PV, Fortes-Silva R, S'anchez-V'azquez FJ, L'opez-Olmeda JF (2016) Daily rhythms of the expression of genes from the somatotropic axis: the influence on tilapia (*Oreochromis niloticus*) of feeding and growth hormone administration at different times. Comp Biochem Physiol C Toxicol Pharmacol 181-182: 27–34. https://doi.org/10.1016/j.cbpc.2015.12.008
40. Tian J, Wu F, Yang CG, Jiang M, Liu W, Wen H (2015) Dietary lipid levels impact lipoprotein lipase, hormone-sensitive lipase, and fatty acid synthetase gene expression in three tissues of adult GIFT strain of Nile tilapia, *Oreochromis niloticus*. Fish Physiol Biochem 41:1–18. https://doi.org/10.1007/s10695-014-0001-1
41. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res 29(9):45–45 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC55695/
42. Elsabagh M, Mohamed R, Moustafa EM, Hamza A, Farrag F, Decamp O, Dawood MAO, Eltholth M (2018) Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*. Aquac Nutr 24(6):1613–1622. https://doi.org/10.1111/anu.12797
43. Dawood MAO, Koshio S (2018) Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. Rev Aquac 10(2):334–350. https://doi.org/10.1111/raq.12163
44. Taoka Y, Maeda H, JO J, Kim S, Park S, Yoshikawa T, Sakata T (2006) Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. Fish Sci 72:755–766. https://doi.org/10.1111/j.1444-2906.2006.01215.x
45. Zaineldin AI, Hegazi S, Koshio S, Ishikawa M, Bakr A, El-Keredy AM, Dawood MAO, Dossou S, Wang W, Yukun Z (2018) *Bacillus subtilis* as probiotic candidate for red sea bream: growth performance, oxidative status, and immune response traits. Fish Shellfish Immunol 79:303–312. https://doi.org/10.1016/j.fsi.2018.05.035
46. Misra CK, Das BK, Mukherjee SC, Pattnaik P (2006) Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture 255:82–94. https://doi.org/10.1016/j.aquaculture.2005.12.009
47. Welker TL, Lim C, Aksoy M, Klesius PH (2012) Use of diet crossover to determine the effects of β-glucan supplementation on immunity and growth of Nile tilapia, *Oreochromis niloticus*. J World Aquacult Soc 43:335–348. https://doi.org/10.1111/j.1749-7345.2012.00569.x
48. Dawood MAO, Koshio S, Ishikawa M, Yokoyama S, El Basuini MF, Hossain MS, Nhu TH, Dossou S, Moss AS (2016) Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish Shellfish Immunol 49:275–285. https://doi.org/10.1016/j.fsi.2015.12.047
49. Iswarya A, Vaseeharan B, Anjugam M, Gobi N, Divya M, Faggio C (2018) β-1, 3 glucan binding protein based selenium nanowire enhances the immune status of *Cyprinus carpio* and protection against *Aeromonas hydrophila* infection. Fish Shellfish Immunol 83:61–75. https://doi.org/10.1016/j.fsi.2018.08.057
50. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008) Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274(1):1–4. https://doi.org/10.1016/j.aquaculture.2007.11.019
51. Guardiola FA, Porcino C, Cerezuela R, Cuesta A, Faggio C, Esteban MA (2016) Impact of date palm fruits extracts and probiotic enriched diet on antioxidant status, innate immune response and immune-related gene expression of European seabass (*Dicentrarchus labrax*). Fish Shellfish Immunol 52:298–308. https://doi.org/10.1016/j.fsi.2016.03.152
52. Dawood MAO, Mohsen M, El-Dakar A, Abdelraouf E, Moustafa E, Ahmed H (2019) Effectiveness of exogenous digestive enzymes supplementation on the performance of rabbitfish (*Siganus rivulatus*). Slov Vet Res 56(22-Suppl):409–419. https://doi.org/10.26873/SVR-779-2019
53. Lauriano ER, Pergolizzi S, Capillo G, Kuciel M, Alesci A, Faggio C (2016) Immunohistochemical characterization of Toll-like receptor 2 in gut epithelial cells and macrophages of goldfish *Carassius auratus* fed with a high-cholesterol diet. Fish Shellfish Immunol 59: 250–255. https://doi.org/10.1016/j.fsi.2016.11.003
54. Carnevali O, de Vivo L, Sulpizio R, Gioacchini G, Olivotto I, Silvi S, Cresci A (2006) Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture 258:430–438. https://doi.org/10.1016/j.aquaculture.2006.04.025
55. Shaheen AA, Eissa N, Abou-El-Gheit EN, Yao H, Wang HP (2014) Effect of probiotic on growth performance and growth-regulated genes in yellow perch (*Perca flavescens*). Glob J Fish Aquac Res 1(2):1–15 https://www.cabdirect.org/cabdirect/abstract/20153094840
56. Falcinelli S, Picchietti S, Rodiles A, Cossignani L, Merrifield DL, Taddei AR, Maradonna F, Olivotto I, Gioacchini G, Carnevali O (2015) *Lactobacillus rhamnosus* lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. Sci Rep 5:9336. https://doi.org/10.1038/srep09336
57. Dossou S, Koshio S, Ishikawa M, Yokoyama S, Dawood MA, El Basuini MF, El-Hais AM, Olivier A (2018) Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, *Pagrus major*. Aquaculture 490:228–235. https://doi.org/10.1016/j.aquaculture.2018.02.010
58. Dossou S, Koshio S, Ishikawa M, Yokoyama S, Dawood MA, El Basuini MF, Olivier A, Zaineldin AI (2018) Growth performance, blood health, antioxidant status and immune response in red sea bream (*Pagrus major*) fed *Aspergillus oryzae* fermented rapeseed meal (RM-Koji). Fish Shellfish Immunol 75:253–262. https://doi.org/10.1016/j.fsi.2018.01.032
59. El Basuini MF, El-Hais AM, Dawood MAO, Abou-Zeid AE, EL-Damrawy SZ, Khalafalla MM, Koshio S, Ishikawa M, Dossou S (2016) Effect of different levels of dietary copper nanoparticles and copper sulfate on growth performance, blood biochemical profiles, antioxidant status and immune response of red sea bream (*Pagrus major*). Aquaculture 455:32–40. https://doi.org/10.1016/j.aquaculture.2016.01.007
60. El Basuini MF, El-Hais AM, Dawood MAO, Abou-Zeid AS, EL-Damrawy SZ, Khalafalla MS, Koshio S, Ishikawa M, Dossou S (2017) Effects of dietary copper nanoparticles and vitamin C supplementations on growth performance, immune response and stress resistance of red sea bream, *Pagrus major*. Aquac Nutr 23(6):1329–1340. https://doi.org/10.1111/anu.12508
61. Abdel-Daim MM, Eissa IA, Abdeen A, Abdel-Latif HM, Ismail M, Dawood MAO, Hassan AM (2019) Lycopene and resveratrol ameliorate zinc oxide nanoparticles-induced oxidative stress in Nile tilapia, *Oreochromis niloticus*. Environ Toxicol Pharmacol 69:44–50. https://doi.org/10.1016/j.etap.2019.03.016

62. Firouzbakhsh F, Noori F, Khalesi MK, Jani-Khalili K (2011) Effects of a probiotic, protexin, on the growth performance and hematological parameters in the Oscar (*Astronotus ocellatus*) fingerlings. Fish Physiol Biochem 37:833–842. https://doi.org/10.1007/s10695-011-9481-4
63. Irianto A, Austin B (2002) Use of probiotics to control furunculosis in rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Dis 25:333–342. https://doi.org/10.1046/j.1365-2761.2002.00375.x
64. Panigrahi A, Kiron V, Satoh S, Watanabe T (2010) Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Fish Physiol Biochem 36:969–977. https://doi.org/10.1007/s10695-009-9375-x
65. Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Sharifian M, Esteban MA (2015) Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. Fish Shellfish Immunol 45(1):27–32. https://doi.org/10.1016/j.fsi.2015.03.029
66. Gobi N, Vaseeharan B, Chen JC, 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 Immunol 74:501–508. https://doi.org/10.1016/j.fsi.2017.12.066
67. Pirarat N, Pinpimai K, Endo M, Katagiri T, Ponpornpisit A, Chansue N, Maita M (2011) Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Res Vet Sci 91(3):92–97. https://doi.org/10.1016/j.rvsc.2011.02.014
68. Van Doan H, Hoseinifar SH, Dawood MAO, Chitmanat C, Tayyamath K (2017) Effects of *Cordyceps militaris* spent mushroom substrate and *Lactobacillus plantarum* on mucosal, serum immunology and growth performance of Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol 70:87–94. https://doi.org/10.1016/j.fsi.2017.09.002
69. Dawood MAO, Koshio S, Ishikawa M, Yokoyama S (2016) Immune responses and stress resistance in red sea bream, *Pagrus major*, after oral administration of heat-killed *Lactobacillus plantarum* and vitamin C. Fish Shellfish Immunol 54:266–275. https://doi.org/10.1016/j.fsi.2016.04.017