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Assessing the Influence of Dietary Supplementation of Lactobacillus acidophilus on the Growth Performance, Biochemical Parameters, Antioxidant Status, and Digestive Enzyme Activity of Whiteleg Shrimp (*P. vannamei*)

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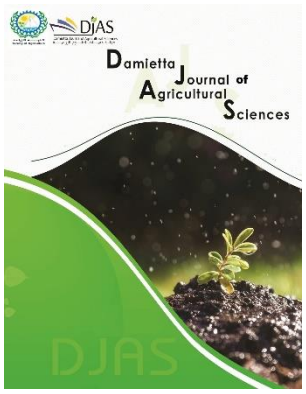

ABSTRACT

This study assesses how dietary supplementation with *Lactobacillus acidophilus* affects the growth performance, biochemical parameters, antioxidant status and digestive enzyme activity of Whiteleg Shrimp (*Panaeus. vannamei*). For 14 weeks, four shrimp groups (20 shrimp/hapa-2m³), each consisting of 240 healthy *P. vannamei*, were given balanced diets that contained 38% crude protein. They were also given supplements containing tested levels of T1, T2, T3, and T4 (0.00, 200, 400, and 600 mg *L. acidophilus*/Kg of diet), respectively. The FCR of the T2, T3, and T4 groups were significantly lower (best) than those of the control group (worst), while the levels of FBW, WG, ADG, SGR, PER and RGR were significantly greater in the *L. acidophilus* groups. When compared to the control group. Antioxidant activity data showed no changes in MDA, TAC, or CAT activities in the group that was fed diets supplemented with *L. acidophilus*, compared to the control, the T3 showed a considerable increase in the predicted digestive enzyme activity (lipase and amylase). However, there was no appreciable variation in the protease levels between the control and *P. vannamei*-treated groups. *L. acidophilus* has been found to significantly influence the production of *HSP70, IL-1β,* and *IGF-1* when included in the diets of *P. vannamei* shrimp. In summary, the results of the study show that diet supplementing with *L. acidophilus* increases *P. vannamei* growth and resistance.

Key words: Key words: dietary supplementation, L. acidophilus, growth performance, biochemical parameters, antioxidant status, digestive enzyme, whiteleg shrimp, P. vannamei

INTRODUCTION

Whiteleg shrimp (*Penaeus vannamei*) are the most farmed animal in the world in terms of the number of individuals, with an annual production of about 6.3 million tons (FAO, 2023), or between 300 and 620 billion individuals (Romero and Autric, 2022). Additionally, during the past 20 years, worldwide production quantities have increased steadily and quickly, averaging 300,000 tons annually (FAO, 2023). China, India, Vietnam, Indonesia, Ecuador, and other nations in central America and southeast Asia are the main producers **(**EUMOFA, 2023; FAO 2009**)**. China, the United States, Europe, and Japan are the primary import markets for shrimp (Globefish, 2023). Shrimp aquaculture productivity is lowered by a number of variables. Both non-pathogenic (environmental factors, nutritional deficiencies, and algal toxins) and pathogenic (bacteria, viruses, parasites, and fungi) diseases are serious problems, and outbreaks cause the largest financial losses in the sector (Toledo *et al*., 2019). Probiotics work in a number of mechanisms. In order to improve intestinal mucosal adhesion, immunological function, the strength of the epithelial shield, and the competitive elimination or decrease of pathogenic adhesions, they can produce anti-pathogenic chemicals (Bermudez-Brito *et al*., 2012). Probiotics including *Bacillus, Dunaliella, Enterobacter, Lactobacillus, Pseudomonas*, and mixed cultures have been shown to have numerous positive effects; shrimp diets have found success with them (Tepaamorndech *et al*., 2019; Interaminense *et al*., 2019; Nimrat *et al*., 2020) Lactic acid bacteria, which possess several

beneficial characteristics needed for probiotic candidates, have been shown in numerous scientific investigations to be highly promising microorganisms for use in aquaculture. *Lactobacillus, Pediococcus, Enterococcus, Streptococcus, Vagococcus, Leuconostoc, Oenococcus, Weissella, Carnobacterium*, and *Tetra genococcus* are lactic acid bacteria that are Gram-positive, gomo- and heterofermentative, optionally anaerobic, and non-sporeforming (Rine *et al*., 2019; Zheng *et al*., 2020). Lactic acid bacteria have antagonistic activity against opportunistic bacteria, viruses, and fungi that cause diseases in fish and shellfish (Doan *et al*., 2021; Ringø, 2020). They are representative of the human microbiota (Yudin *et al*., 2018) as well as the fish/mollusk microbiota (Ringø *et al*., 2018; Sergaliev *et al*., 2019) An excellent example of a probiotic that enhances growth performance, nutritional utilization, and disease control is *Lactobacillus acidophilus*, which plays a vital function as a biological disease control agent and health-promoting agent (Adeshina *et al*., 2020 and Hassan *et al*., 2021). As well, *Lactobacillus acidophilus* is a lactic acid bacterium that produces a variety of metabolic products, primarily lactic acid, which inhibits microbial growth. It also produces other extracellular products, such as hydrogen peroxide, bacteriocins, and short-chain fatty acids, which are known to obstruct the spread of microbes (Va´zquez *et al*., 2005). By assessing growth performance, feed utilization, biochemical blood parameters, digestive enzymes and gene expression, the study aimed to determine the probiotic effect of *L. acidophilus* on whiteleg shrimp (*P.vannamei*) culture and assess the health state of the species.

3. MATERIALS AND METHODS:

Experimental Diets and *P. vannamei* rearing:

The experimental work for this study was conducted on a private commercial farm in Shatta, Damietta City, Damietta Governorate, Egypt, in cooperation with the Animal, Poultry, and Fish Production Department of the Faculty of Agriculture at Damietta University in Egypt. 240 healthy young *P. vannamei* shrimp weighing 3.20±0.056g were used in this study. The juveniles were randomly assigned to 12 hapa, or four treatments, after being moved to the experimental enclosures (hapa) on the same farm. To lessen stress during transit, the juveniles were not fed. Each treatment consisted of three hapa (1 x 2 x 1.25 m) with 20 *P. vannamei* juveniles each. They were fed diet that contained 37% CP at a feeding rate of 5% of fish total biomass in each hapa. The *P. vannamei* were given the examined diets by hand twice a day, at 9:00 and 15:00, and the quantity of feed they ingested each day was recorded. *P. vannamei* were taken from each hapa every two weeks, weighed, and the feed volume was adjusted to account for changes in body weight throughout the study. The commercial product of Metabolic company, contained 2.5×10^{11} CFU/g of *L. acidophilus* (DSM 20079), which has been employed as a biotic-safe food additive, in four isocaloric and isonitrogenous diets.

The first group was given a commercial diet free of feed additives as a control group (T1) The other treatment groups consisted of a full diet supplemented with 200 mg of *L. acidophilus* (T2), 400 mg of *L. acidophilus* (T3), and 600 mg of *L. acidophilus* (T4) per kilogram of diet respectively. The rearing water temperature remained consistent over the course of the 14-week feeding trial, which was perfect for *P. vannamei* production. For the shrimp groups, a natural photoperiod consisting of 12 hours of light and 12 hours of darkness was maintained. The rearing water temperature was measured daily between 24.8 and 25.6 °C, the salinity between 28.62 and 29.28 ppt, the dissolved oxygen levels between 3.9 and 4.3 mg/L, and the pH between 7.61 and 7.87. The mean values for the second anticipated water quality criterion each 2 weeks were ammonia (NH_3+) from 0.055 to 0.056 mg/L, nitrite (NO_2-) from 0.047 to 0.049 mg/L, and nitrate (NO_3-) from 0.050 to 0.056 mg/L. These values are within the acceptable ranges required for *P. vannamei* shrimp growth, according Adiwidjaya *et al*. (2003).

Diet formulation and chemical analysis:

The experimental diets were prepared by thoroughly mixing the dry ingredients and adding 200 milliliters of water for each kilogram of diet. Once the mixture reached a paste consistency, it was pelleted using a laboratory pellet machine with a 1 mm diameter. mix 30 ml of Nutri-B Gel (binding agent) with 4 ml of water and the feed additive (*L. acidophilus*). Then, blend this mixture evenly with 1kg of shrimp diet. The pellets were stored in plastic bags at 4 °C until they were needed after drying at room temperature. The developed diets were designed to meet *P. vannamei* shrimp nutritional requirements as listed in Table 1 and suggested by Elkin *et al*. (2007) The chemical compositions of formulated feed, including the concentrations of moisture, dry matter, crude protein, crude fat, and ash, have been analyzed

using the AOAC (2000) standard applicable techniques.

Parameters of Feed Efficiency:

Throughout this experiment, shrimp in each treatment were regularly weighed every two weeks in order to compute growth indices and feed efficiency evaluations. They were starved for a full day prior to taking weight samples using the following formulas:

➢Weight gain (WG, g/ fish) = FW (g)-IW (g).

➢Daily weight gain (DWG, g/ fish/ day) = WG (g) /P (days) .

➢Specific growth rate (SGR, % day -1) = (Ln FW-Ln IW) /P) × 100 .

Feed conversion ratio (FCR, g / g) = TFI (g) /WG (g) .

➢Relative growth rate (RGR, %) = (WG/IW) × 100 .

➢Protein efficiency ratio (PER, g/g) = WG (g)/PI (g) .

➢Number of fish harvested per hapa / number of stocked fish per hapa ×100 is the survival rate percentage (%) per hapa.

Where: Final weight (FW) and beginning weight (IW) P: number of trial days: Total feed intake, or TFI, is determined by adding up all of the feed that each *P. vannamei* consumed during the trial period: by using the following formula to determine your protein intake: TFI × CP (dietary crude protein content).

Hemolymph Sampling Method:

Following a 14-weeks experimental investigation, all *P. vannamei* shrimp were fasted for 24 hours prior to sampling. Six shrimp were randomly selected from each treatment group for this purpose. According to Adeshina *et al*. (2016), 95 mg L^{-1} of clove oil (Oleum, Cairo, Egypt) was used to anesthetize each *P. vannamei* sample in three minutes. 200 µL of hemolymph was extracted separately from the ventral sinus at the base of the first abdominal segment using a 3 mL syringe that had been cleaned with cooled 5% potassium oxalate in isotonic saline anticoagulant solution (Mercier *et al*., 2006). After centrifuging at 800 g for 10 minutes at 4°C, hemolymph was separated and stored at -75°C for further examination.

Serum Biochemical Measurements:

Total protein (TP) and albumin (ALB), two components of serum proteins, were measured using a colorimetric method. Meanwhile, the globulin (GLOB) level was calculated by subtracting the ALB value from the TP. the manufacturer's protocol was followed when assessing the AST, ALT, and glucose using kits (Biodiagnostic, Giza, Egypt).

Antioxidant Activity measurements:

Superoxide dismutase (SOD), catalase, and CAT were among the enzymatic antioxidant activities of the muscle samples that were evaluated using commercial kits (Shimadzu, Kyoto, Japan) in accordance with the manufacturer's instructions. SOD activity was assessed using the auto-oxidation of pyrogallol, as described by Marklund and Marklund (1974). Claiborne (1985) explained that after the H2O2 reduction, the CAT activity was detected at 240 nm. The enzyme activity units were reported as specific activities (IU/mg protein). Malondialdehyde (MDA) was quantified using the method described by Draper and Hadley (1990) for generating TBARS by an acid-heating reaction in order to assess lipid peroxidation using MDA nmol /mg is used to represent protein equivalents.

Digestive enzymes protocol:

According to Jiang (1982) and Worthington (1993), iodine was utilized to measure amylase activity in order to detect non-hydrolyzed starch. Lipase activity was determined by measuring the fatty acids generated by the enzymatic hydrolysis of triglycerides in a stabilized emulsion of olive oil, following the procedure outlined by Borlongan (1990) and Jin (1995).

Gene Expression:

Following the dissection of the *P. vannamei* shrimp, 10 liver samples were extracted from each group and placed in sterile Eppendorf tubes to be preserved in liquid nitrogen for RNA isolation. Total RNA extraction was performed using 100 milligrams of Trizol (iNtRON Biotechnology, Seongnam-Si, Korea), as instructed by the manufacturer. Through the use of the Nanodrop (UV-Vis spectrophotometer Q5000, Quawell, San Jose, CA, USA), the extracted RNA's purity and amount were confirmed. Using a SensiFASTTM cDNA synthesis kit (Bioline, Nottingham, UK), samples with an OD260/OD280 of 1.8 were used for cDNA synthesis in accordance with the manufacturer's instructions. With b-actin acting as a housekeeping gene, the selected genes were amplified using primers specific to heat shock protein 70 (HSP70), interleukin-1 (IL-1b), and insulin-like growth factor I (IGF-I) (Table 2). Amplification was carried out using the TOP real™ preMIX SYBR Green qPCR master mix (Enzynomics, Daejeon, Korea) on a Stratagene

MX300P PCR apparatus. The data was evaluated using the 2^-ΔΔCT approach (Livak and Schmittgen, 2001).

Statistical analysis:

The homogeneity and normality of the data were evaluated using the Shapiro-Wilk test. All calculated and estimated data were also subjected to one way ANOVA statistical analysis approach, and mean differences were verified using Duncan's new multiple range test (DMRT) at ($P \leq 0.05$) was selected as the significant criterion.The data are shown as mean± SE values, and all statistical analyses were performed using the system (**SAS, 2012**).

4. RESULTS:

Shrimp fed on diet supplemented with *L. acidophilus* had significantly higher levels of FW, WG, SGR, and RGR than the control group ($p < 0.05$). The T2, T3, and T4 groups' FCR and PER were considerably lower than the control group's ($p < 0.05$). When comparing T3 to T4, the changes in FW, WG, SGR, and RGR were not statistically significant (Table, 3); T3 and T4 recorded the greatest final BW (30.73 and 30.70g), followed by T2 (27.01), while the control had the lowest final BW (22.84 g). Body weight means showed a significantly differences ($P<0.05$). *P. vannamei* daily weight gain (ADG) was impacted by L. acidophilus; T3 had the highest ADG (0.248 g), followed by T4 and T2 (0.246 and 0.208g respectively), while the control group had the lowest (0.168 g). Body weight averages showed a significantly differences ($P<0.05$). *L. acidophilus* effect on *P. vannamei* specific growth rate (SGR) and relative growth rate (RGR) was observed in T3 (2.31%/day and 861.35%), while T1 (control) showed the lowest SGR and RGR values (2.05%/day and 613.38 %), respectively. The differences between the means were significant ($P<0.05$). Furthermore, as compared to the control, the FCR values decreased dramatically with treatments T2, T3, and T4. Additionally, SR (%) was considerably higher ($P < 0.05$) in all experimental levels of treatment when compared to the T1 group; the shrimp groups in T3 had the greatest SR%.

Dietary *L. acidophilus* at varying dosages did significantly raise serum TP in comparison to the control (Table 4). Furthermore, the concentrations of ALB and GLOB were considerably raised by the presence of all meal supplements and their interaction. Meanwhile, ALT and AST levels significantly decreased in the *P. vannamei* group that was exposed to *L. acidophilus*.

Antioxidant and immunological responses:

results in Table 5 demonstrate that the dietary supplementation of *L. acidophilus* significantly ($P\leq0.05$) increased immunological and antioxidant parameters. Specifically, serum phagocytic activity, IgG, IgA, and glucose levels were all significantly ($P < 0.05$) elevated by all of the diets. The highest amounts were found in *P. vannamei* shrimp that were supplemented with T3 and T4. According to the results of the antioxidant activity, the group that was fed diets supplemented with *L. acidophilus* did not exhibit any changes in MDA, TAC, or CAT activities when compared to other *P. vannamei* shrimp groups. SOD levels, however, differed significantly ($P\leq0.05$) between the *P. vannamei* shrimp diets supplemented with fortified feed and the control group (T1).

Digestive enzyme activities:

The effects of feed additives on digestive enzyme activity (amylase, lipase, and protease) under *P. vannamei* shrimp aquaculture conditions are described in Table 6. T3 group demonstrated a significant increase ($P < 0.005$) in estimated digestive enzyme activities (lipase and amylase) in comparison to the control shrimp raised in enclosures (hapa). Nonetheless, there was no discernible difference in the levels of protease between the *P. vannamei* diets supplemented with varying concentrations of *L. acidophilus* and the control group.

Gene transcription:

The information in **Figure 1** showed how feed additives affected the transcription of the target genes for *P. vannamei*. It has been found that adding L. acidophilus to the feed diets of *P. vannamei* significantly regulates the transcription of *HSP70, IL-1β, and IGF-1*. Specifically, the *P. vannamei* group fed diets supplemented with 600 mg L. acidophilus exhibited a highly significant ($P <0.05$) increase in *HSP70, IGF-1,* and *IL-1β* in comparison to the other treatment groups (**Fig 1**)

Table 1. Formulation of feed ingredients and proximate chemical composition for the experimental diets

Ingredients	Control	Diet 1	Diet 2	Diet 3
Fishmeal (crude protein, 62%)	27	27	27	27
Corn gluten meal (crude protein, 60%)	6	6	6	6
Starch (crude protein, 2.5%)	6	5.8	5.6	5.4
Soybean meal (crude protein, 48%)	42	42	42	42
Fish oil	9.6	9.6	9.6	9.6
Soybean oil	6.4	6.4	6.4	6.4
Mineral and Vitamin premix[a]	3	3	3	3
L. acidophilus mg/kg	0	0.02	0.04	0.06
Sum	100	100	100	100
Moisture	6.75	6.72	6.77	6.74
Ash	12.42	12.4	12.45	12.41
Crude protein	37.12	37.02	37.07	37.09
Crude lipid	12.12	12.09	12.13	12.1
Fibre	6.85	6.83	6.8	6.86
NFE[b]	31.49	31.66	31.55	31.54
Gross energy, MJ/kg[c]	453.69	453.54	453.74	453.53

[a] Giving, per kilogram of the mixture: Vitamin E, 5.8 g; vitamin K3, 3.3 g; thiamin, 3.3 g; riboflavin, 6.6 g; pyridoxine (as pyridoxine hydrochloride), 3.3 g; niacin, 16.6 g; folic acid, 3.3 g; vitamin B12 (cyanocobalamin), 0.01 g; D-biotin, 0.1 g; vitamin c (ascorbic acid), 33.3 g; calcium pantothenate, 13.3 g; copper sulfate, 3 g; I, 0.4 g; Co, 0.3 g; Mn, 10 g; zinc oxide, 30 g; sodium selenite, 0.08 g; calcium, 0.8 g. b GE estimates were calculated by multiplying the energy content of the preceding by the protein, fat, and carbohydrate content of feed raw materials: GE is equal to 21.62 kJ/g of protein, 39.52 kJ/g of fat, and 17.2 kJ/g of carbohydrates. c NFE = 100- (CP + EE + CF + Ash).

Table 2.: Particular primer sequences and accession numbers utilized in RT-qPCR analysis.

Target gene	Forward	Target gene	Forward
β-actin	ACGGACAGGTCATCACCACG	GACGAGAAGCATTTGCGGTG	AF384096.1
IGF1	GTGCGATGCCATGCTGTATCA	AGCAGCTCAGCAGCTTTGGA	EF563837.1
HSP70	AAATGTTCTGCCGCATCATCA	CCCCAACCTTTATTGTCCAAT	EU805481.1
IL-1β	CTCGGGCTGAACAACAGCACT	CCTCCATTAACACTGCTCCAC	115592467

Table 3. Growth performance and feed utilization parameters, and mortality rate of *P. vannamei* fed diets supplemented with *L. acidophilus*

parameters	Treatments				P-Value
	T1	T2	T3	T4	
Initial weight (g)	3.20±0.056	3.30±0.043	3.19±0.06	3.31±0.046	0.52
Initial length (cm)	5.50±0.027	5.49 ±0.03	5.51±.03206	5.54±.03579	0.677
Final weight (g)	22.84±0.061[c]	27.01±0.14[b]	30.73±0.13[a]	30.70±0.19[a]	0.0001
Final length (cm)	14.70±0.07c	15.45±0.06b	16.01±0.07a	16.02±0.14a	0.0001
Weight Gain (g)	19.64±0.060c	23.70±0.14b	27.51±0.13a	27.39±0.19a	0.0001
ADG g/d	0.168±0.0006c	0.208±0.001b	0.248±0.001a	0.246±.002a	0.0001
SGR (%/d)[2]	2.05±0.003d	2.14±0.005c	2.31±0.005a	2.27±.006b	0.0001
RGR(g/g)	613.38±0.02d	717.78±0.04c	861.35±0.04a	827.23±.06b	0.0001
TFI (g fish^{-1})	27.99±0.72c	30.34±0.46b	35.34±0.24a	35.93±0.21a	0.0001
FCR (g/g)	1.43±0.037a	1.26±0.013b	1.29±0.02b	1.31±0.009b	0.0001
FE (G:F)	1.03±0.34	0.79±0.016	0.78±0.005	0.76±0.007	0.654
PER (%)	2.46±0.82	1.88±0.04	1.86±0.013	1.82±0.018	0.71
SR%	90.00±0.62d	92.50±0.74c	98.60±0.59a	95.00±0.060b	0.023

T1: CTR, *P. vannamei* group fed basal diet; T2: *P. vannamei* group fed diet supplemented with 200 mg L. acidophilus per kg; T3: *P. vannamei* group fed diet supplemented with 400mg L. acidophilus per kg; T4: *P. vannamei* group fed diet supplemented with 600mg L. acidophilus per kg; a,b,c,d: Values within the same column having different superscripts are significantly different ($P < 0.05$). Data were presented as the mean ± mean pool standard error (PSE). IW=Initial weight (g); FW= Final weight (g); WG= Weight gain (g); DG= daily gain; SGR= Specific growth rate (%); RGR = Relative growth rate (g/g); SR%= Survival rate. TF=Total consumed feed (g); FCR= Feed conversion ratio (g/g); FE= Feed efficiency; PER= Protein efficiency ratio

Table 4. Biochemical parameters of *p. vannamei* fed diets supplemented with *L. acidophilus*

parameters	Treatments				P-Value
	T1	T2	T3	T4	
TP	6.64±0.27^{b}	5.86±0.32^{b}	8.29±0.31^{a}	8.57±0.06^{a}	0.0001
ALB	3.72±0.16^{b}	3.42±0.15^{b}	5.27±0.06^{a}	5.41±0.14^{a}	0.0001
GLO	2.99±0.10^{ab}	2.44±0.18^{b}	3.02±0.24^{a}	3.16±0.08^{a}	0.061
AST	44.69±2.19^{a}	43.36±2.16^{c}	32.46±1.62^{b}	24.42±2.52^{c}	0.0001
ALT	78.66±1.36^{a}	77.93±1.03^{a}	65.91±1.54^{b}	49.39±1.14^{c}	0.0001

TP=total protein; ALB=albumin; GLOB=globulin; ALT=alanine transaminase; AST=aspartate aminotransferase.

Table 5. Immunity response and Redox status of *P. vannamei* fed diets supplemented with *L. acidophilus*

parameters	Treatments				P-Value
	T1	T2	T3	T4	
Lysozyme	9.32±1.14c	14.18±1.36b	20.56±0.98a	21.08±1.09a	0.013
Phgocytic activity	17.11±.71^{b}	16.29±3.53^{b}	22.97±2.55^{ab}	29.24±.53^{a}	0.011
IgG	1.43±0.05^{c}	1.95±0.101^{b}	2.57±0.14^{a}	2.94±0.17^{a}	0.0001
IgA	0.97±0.05^{b}	1.27±0.11^{b}	1.72±0.18^{a}	1.71±0.16^{a}	0.01
IgM	1.29±0.51c	1.42±0.38c	2.77±0.29b	3.64±0.35a	0.011
Glucose mg/dL	60.82±1.16^{b}	62.76±1.47^{b}	82.26±2.03^{a}	83.11±1.86^{a}	0.009
Redox status					
MDA	4.97±0.72	4.68±1.18	3.37±.67976	3.48±0.90	0.51
TAC	21.14±0.79	24.46±1.13	24.56±2.26	26.45±2.16	0.25
SOD	23.27±1.78^{ab}	17.91±1.85^{b}	27.64±2.18^{a}	29.18±2.27^{a}	0.017
CAT	3.80±0.67	3.6267±0.69	4.85±0.67	4.96±0.76	0.442

IgG= Immunoglobulin G; IgA= Immunoglobulin A; IgM, immunoglobulin M; MDA=malonaldehyde; TAC= Total antioxidant capacity; SOD=super oxide dismutase, CAT=catalas.

Table 6. digestive enzymes activities of *P. vannamei* fed diets supplemented with *L. acidophilus*

parameters	Treatments				P-Value
	T1	T2	T3	T4	
Amylase	243.01±12.10^{c}	298.48±23.94^{bc}	399.80±7.49^{a}	361.37±49.97^{ab}	0.021
Lipase	312.76±14.54^{b}	331.10±18.38^{b}	411.36±34.97^{a}	393.94±53.69^{b}	0.199
proteases	494.47±14.42	504.83±15.57	502.35±20.48	512.56±14.15	0.003

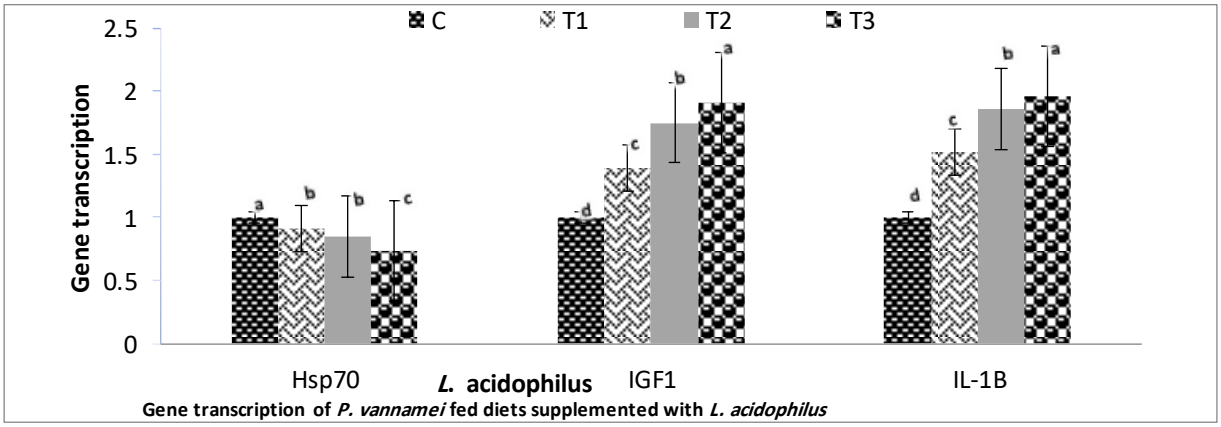

Figure 1: *HSP70* (heat shock protein 70), *IGF-1* (insulin-like growth factor-1), and *IL-1β* (interleukin-1 beta genes) are displayed in the livers of *P. vannamei* shrimp that were fed diets supplemented with varying concentrations of *L. acidophilus* for 14 weeks. Means ± S.E. showed differences between groups that were significant ($P < 0.05$), as indicated by the bars.

5.DISCUSSION

Fish health and performance have been cited to be positively impacted by dietary probiotic treatments (Hoseinifar *et al*., 2014; De *et al*., 2014). Numerous studies on various facets of probiotic administration in fish and shellfish aquaculture have been conducted in recent decades (Hoseinifar *et al*., 2014; Daniels and Hoseinifar, 2014). According to Merrifield et al. (2014), lactic acid bacteria are a type of bacteria that are frequently tested as probiotics in fish and have shown encouraging outcomes since 2007, not now. In this investigation, dietary probiotics (*L. acidophilus*) enhanced feed intake, feed utilization, and overall growth performance. Fish bioavailability and feed utilization may be linked to the noticeably improved development seen in this study. By altering the stomach and producing digestive enzymes, probiotics have been shown to improve feed digestion (Merrifield *et al*. 2010; Welker and Lim 2011). When given dietary probiotics and a lower feed conversion ratio, Nile tilapia (Abdel-Tawwab *et al*. 2008; Opiyo *et al*. 2019), common carp (Hassaan *et al*. 2018), and rainbow trout (Yanbo and Zirong 2006; Soltan *et al*. 2017) have demonstrated enhanced growth performance. The results of this study are consistent with those of Adel *et al*. (2017), who found that dietary probiotics release vitamins and amino acids to fish, promote nutrient digestion and breakdown, provoke gut microbiota, and inhibit harmful bacteria. Further research on the immune modulatory function of these different probiotics has become necessary due to the positive and improved performance of fish fed probiotics in terms of growth performance, body composition, immune response, protection against pathogens, and survival rate (Abumourand *et al*. 2013; Araujo *et al*. 2015; Adeshina *et al*. 2018a). The growth, antioxidant capability, and gene-related immunological profiles of juvenile *P. vannamei* were investigated in this work. Sizeable fish must be produced, and optimal weight should be attained early, in order to close the gap between the supply and demand of fish and crustaceans (Adeshina *et al*. 2018b). In this study, mean growth rate of *P. vannamei* fed diets supplemented with *L. acidophilus* was superior to that of the shrimp group fed a control diet. Probiotic-fortified diets improved the growth performance of *P. vannamei* (Faramarzi *et al*. 2011; Can *et al*. 2012). It is also noteworthy that the inclusion of *L. acidophilus* as a dietary supplement led to improved growth performance and feed consumption (Dimitroglou *et al*. 2010; Zhou *et al*. 2010; Abdel-Tawwab *et al*. 2018). There is a widespread belief that feed additives are the main factor in boosting the blood biochemical variables of aquatic animals (Femi-Oloye *et al*., 2020). The best dosage for raising blood protein in this case was 600 mg *L. acidophilus*/kg diet, which illustrates the advantageous function of probiotics in preserving shrimp immunity (Dawood *et al*., 2019). In terms of liver function, the breakdown of amino acids during catabolism is aided by liver enzymes, particularly AST and ALT, whose increased levels in blood serum are thought to be a good sign of liver impairment. The current study generally concurred with Adeshina *et al*. (2018b), who found that adding commercial probiotics including *B. subtilis* and *B. licheniformis* to tilapia diets at a dosage of 1 or 1.5 g /kg led to a notable reduction in liver enzyme activity. This study unequivocally demonstrated that when *L. acidophilus* levels in shrimp diets increase, particularly at a concentration of 600 mg/kg diet, the activities of ALT and AST drop. Since TG stores extra calories and provides the fish with energy, the significant increase in TG in response to the dietary addition of Lactic acid bacteria in this study is compelling evidence of the beneficial effects of probiotic supplementation on lipid metabolism (Chizhayeva et al. *et al*., 2022). In this sense, this study confirms that probiotics are crucial for enhancing fish antioxidant profiles. Reactive oxygen species (ROS) have been found to be produced by the metabolic processes of animals. Oxidative stress happens when the cells' redox capacity is unable to counteract the excessive production of ROS (Naiel *et al*., 2021). Aquatic animals redox balance and reaction to oxidative stress are commonly assessed using antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) (Naiel *et al*., 2021). SOD is the first line of defense because it converts superoxide to hydrogen peroxide and oxygen (Li *et al*., 2014). However, CAT facilitates the breakdown of hydrogen peroxide (Wang *et al*., 2017). Malondialdehyde (MDA) is thought to be a biomarker of lipid peroxidation and cell damage, in contrast to the antioxidant activity demonstrated by SOD and CAT levels (Tang *et al*., 2017). The immunological status and physiological processes in fish tissue are closely linked to the antioxidant defense system that is preserved by antioxidant state (Hoseinifar *et al*., 2020). In this study, 600 mg Lac/kg diet was the ideal dosage for *L. acidophilus*, resulting in MDA levels and an increase in total antioxidant capacity. Moreover, at 600 mg *L. acidophilus*/kg diet, SOD and CAT activities peaked. These results were consistent with those of Fath El-Bab *et al*. (2022), who discovered that, in comparison to the control group, the MDA content was lowest and the SOD and CAT levels were highest when *B. coagulans* was added to the diets of Nile tilapia. Furthermore, Yang *et al*. (2010) showed that the SOD and CAT activity of shrimp given diets treated with *Rhodosporidium paludigenum* yeast

was noticeably increased. The current findings about digestive enzymes can be explained by probiotics potential to enhance shrimp growth performance via two primary pathways. First, by simplifying nutrients so that the gut can more readily absorb them into the bloodstream. Second, probiotics have been shown to activate growth hormone (GH), which is a powerful stimulant of intestinal epithelial cells' transport routes (Yan and Charles, 2018; Petro-Sakuma *et al*., 2021). In the same sense, better feed consumption and nutrient digestibility may be linked to the growth performance. According to the findings of this study, adding *L. acidophilus* at 400 and 600 mg/kg diet may cause the release of digestive enzymes like lipase, amylase, and proteases, respectively. This could increase feed digestibility, feed efficiency, and average daily gain. The current findings were consistent with those of Zheng *et al*. (2018), who demonstrated a noteworthy rise in the hepatopancreatic activities of lipase, amylase, and pepsin in juvenile Pacific white shrimp that were given 10^9 CFU mL^{-1} of L. plantarum. According to Ibrahim *et al*. (2021), cytokines, like *IL-1β*, are essential for controlling the immunological response. Their expression can be used to predict differences in immunological response, according to a number of studies (Do Huu *et al*., 2016; Dawood *et al*., 2017; Dawood *et al*., 2020a). The interaction between intestinal epithelial cells and beneficial bacteria cells is responsible for the immunomodulatory effects of probiotics (Dou *et al*., 2023). By fortifying epithelial junctions, creating mucosal immunoglobulins, generating antimicrobial peptides, regulating inflammatory responses, and eventually boosting the immune response, this interaction may improve mucosal immunity in the gut (Ai *et al*., 2007). In contrast to other treatment groups, a 600 mg/kg diet of *L. acidophilus* considerably increased the regulation of *IL-1β* in the current investigation. Peptidoglycans and lipopolysaccharides have been shown to increase the transcription of proinflammatory cytokines (Hasan *et al*., 2019). Furthermore, zebrafish *IL-1β* is upregulated by probiotics, according to Rodríguez *et al*. (2009). Omar *et al*. (2024) gene analysis revealed that *O. niloticus* fed probiotic had higher levels of interleukin 1 beta (*IL-1B*). In sea bream raised in certain settings, elevated immunity is associated with increased transcription of *IL-Iβ*. Additionally, this gene's overexpression in response to diets supplemented with *B. coagulans* emphasizes how probiotics might lower inflammation by drawing in and activating neutrophils in afflicted areas, improving the immune system and the general health of fish (McGeachy *et al*., 2009). Fish cells normally express heat shock protein 70 (*HSP70*) in reaction to environmental stimuli (Ming *et al*., 2010). Unfavorable rearing conditions have been associated with elevated serum levels of cortisol and glucose, which may trigger glycolysis in muscle and liver tissues, generating glucose as an energy source for stressed fish (Dawood *et al*., 2020b). Furthermore, prior research has demonstrated that fish lipid metabolism can be impacted by dietary probiotic consumption (Bell *et al*., 1999). Additionally, it was discovered that *P. vannamei* fed diets containing lactic acid bacteria had decreased levels of *HSP70* transcription. Previous reports (Fu *et al*., 2019; Dou *et al*., 2023; Omar *et al*., 2024) are consistent with the current findings. This demonstrates how probiotics affect *P. vannamei* overall reaction to environmental stresses.

6.CONCLUSION

Ultimately, these results showed that lactobacillus acidophilus treatment as a probiotic may enhance *P. vannamei* shrimp performance. Also, by improving feed utilization, antioxidant capacity, and gene expression as well as by changing some related biochemical parameters, feed additive supplementation may improve fish health.

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AUTHORS CONTRIBUTION

Ahmed F. Fath El-Bab: General supervision, Conceptualization, Investigation, Methodology. Fayza A, Al-Kazzaz: Formal analysis, Investigation, Follow-up, Writing - original draft. Asem A Amer: Writing, Follow-up, Methodology, original draft. Ibrahim A. Abu El-Naser: Formal analysis, Supervision, Writing, Follow-up, Methodology, original draft.

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الملخص العربي

تقييم تأثير المكملات الغذائية من اللاكتو باسيلاس اسيدوفيلاس على أداء النمو، والمعايير الكيميائية الحيوية، وحالة مضادات الأكسدة، ونشاط إنزيمات الهضم في الجمبرى الفانمي

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1 قسم الانتاج الحيوانى و الداجني و السمكي- كلية الزراعة-جامعة دمياط

2 المعمل المركزي لبحوث الثروة السمكية - وحدة بحوث بسخا - كفر الشيخ

تمت دراسة تأثير المكملات الغذائية المحتوية على اللاكتو باسيلاس اسيدوفيلاس على أداء النمو، والمعايير الكيميائية الحيوية، وحالة مضادات الأكسدة، ونشاط الإنزيمات الهضمية لدى الجمبرى الفانمي. على مدار 14 أسبوعًا، تم تغذية أربع مجموعات من الجمبرى الفانمي (20 إصبعية / هابه بمساحة -2م³) علي وجبات غذائية متوازنة تحتوي على 38% بروتين خام. كما أُعطيت مكملات تحتوي على مستويات مُختبرة بمعدل (0.00، 200، 400، و600 ملجم من اللاكتو باسيلاس اسيدوفيلاس/كجم عليقة) على التوالي. أظهرت النتائج أن معدل التحويل الغذائي ومعدل كفاءة البروتين للمجموعات الثانية، والثالثة والرابعة أقل بشكل ملحوظ من مثيلاتها فى المجموعة الضابطة، بينما كانت مستويات وزن الجسم النهائي، الوزن المكتسب، معدل الزيادة اليومية، معدل النمو النوعى ومعدل النمو النسبى أعلى بشكل ملحوظ في مجموعات اللاكتو باسيلاس اسيدوفيلاس بالمقارنة مع المجموعة الضابطة، لم تُظهر بيانات نشاط مضادات الأكسدة أي تغيرات في أنشطة المالونديالدهيد أو أنشطة السعة الكلية لمضادات الأكسدة أو انزيم الكتاليزفي المجموعة التي تناولت علائق مُكمّلة ببكتريا اللاكتو باسيلاس اسيدوفيلاس. وبالمقارنة مع المجموعة الضابطة، أظهرت المعاملة الثالثة زيادة ملحوظة في نشاط إنزيمي الهضم المتوقع (الليبيز والأميليز). ومع ذلك، لم يكن هناك تباين ملحوظ في مستويات البروتيز بين المجموعة الضابطة والمجموعات المعاملة ببكتريا اللاكتو باسيلاس اسيدوفيلاس للجمبرى الفانمي. وقد وُجد أن بكتريا اللاكتو باسيلاس اسيدوفيلاس تؤثر بشكل كبير على إنتاج جينات بروتين الصدمة الحرارية، شبيه الانسولين وانتركولين 1 بيتا عند إضافته في علائق الجمبرى. باختصار، تُظهر نتائج الدراسة أن إضافة اللاكتو باسيلاس فى الغذاء تزيد من نمو ومقاومة الجمبرى الفانمي.