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DOSE-RESPONSE CURVES FOR THE EFFECTS OF *LACTOBACILLUS PLANTARUM* ON GROWTH PERFORMANCE, FEED UTILIZATION, AND HEALTH STATUS OF *LITOPENAEUS VANNAMEI* SHRIMP. OPTIMIZING THE ECONOMIC EFFICIENCY OF SUPPLEMENTATION

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Abstract

A 14-week feeding trial was conducted to employ polynomial regression analysis to establish the optimal dosage of *Lactobacillus plantarum* (Lac) for enhancing the growth performance, digestive enzyme activities, and blood biochemical, redox balance, and immunity response of *Litopenaeus vannamei* shrimp (initial body weight = 2.94±0.03 g). A total of 240 healthy *Litopenaeus vannamei* shrimp were randomly distributed into four equal groups and were fed diets containing 0, 200, 400 and 800 mg Lac/kg diet respectively for 98 days. Increasing the levels of dietary Lac cubically improved growth performance and feed utilization (P<0.01), the optimal doses were established at 600 and 650 mg Lac/kg diet, respectively. Muscles thickness decreased significantly in all treated group compared to the control (P<0.05). The dietary treatment quadratically affected total protein (P<0.0001), triglycerides (P<0.0001), and cortisol (P=0.0097), the optimal responses were observed at 650, 700, and 600 mg Lac/kg diet, respectively. Meanwhile the activities of liver enzymes (ALT and AST), the levels of blood urea and digestive enzymes (amylase and proteases) were cubically enhanced by the treatment, the optimal dosages were at 600 and 650 mg Lac/kg diet for liver enzymes, and urea concentration, respectively and at 650 and 700 mg Lac/kg diet for the activities of amylase and protease, respectively. With regards to redox balance, increasing the levels of Lac caused a quadratic decrease in the levels of malondialdehyde (P=0.0398) and a cubic increase in the activities of superoxide dismutase (P=0.0265), and catalase (P=0.0163), the corresponding dose–response curves showed that the optimal dose was at 650 mg/kg diet. However, the levels of total antioxidant capacity were in a quadratic increase (P=0.0372), maximizing at a level of 600 mg Lac/kg diet. Concerning the immunity response, both lysozyme and IgM were significantly affected by the dietary treatment (P=0.0002 and 0.0001, respectively), maximizing at 600 and 650 mg Lac/kg diet, respectively. Dietary supplementation of Lac had significant and substantial impacts on the economic efficiency (P<0.0001). In conclusion, the dietary inclusion of 600–700 mg Lac/kg diet can be used as an effective and practical feeding strategy to enhanced growth performance, feed efficacy, redox balance and nonspecific immune responses in *Litopenaeus vannamei* shrimp.

Key words: shrimp, *Lactobacillus plantarum*, growth performance, feed utilization, redox status, immunity

Pacific white shrimp, scientifically known as *Litopenaeus vannamei*, is the most important mariculture shrimp species worldwide due to its ability to thrive in a wide range of environmental conditions, high economic significance, and rapid growth (Wang et al., 2015). Feed cost represents a substantial portion of fish production costs (Khanjani et al., 2024). The low feed utilization in shrimp culture poses the serious economic loss worldwide (Martinez-Cordova et al., 2002). Therefore, it is imperative for both scientific researchers and aquaculture industries to prioritize enhancing the growth performance of shrimp and optimizing feed efficiency.

Probiotics are defined as living microorganisms that when consumed in appropriate quantities, confer health benefits to the host by balancing intestinal microbes (El-Raghi et al., 2024). While probiotics have been widely utilized in the cattle, poultry, and human sectors, their application in the aquaculture industry, particularly in shrimp is considered relatively new. Recently, probiotics have emerged as an ecofriendly health management strategy to improve growth performance, feed utilization, and digestibility of dietary ingredients in aquaculture as well as promote the immune response of shrimp (Hai, 2015; Yousuf et al., 2022).

Lactobacillus plantarum is a type of bacteria that is catalase-negative, gram-positive, rod-shaped, and non-spore forming. It belongs to the group of lactic acid bacteria (LAB) and is classified as a facultative anaerobic bacterium (Jeong et al., 2022). *L. plantarum* has attracted significant attention as a live diet supplement in various applications. It can effectively colonize the gut of host and enhances feed utilization by inducing growth factors

such as vitamins, fatty acids, cofactors and amino acids. Additionally, *L. plantarum* can also augment the activities of digestive enzymes in the target animal, leading to improved nutrient absorption and growth performance of the host (Duan et al., 2017; Kewcharoen and Srisapoome, 2019).

Previous studies have provided evidence of the potent effects of *L. plantarum* in various species. These studies have noted that the supplementation of *L. plantarum* can improve the growth performance and feed utilization in *Litopenaeus vannamei* (Kongnum and Hongpattarakere, 2012) and *Macrobrachium rosenbergii* (Dash et al., 2014). Additionally, it has been observed to increase the activities of digestive enzymes in *Portunus pelagicus* (Talpur et al., 2013) and enhance the immunity status, disease resistance, and survival rates in *Marsupenaeus japonicus* (Maeda et al., 2014) and *L. vannamei* (Kongnum and Hongpattarakere, 2012). Furthermore, there is some information suggesting that *L. plantarum*, even in its heat-killed form, has shown promising results in improving growth performance of teleost models such as amberjack (*Seriola dumerili*) (Dawood et al., 2015 a) and red seabream (*Pagrus major*) (Dawood et al., 2015 b, c). Likewise in crustacean models such as *M. japonicus* (Tung et al., 2010) and *M. rosenbergii* (Dash et al., 2015). Therefore, the objective of this study is to use the polynomial regression to determine the ideal dosage of *L. plantarum* to optimize the growth performance, digestive enzyme activity, and blood biochemical parameters, redox status, and immunity response of *Litopenaeus vannamei* shrimp.

Material and methods

The present study was conducted at a private farm (Shatta, Damietta Governorate, Egypt), in cooperation with Animal, Poultry, and Fish Production, Faculty of Agriculture, Damietta University. The commercial growth promoter (metabolic) was obtained from free trade Egypt Company, Egypt, containing 2×10^{-11} *Lactobacillus plantarum* CFU/g as a safe feed additive.

Experimental design and rearing conditions

A total of 240 healthy *Litopenaeus vannamei* shrimp with initial body weight = 2.94±0.03 g) were obtained from Al-Ikhlas Fish Resources Company, a private shrimp propagation center in Damietta city, Egypt. Upon their arrival, the shrimps underwent a disinfection process for 10 days using formalin at a concentration of 100 mg/L, following the husbandry conditions according to Ghaffarizadeh et al. (2022). The basal diet (control diet) was obtained from Skretting Egypt for Animal Nutrition, 10th of Ramadan, Belbies, El Sharqia 00202, Egypt.

Lactobacillus plantarum (Lac) was incorporated into the basal diet at 4 different levels: 0 (control), 200, 400, and 800 mg Lac/kg diet by thoroughly mixing the dry ingredients of each diet. Subsequently, 200 ml of water was added per kg diet, the mixture (ingredients and water) was blended to make a paste of each diet. The pastes were then pelleted using a laboratory pellet machine with a 1 mm diameter die; the resulting wet pellets were dried until the full drying at room temperature. The diets were stored in plastic bags in refrigerator (4°C) until they were ready to be used. A total of 240 healthy *Litopenaeus vannamei* shrimp were randomly distributed into twelve hapa nets (20 shrimp/hapa). The 1st group received the basal diet and served as a control while the 2nd, 3rd, and 4th groups were fed diets containing Lac at levels of 200, 400 and 800 mg/kg diet respectively. This feeding regimen was carried out for a period of 14 weeks (98 days).

Table 1. Formulation and chemical composition of the basal diet (% dry matter)

Ingredients	Tested diets[#]			
	Lac0	Lac200	Lac400	Lac800
Fishmeal (crude protein, 62%)	38	38	38	38
Starch (crude protein, 2.5%)	6	5.8	5.6	5.4
Corn gluten meal (crude protein, 60%)	5	5	5	5
Soybean meal (crude protein, 48%)	32	32	32	32
Soybean oil	6.4	6.4	6.4	6.4
Fish oil	9.6	9.6	9.6	9.6
Mineral[1] and vitamin premix[2]	3	3	3	3
Lactobacillus plantarum 2×10^{11} Cfu/g	0	0.2	0.4	0.8
Total	100	100	100	100
Moisture	7.01	7.04	6.99	7.03
Crude protein	40.55	40.63	40.58	40.52
Ash	13.75	13.79	13.81	13.73
Fiber	7.65	7.69	7.74	7.72
Crude lipid	10.03	10.05	10.02	10.07
NFE[3]	28.02	27.84	27.85	27.96
Gross energy, MJ/kg	355.3325	356.1379	355.7774	355.8287

[1]Composition of mineral premix kg^{-1}: zinc, 40 g; manganese, 53 g; 2.7 g; iodine; iron, 20 g; copper, cobalt, 70 mg, 0.34 g; selenium, 70 mg; and calcium carbonate as carrier up to 1 kg.

[2]Composition of vitamin premix kg^{-1}: vitamin D_3, 2,000,000 IU; vitamin A, 8,000,000 IU; vitamin E, 7,000 mg; vitamin B_1, 700 mg; vitamin B_2, 3,500 mg; vitamin K_3, 1,500 mg. vitamin B_6, 1,000 mg; biotin, 50 mg; vitamin B_{12}, 7 mg; pantothenic acid, 7,000 mg; folic acid, 700 mg; nicotinic acid, 20,000 mg.

[3]NFE = 1000 – (Crude Protein + Crude Fiber + Crude Lipids + ash).

[#]Lac0, Lac200, Lac400, and Lac800 indicate 0-, 200-, 400-, and 800-mg *Lactobacillus plantarum*/kg diet, respectively.

Throughout the feeding trial period, the shrimp rearing conditions and the water physiochemical features (mean ±SD) were preserved at the optimum conditions for shrimp; hapa dimensions: 1×2×1 m, photoperiod 12 h L:12 h D, water temperature (19.03±0.21°C), pH (8.03 ±0.06), salinity (28.7±0.1), NO_2 (0.058±0.001 mg/L), NO_3 (0.06±0.001 mg/L), NH_3 (0.06±0.001 mg/L), O_2

(4.76±0.15 mg/L), K (2.56±0.05 mg/L), Na (209.33±0.57 mg/L), Mg (51.43±0.25 mg/L), Ca (62.3±0.1 mg/L), SO_4 (163.33±0.57 mg/L), CL (165.66±0.57 mg/L), and HCO_3 (2.13±0.05 mg/L). Proximate analyses of the tested diets were illustrated in Table 1, and the chemical composition of formulated diet samples was estimated according to AOAC (2000) procedures.

Growth performance and feed utilization

Hand feeding was employed to prevent any food from remaining on the bottom of each hapa, any unconsumed food was promptly excluded from each hapa 30 minutes after each feeding period. Feed intake (FI) was obtained by subtracting the dry mass of the leftover diet from the total amount of feed provided. The initial and final body weights of the shrimp were recorded to determine their growth performance. Additionally, feed conversion ratio (FCR), specific growth rates (SGR), and protein efficiency ratio (PER) were calculated using the following formulas:

Weight gain (WG) = final body weight (FBW, g) - initial body weight (IBW, g)

FCR = feed intake (g)/weight gain (g)

SGR (% /day) = 100 × [ln FBW (g) - ln IBW (g)/duration (day)]

PER = [FBW− IBW]/Protein intake (PI)

Biochemical parameters in hemolymph plasma

The metabolic response of shrimp was determined by analyzing their hemolymph. Individual hemolymph samples (200 µL) were collected from the ventral sinus at the base of the first abdominal segment using a 3 mL syringe rinsed with a cooled 5% potassium oxalate in isotonic saline anticoagulant solution (Mercier et al., 2006). Then, the collected hemolymph was centrifuged for 10 minutes at 800 g at 4°C to separate the plasma, which was stored for further analysis at –75°C. Blood protein, liver enzymes (AST and ALT), urea, glucose, creatinine, cortisol, and triglyceride were evaluated using commercial kits (Biodiagnostic, Giza, Egypt) following the manufacturer's instructions. The activity of amylase was determined following the methods described by Jiang (1982) and Worthington (1993), using iodine to visualize non-hydrolyzed starch. Lipase activity was determined by measuring the fatty acids released through enzymatic hydrolysis of triglycerides in a stabilized emulsion of olive oil, according to the method outlined by Borlongan (1990) and Jin (1995).

Enzymatic antioxidant activities, including superoxide dismutase (SOD) and catalase (CAT), were evaluated in the muscle samples using commercial kits (Shimadzu, Kyoto, Japan) following the manufacturer's instructions. SOD activity was measured by assessing the auto-oxidation of pyrogallol, as outlined by Marklund and Marklund (1974). CAT activity was detected by measuring the H_2O_2 reduction at 240 nm according to Claiborne (1985). The units of these enzymes activities were obtained as specific activities (IU/mg protein). To assess lipid peroxidation, the levels of malondialdehyde (MDA) were measured using the method described by Draper and Hadley (1990). This included generating thiobarbituric acid reactive substances (TBARS) through an acid-heating reaction to evaluate lipid peroxidation. The equivalent amounts of MDA were expressed as nmol/mg protein. The levels of immunoglobulin M (IgM) and the lysozyme activity were quantified using an Elisa kit assay (MyBioSource, San Diego, CA, USA) following the instructions provided by the manufacturer.

Microscopic examination

Muscle samples from examined groups were put in 10% formalin fixative for 24 hours. Subsequently, they underwent a series of treatments including increasing alcohol concentrations, followed by xylene, and immersion in paraffin. Paraffin blocks were used to obtain 5-µm sections, which were then rehydrated and deparaffinized. The sections were stained with eosin and hematoxylin for light microscopy study of muscles (Olympus, CX30, Tokyo, Japan). Images of the muscle histology were captured using a digital camera, using software J image (type 1, 50b. US). The measurements were taken from three shrimp from each group.

Economic efficiency

Economic efficiency (EE, %) was calculated in percentage (%) by United States Dollars (USD) based on the prevailing prices of 1 kg feed and 1 kg body weight during the experimental period: Price of 1 kg/body weight on selling was 6.153 USD, 1 kg feed cost was 0.692 USD, and 1 g Lac was 0.0005 USD. The prices used were collected in Egyptian pounds from the local market, thereafter they were converted based on the official exchange rate. The following economic items were calculated:

Total feed costs = [(total feed intake / the number of shrimp) × price per kilogram of feed]

Net revenue (NE) = [price per kilogram of shrimp – total feed costs]

EE, % = [net revenue/total feed costs] × 100

Statistical analysis

The normality and homogeneity of data were assessed using Levene and Shapiro–Wilk tests. Furthermore, all estimated and computed data were statistically analysed by the general linear model of statistical analysis system (Proc GLM; SAS, 2012), and the differences between the means were determined by Tukey's range test. The results are presented as least square mean ± pooled standard error. Additionally, a polynomial regression analysis was performed to test the relationship between dietary levels of Lac (0, 200, 400 and 800 mg/kg diet) and only different significant parameters. GraphPad Prism software 9.0 (GraphPad, USA) was used to fit the dose-response curves. The tested level of significance was set at $P<0.05$.

Table 2. Concentration-dependent effects of *Lactobacillus plantarum* on growth performance and feed efficiency in *Litopenaeus vannamei* shrimp; data are expressed as least square means ±standard errors (SEM)

Items	Treatments (TRTS)				SEM	P-value			
	Lac0	Lac200	Lac400	Lac800		TRTS	*Lin.*	*Quad.*	*Cub.*
IBW (g)	2.89	2.99	3.00	2.88	0.06	0.3602	0.4236	0.8623	0.1116
FBW (g)	20.41 c	26.41 b	27.52 a	27.80 a	0.19	<.0001	<.0001	<.0001	<.0001
ADG (g)	0.17 d	0.23 c	0.25 b	0.25 a	0.01	<.0001	0.8616	<.0001	<.0001
SGR (g/day)	1.39 b	1.45 a	1.46 a	1.47 a	0.01	<.0001	<.0001	<.0001	<.0001
FCR (g feed/g gain)	1.73 a	1.58 a	1.32 b	1.36 b	0.01	<.0001	<.0001	0.1286	0.0035
PER (g/gain)	1.76 a	1.35 c	1.62 ab	1.50 b	0.01	<.0001	<.0001	<.0001	<.0001

IBW, initial body weight; FBW, final body weight; ADG, average daily gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; a, b, c – means within a row without a common letter differ at P<.05. Lac0, Lac200, Lac400, and Lac800 indicate 0-, 200-, 400-, and 800-mg *Lactobacillus plantarum*/kg diet; respectively. Lin., linear response; Quad., quadratic response; Cub., cubic response.

Results

Growth performance and feed utilization

The effects of dietary addition of *Lactobacillus plantarum* (Lac) on growth performance of *Litopenaeus vannamei* shrimp are illustrated in Table 2. The values of average daily gain (ADG) and specific growth rate (SGR) were significantly higher in Lac treated groups compared to the control (P<0.05).Regression analysis indicated the increased levels of Lac cubically affected both ADG and SGR, the optimal dose was 650 mg Lac/kg diet (Figure 1 A and B). With regard to feed efficiency, both feed conversion ratio and protein efficiency ratio were affected significantly as response to the dietary treatment (P<0.000), being in a cubic decrease with the increased levels of Lac (P=0.0035 and <0.0001, respectively), the optimal doses were established at the level 600 mg/kg diet (Figure 1 C and D).

Morphometric structures of muscles

Representative photomicrographs of shrimp muscle are presented in Figure 2. The photomicrograph of the control group showed degenerative changes characterized by swollen muscle fibers (thin arrow) and necrosis in myofibril invaded with many inflammatory cells as granular hemocytes admixed with fibroblasts (thick arrow). Minimal muscle degeneration (thin arrow) and a limited amount of edema (a star) are observed in the Lac200. However, in the Lac400 group, there was a normal histological appearance of the myofibrils. Also, the photomicrograph in the Lac800 group illustrates a normal histological appearance of the muscle, indicating no observable abnormalities or changes. Generally the muscles thickness decreased significantly in all treated group compared to the control (P<0.05)

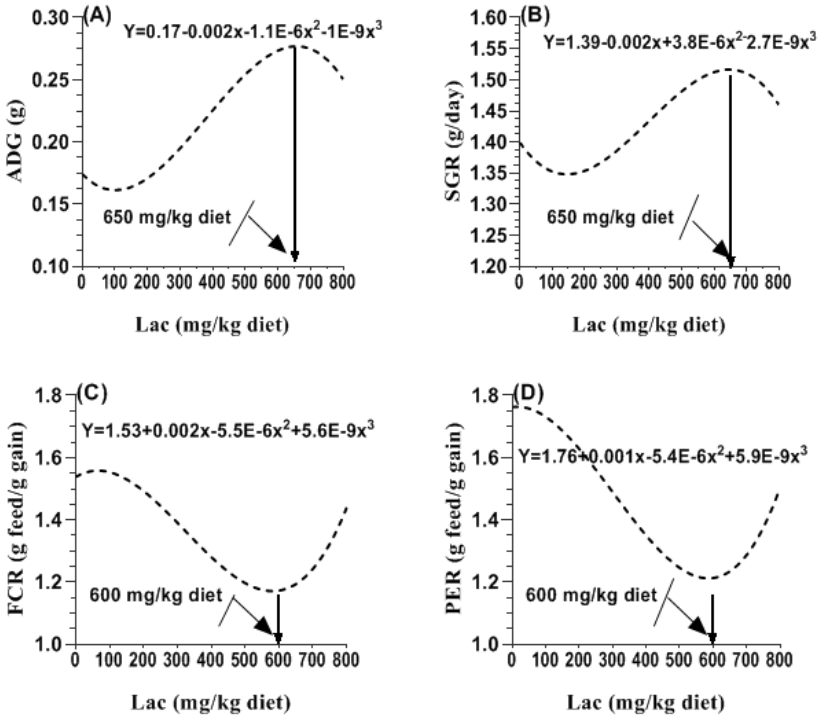

Figure 1. A polynomial regression analysis between dietary levels of *Lactobacillus plantarum* (Lac) and growth performance (average daily gain-ADG-A; specific growth rate-SGR-B), and feed efficiency (feed conversion ratio-FCR-C; protein efficiency ratio-PER-D)

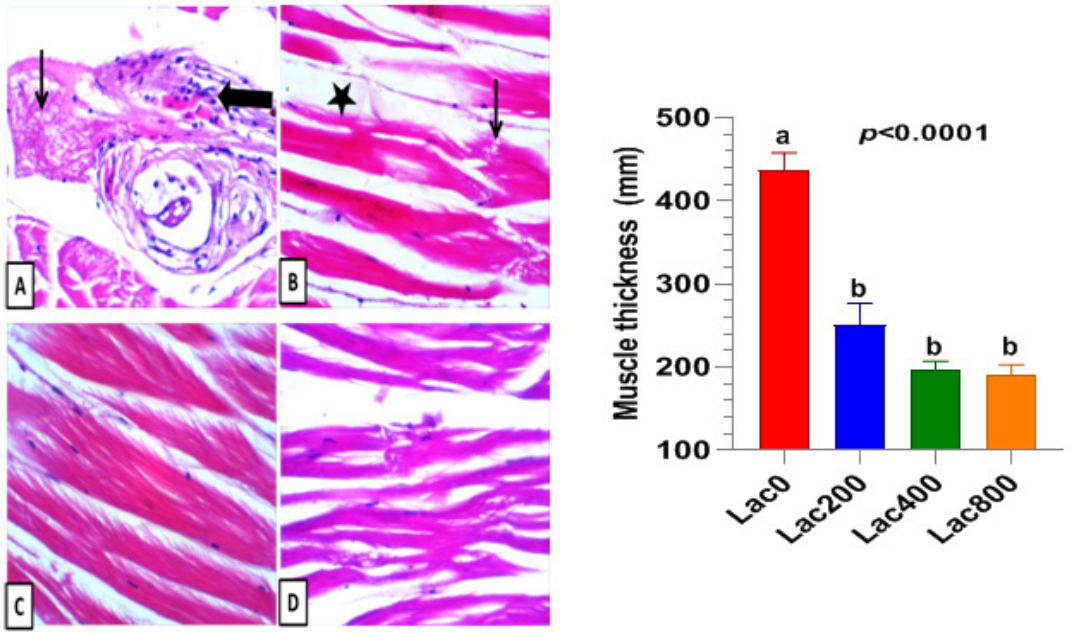

Figure 2. Histological description for transversal section photomicrograph of the muscles of *Litopenaeus vannamei* shrimp. Lac0 (A), Lac200 (B), Lac400 (C), and Lac800 (D) indicate 0-, 200-, 400-, and 800-mg *Lactobacillus plantarum*/kg diet. Image magnification=400×. Different letters indicate significant differences in muscle thickness among the treatments using one way ANOVA with Tukey adjustment

Table 3. Concentration-dependent effects of *Lactobacillus plantarum* on biochemical variables, and digestive enzymes in *Litopenaeus vannamei* shrimp; data are expressed as least square means ±standard errors (SEM)

Items	Treatments (TRTS)				SEM	P-value			
	Lac0	Lac200	Lac400	Lac800		TRTS	*Lin.*	*Quad.*	*Cub.*
TP (g/dL)	5.46 c	6.46 b	8.39 a	8.11 a	0.13	<.0001	0.4261	<.0001	0.0974
ALT (IU/L)	81.46 a	80.80 a	78.35 b	77.36 b	0.21	<.0001	<.0001	0.0011	0.0023
AST (IU/L)	50.54 a	47.43 b	47.10 b	46.89 b	0.15	<.0001	<.0001	<.0001	0.0065
Creatinine (mg/dL)	0.33	0.32	0.31	0.29	0.11	0.0662	0.0122	0.6438	0.7296
Urea (mg/dL)	1.86 a	1.77 ab	1.54 b	1.65 b	0.04	0.0107	0.5768	0.6170	0.0016
Glucose (mg/dL)	60.13	62.00	48.87	82.35	12.74	0.3716	0.3753	0.2503	0.3114
TG (mg/dL)	65.17 d	71.36 c	89.20 a	83.76 b	1.06	<.0001	<.0001	<.0001	0.9946
Cortisol (µg/dL)	76.94 a	65.20 b	58.56 c	60.26 c	1.07	0.0219	0.0682	0.0097	0.2941
Amylase (U/g)	267.30 b	270.41 b	404.79 a	415.25 a	18.60	0.0207	0.1398	0.1821	0.0075
Lipase (U/g)	312.02	330.00	409.22	392.56	34.11	0.2060	0.0686	0.6253	0.3333
Proteases (U/g)	493.16 b	496.61 b	543.13 b	619.85 a	14.88	0.0038	0.0229	0.0204	0.0045

TP, total protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglycerides; a, b, c – means within a row without a common letter differ at P<.05. Lac0, Lac200, Lac400, and Lac800 indicate 0-, 200-, 400-, and 800-mg *Lactobacillus plantarum*/kg diet; respectively. Lin., linear response; Quad., quadratic response; Cub., cubic response.

Blood biochemical

As illustrated in Table 3, the majority of blood parameters were statistically (P<0.05 or 0.0001) affected by the *Lactobacillus plantarum* (Lac) treatment. The concentration of total protein increased quadratically as response to the treatment, the optimal dose was 650 mg Lac/kg diet (P<0.0001; Figure 3 A). With regard to liver function, the activities of ALT and AST decreased significantly in all treated groups compared to the control (P<0.05), the dose response curve showed that the optimal dose was at 600 mg Lac/kg diet (Figure 3 B and C). For kidney function, the concentration of blood urea was cubically affected by the treatment and the optimal dose was established at 600 mg Lac/kg diet (P=0.0016; Figure 3 D). Non-significant differences were observed in the levels of blood glucose and creatinine between the control and all treated groups (P>0.05). Triglycerides quadratically increased as response to the Lac treatment, showing the optimal dose at 700 mg Lac/kg diet (P<0.0001; Figure 3 E). However the levels of cortisol quadratically decreased in all treated groups compared to the control, the polynomial regression analysis indicated that the optimal level was at 700 mg/kg diet (P=0.0087; Figure 3 F). Both amylase and protease concentration were cubically increased as response to the Lac treatment (P=0.0075 and 0.0045, respectively) and the optimal doses were established at the levels of 650 and 700 mg Lac/kg diet, respectively (Figure 3 G and H).

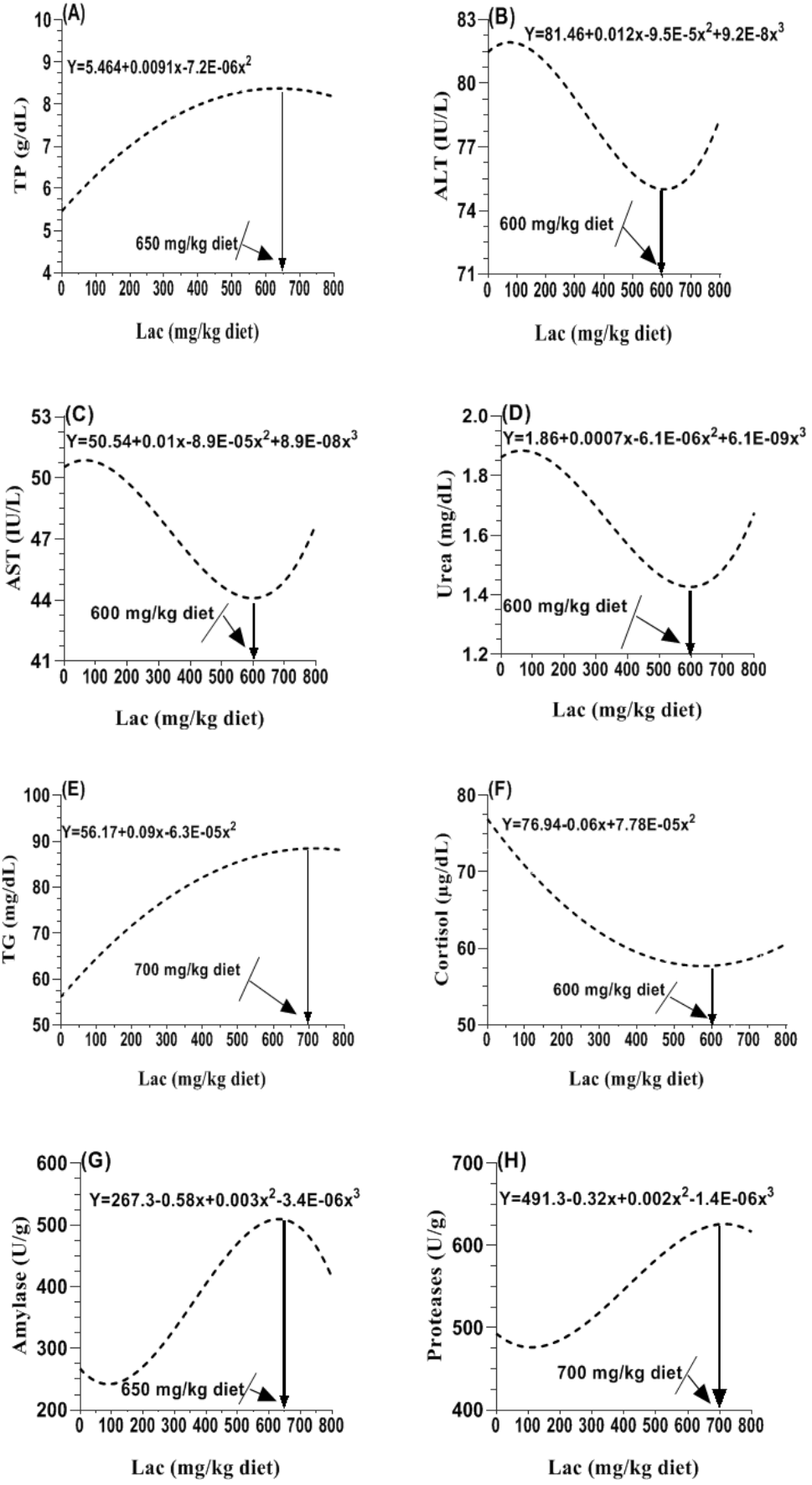

Figure 3. A polynomial regression analysis between dietary levels of *Lactobacillus plantarum* (Lac) and total protein (TP-A), alanine aminotransferase (ALT-B); aspartate aminotransferase (AST-C), triglycerides (TG-D), cortisol (E), urea (F), amylase (G), and protease (H)

Redox status and immunity response:

The effects of various levels of *Lactobacillus plantarum* (Lac) on antioxidant capacity and immunity response in *Litopenaeus vannamei* shrimp are shown in Table 4. With respect to lipid peroxidase, increasing the levels of Lac caused a quadratic decrease in the levels of MDA (P=0.0398). Corresponding dose–response curves showed that the optimal dose was at 650 mg Lac/kg diet (Figure 4 A). However, the levels of TAC were in a quadratic increase (P=0.0372), maximizing at the level of 600 mg Lac/kg diet (Figure 4 B). Regarding the antioxidant enzymes, the dietary treatment cubically affected both SOD and CAT activities (P=0.0265 and 0.0163, respectively), and the optimal Lac dose was at 650 mg Lac/kg diet (Figure 4 C and D). Concerning the immunity response, both lysozyme and IgM were significantly affected by the treatment (P=0.0002 and 0.0001, respectively). Regression analyses indicated that the optimal doses were established at 600 and 650 mg/kg diet, respectively (Figure 5 A and B).

Table 4. Concentration-dependent effects of *Lactobacillus plantarum* on redox balance, and immunity response in *Litopenaeus vannamei* shrimp; data are expressed as least square means ±standard errors (SEM)

Items	Treatments (TRTS)				SEM	P-value			
	Lac0	Lac200	Lac400	Lac800		TRTS	*Lin.*	*Quad.*	*Cub.*
Redox status									
MDA (nmol/mg)	4.397 a	3.86 a	2.70 b	2.79 b	0.02	0.0244	0.0672	0.0398	0.3816
TAC (µmol/g)	19.31 b	23.70 ab	25.75 a	23.91 ab	0.87	0.0488	0.0515	0.0372	0.5223
SOD (U/mg)	20.53 b	23.18 ab	29.92 a	30.48 a	1.91	0.0126	0.0122	0.1089	0.0265
CAT (U/mg)	2.90 b	3.11 b	4.14 a	4.27 a	0.18	0.0015	0.0005	0.3930	0.0163
Immunity response									
Lysozyme	8.12 c	15.69 b	23.93 a	21.32 a	1.26	0.0002	0.1144	<.0001	0.7488
IgM (ng/mL)	1.34 c	1.39 c	2.81 b	3.71 a	0.12	0.0001	0.5331	0.5643	<.0001

MDA, malondialdehyde; TAC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; IgM, immunoglobulin M; a, b, c – means within a row without a common letter differ at P<.05. Lac0, Lac200, Lac400, and Lac800 indicate 0-, 200-, 400-, and 800-mg *Lactobacillus plantarum*/kg diet; respectively. Lin., linear response; Quad., quadratic response; Cub., cubic response.

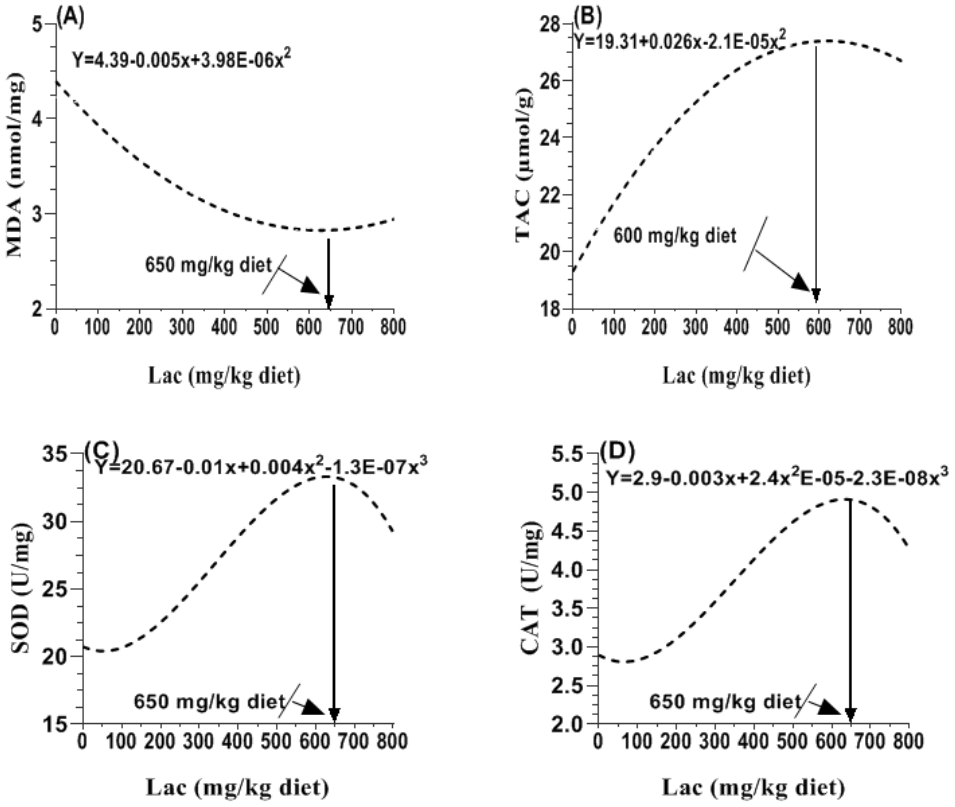

Figure 4. A polynomial regression analysis between dietary levels of *Lactobacillus plantarum* (Lac) and malondialdehyde (MDA-A), total antioxidant capacity (TAC-B); superoxide dismutase (SOD-C), and catalase (CAT-D)

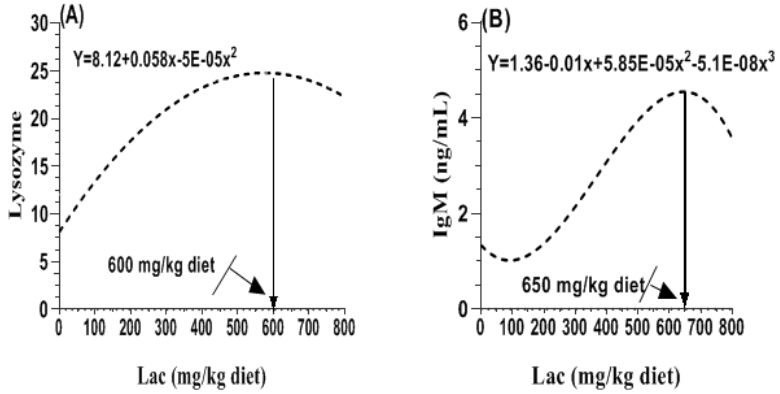

Figure 5. A polynomial regression analysis between dietary levels of *Lactobacillus plantarum* (Lac) and lysozyme (A), immunoglobulin M (IgM-B)

Table 5. Concentration-dependent effects of *Lactobacillus plantarum* on economic efficiency of *Litopenaeus vannamei* shrimp; data are expressed as least square means ± standard errors (SEM)

Items	Treatments (TRTS)				SEM	P-value
	Lac0	Lac200	Lac400	Lac800		
Live body weight (g)	20.41 b	26.41 a	27.52 a	27.80 a	0.19	<0.0001
Body weight gain (g)	17.52 b	23.42 a	24.52 a	24.92 a	0.14	<0.0001
Total revenue (USD)	0.107 b	0.144 a	0.150 a	0.153 a	0.01	<0.0001
Feed intake (g)	30.32	33.04	32.41	33.92	1.26	0.1341
Feed costs (USD)	0.020	0.023	0.022	0.023	0.00	0.0881
Lac cost (USD)	0.00	7.40E-06	3.42E-06	8.77E-06	0.00	<0.0001
Total cost (USD)	0.0209	0.026	0.022	0.023	0.00	0.4172
Net revenue (USD)	0.087 b	0.118 a	0.128 a	0.130 a	0.01	<0.0001
Economic efficiency	4.350 b	4.536 b	5.817 a	5.649 a	0.16	<0.0001
Relative economic efficiency	100	104.29	133.72	129.87		

a, b, c – means within a row without a common letter differ at P<.05. Lac0, Lac200, Lac400, and Lac800 indicate 0-, 200-, 400-, and 800-mg *Lactobacillus plantarum*/kg diet; respectively.

Economic efficiency

Results of economic efficiency are presented in Table 5. The addition of *Lactobacillus plantarum* (Lac) in shrimp diets had a significant effect on economic efficiency. As the levels of Lac in the basal diets increased, there was a pronounced increase in net revenue, economic efficiency, and relative economic efficiency, indicating a more profitable production system.

Discussion

In this study, the dietary supplementation of shrimp diets with *Lactobacillus plantarum* (Lac) significantly improved growth performance (average daily gain and specific growth rate) as well as feed efficiency (lower feed conversion ratio and protein efficiency ratio). Dose response curves indicated that the optimal doses were 650 mg Lac/kg diet for growth performance and 600 mg Lac/kg diet for feed utilization, which is in general agreement with the findings of Du et al. (2022) who observed that *Litopenaeus vannamei* shrimp that were orally administered with *L. plantarum* at a dose of 5×10^8 CFU g^{1} for 4 weeks showed significant improvements in growth performance and feed efficiency. The current study can be further reinforced by examining the morphometric structures of muscles. The muscles thickness exhibited a significant decrease in all groups that were supplemented with probiotic compared to the control (P<0.05). Interestingly, the photomicrographs revealed a normal histological appearance in the muscles of shrimp that received 400 or 800 mg Lac/kg diet, providing additional evidence to support the potential effects of probiotic supplementation on muscle integrity and structure. The present findings can be attributed to the capability of probiotics to promote shrimp growth performance through two main mechanisms. Firstly, by breaking down nutrients into simpler forms that are more easily absorbed by the gut into the bloodstream. Secondly, probiotics are known to activate growth hormone (GH) which plays a potent role in stimulating the transport pathways within the intestinal epithelial cells (Yan and Charles, 2018; Petro-Sakuma et al., 2021). In the same context, the growth performance could be related to improved nutrient digestibility and

efficient feed utilization. In the current study, the dose response curves suggested that the addition of *L. plantarum* at levels of 650 and 700 mg/kg diet can induce the secretion of digestive enzymes such as amylase and proteases, respectively, which might improve the nutrient digestibility and thus feed efficiency and average daily gain. The present results corresponded with the results of Zheng et al. (2018) who showed significant increase in the activities of amylase, lipase, and pepsin in hepatopancreas of juvenile Pacific white shrimp that received *L. plantarum* at a dose of 10^9 CFU mL^{-1}.

According to Femi-Oloye et al. (2020), there is a common assertion that feed supplements play a primary role in stimulating the blood biochemical variables of aquatic animals. Herein, the dietary addition of 650 mg Lac/kg diet was observed to be the optimal dose for increasing blood protein which reflects the beneficial role of probiotics in maintaining shrimp immunity (Dawood et al., 2019). Regarding liver function, liver enzymes, specifically AST and ALT, are involved in the breakdown of amino acids during catabolism and their elevated levels in blood serum are considered a reliable indicator of liver damage. The present study clearly confirmed a cubic decrease in the activities of ALT and AST with increasing the levels of Lac in shrimp diets, particularly at a dose of 600 mg/kg diet, indicating that the probiotic supplementation can enhance the liver function of shrimp, which was in general agreement with El-Raghi et al. (2024), who reported that the addition of commercial probiotic containing *B. subtilis* and *B. licheniformis* at a level of 1 or 1.5 g IMB/kg diets in tilapia diet resulted in a significant decrease of liver enzymes activity. Triglycerides (TG) store unused calories and provide the fish body with energy; the significant increase in TG as a response to the dietary inclusion of Lac in this study serves as compelling evidence of the beneficial impacts of probiotic supplementation on the lipid metabolism (Fath El-Bab et al., 2022).

Animals' metabolic activities have been observed to produce reactive oxygen species (ROS). When the cells' redox capacity cannot overcome the excessive generation of ROS, oxidative stress occurs (El-Raghi et al., 2023). To assess the redox balance and response to oxidative stress in aquatic animals, antioxidant enzymes such as catalase (CAT) and super oxide dismutase (SOD) are commonly used (Naiel et al., 2021). SOD acts as the primary line of defense, converting superoxide into hydrogen peroxide and oxygen (Li et al., 2014). However, CAT facilitates the breakdown of hydrogen peroxide (Wang et al., 2017). The level of malondialdehyde (MDA) is considered a biomarker of cell damage and lipid peroxidation, and it is opposite to the antioxidant activities indicated by SOD and CAT levels (Tang et al., 2017). The physiological processes in fish tissue and their immunity status are closely linked to the antioxidant defense system maintained by antioxidant status (Hoseinifar et al., 2020). In this study, increased levels of Lac resulted in a quadratic increment in total antioxidant capacity and a quadratic reduction in MDA levels, the ideal dosage was at 600 mg Lac/kg diet. Additionally, the cubic increase was observed for SOD and CAT activities, reaching their peak at 650 mg Lac/kg diet. These results were similar to the findings of Fath El-Bab et al. (2022), who reported that the addition of β-glucan and/or *B. coagulans* in Nile tilapia diets resulted in the lowest MDA content and the highest levels of SOD and CAT compared to the control group. Also, Yang et al. (2010) showed a significant increase in the activities of SOD and CAT in shrimps fed diets treated by yeast *Rhodosporidium paludigenum*. In the entire context, cortisol and uric acid in blood serum are considered good indicators of oxidative stress in animals (Eslamloo et al., 2012; Essawi et al., 2021; Yokv et al., 2007). Regression analysis indicated that there was a cubic and quadratic decrease in the levels of urea and cortisol, respectively in shrimp when fed diets with increasing levels of Lac, and the optimal dose was 600 mg Lac/kg diet, indicating a potential mitigation of oxidative stress.

Lysozyme activity has garnered considerable attention as an immunity modifier (Salaah et al., 2022). Lysozyme is an enzyme plays a crucial role in breaking down the cell walls of bacteria by enhancing the efficiency of protein or nucleic acid extraction (Whang et al., 2011). Our research findings concluded that the dietary addition of 600 mg Lac/kg diet was the optimal dosage to increase lysozyme activity in *Litopenaeus vannamei* shrimp which could potentially be attributed to the proliferation of phagocytes or heightened release from lysosomes (El-Raghi et al., 2024).

Additionally, immunoglobulin M (IgM) is the initial antibody produced by the body when it fights a new infection (Lee et al., 2022). It plays crucial roles in sustaining B cell survival and preserving lymphoid tissues architecture (Michaud et al., 2020), which was increased cubically with increasing the levels of *L. plantarum* in shrimp diets in the current study, maximizing at 650 mg Lac/kg diet. These findings provide support for the beneficial role of *L. plantarum* in activating immune responses in shrimp. Notably, probiotics exhibit several important mechanisms, including competitive exclusion of pathogenic bacteria, enhancement of epithelial barrier function, production of anti-microbial substances, inhibition of pathogen adhesion, regulation of the innate immune system, and improved adherence to the intestinal mucosa (Bermudez-Brito et al., 2012).

Finally, the increased demand for animal protein in Egypt as well as increasing challenges faced in fish farming, such as high feed prices, have prompted the use of new approaches to improve fish production. One such approach is the utilization of new substances that enhance feed utilization and maintain water quality. The incorporation of probiotics into fish diets offers the potential to reduce the reliance on antibiotics and synthetic drugs in fish diets (Amiin et al., 2023). In this study there was a significant increase in the economic efficiency of Lac supplemented groups compared to the control. The pre-

sent results corresponded with Abareethan and Amsath (2015) who indicated that the use of probiotics results in considerable decrease in feed costs of fish, which plays an important role in assessing their practicality and viability.

Conclusion

This study provides compelling evidence supporting the useful effects of including *Lactobacillus plantarum* (Lac) in shrimp diets. Fortification of basal diets with 600–700 mg Lac/kg diet demonstrated significant improvements in growth performance, feed utilization, blood biochemical, redox balance, and immune response of *Litopenaeus vannamei* shrimp. These findings indicate the potential for a more economical production system.

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Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics statement

Animal care and maintenance were conducted following the guidelines of the Egyptian Research Ethics Committee and the instructions contained in the Guide for the Care and Use of Laboratory Animals (2011).

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