Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 26 No. 4 Ser. No. 93 2025

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Effects of tamarind pulp powder supplementation on growth performance, haemato-immunological parameters, and immune-related gene expression in pacific white leg shrimp (*Litopenaeus vannamei*)

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10.22099/ijvr.2025.51831.7714

(Received 2 Dec 2024; revised version 21 Jul 2025; accepted 16 Sept 2025)

Abstract

Background: Application of dietary phytobiotics against bacterial pathogens in shrimp farming is considered a promising alternative to antibiotics. **Aims:** The present study investigated the impact of tamarind pulp-powder (TPP) supplemented diets on growth, survival, immune responses, resistance to *Vibrio harveyi*, and immune gene expression in *Litopenaeus vannamei*. **Methods:** A 49-day feeding trial was conducted using *L. vannamei* (mean weight 5.60±0.45 g) fed with TPP-supplemented diets containing 0 (T0), 1 (T1), 5 (T2), and 10 (T3) g/kg diets at 3% body weight. Growth performance viz., net weight gain (NWG) and feed conversion ratio (FCR) were recorded on weekly basis and immune responses viz., serum protein (SP), Prophenoloxidase (PPO), and lysozyme (LZ) activities were analyzed at the end. A challenged test conducted for 96 h with *V. harveyi* (1×10^5 CFU/ml) to assess mortality. After the challenge test, enzymatic activities and immune genes viz., toll-like receptor and lysozyme were analyzed. **Results:** Shrimp fed with the T2 diet (5 g/kg TPP) showed significantly ($P< 0.05$) higher NWG, improved FCR, and survival compared to other groups. Total hemocyte count, SP, PPO, and LZ activities were significantly higher in T2 shrimp. Reduced levels of enzymatic activities and upregulated expression of immune-related genes were also observed in T2. During the challenge, T2-fed shrimp achieved the highest survival rate (86%), confirming enhanced resistance to *V. harveyi*. **Conclusion:** A dietary inclusion level of 5 g/kg TPP was found optimal for improving growth, immunity, and resistance against vibriosis in *L. vannamei*, highlighting its potential as a phytobiotic alternative to antibiotics in shrimp aquaculture.

Key words: Growth parameters, immune gene expression, Immune response, *Vibrio harveyi*

Introduction

White shrimp, *Litopenaeus vannamei*, is extensively farmed across various regions globally. Its suitability stems from its rapid growth, ability to thrive in high-density environments, and resilience against diseases. It is ideal for semi-intensive and intensive cultivation methods in the grow-out phase (Yu *et al*., 2024). Shrimp farming has grappled with persistent challenges from viral and bacterial diseases such as Taura Syndrome Virus (TSV) and White Spot Syndrome Virus (WSSV), alongside bacterial infections like vibriosis triggered by *Vibrio alginolyticus* and *Vibrio harveyi* for an extended duration. The *Vibrio* genus poses significant challenges in aquaculture, contributing to early mortality syndrome (EMS) linked to *V. parahaemolyticus* (Lightner *et al*., 2012; Tran *et al*., 2013). Additionally, it's associated with conditions like white gut and white fecal syndrome, attributed to *V. parahaemolyticus*, *V. fluvialis*, *V. alginolyticus*, and *V. mimicus* (Limsuwan, 2010; Mastan, 2015). Moreover, *Vibrio* spp. have been identified as the cause of anorexia, lethargy, reduced growth rates, muscular necrosis, ultimately leading to high mortality rates (Chiu *et al*., 2007; Lafferty *et al*., 2015). Consequently, antibiotics have been in extensively used in intensive farming systems. They serve a dual purpose, functioning to not only prevent and manage infections but as growth enhancers and stabilizers of intestinal microflora (Van Hai, 2015). Yet, the build-up of unmetabolized molecules in the environment, stemming from excreted feces of aquatic animals, can foster the development of antibiotic-resistant bacteria (Dawood and Koshio, 2018). Furthermore, since the antibiotics utilized in aquatic animal farming are often the same ones employed to treat human diseases, prolonged use of these antibiotics in aquaculture might lead to potential public

health concerns. This scenario raises the risk of transferring bacterial species, which could be pathogenic to humans (Pourmozaffar *et al*., 2017). Extensive discussions have taken place regarding numerous strategies for developing antibiotic alternatives in shrimp aquaculture. Hence, researchers have heightened their endeavors to explore alternative approaches in combating bacterial infections, such as the utilization of non-nutritive feed additives, including herbal-based products known as phytobiotics (Labrador *et al*., 2016; Palanikumar *et al*., 2020). Herbal feeds like turmeric, ginger, tamarind, and garlic possess the ability to improve nutrient digestibility, promote animal growth, enhance health, and offer a diverse array of pharmacological benefits. These include regulating digestion and metabolism, bolstering antioxidant defenses, and fortifying immune functions (Citarasu, 2010; Asimi and Sahu, 2013; Van Hai, 2015; Palanikumar *et al*., 2020). Moreover, with the rising demand for environmentally friendly shrimp farming practices, there has been a notable increase in attention towards herbs and herbal feed additives (Direkbusarakom, 2004). Tamarind, derived from the tropical fruit of *Tamarindus indica*, is a member of the *Fabaceae* family, predominantly found in Asia and prized for its pulp. The tamarind fruit contains about 55% pulp, 34% seeds and 11% shell pod. Every part of *T. indica* tree serves nutritional and medicinal purposes (Kumar and Bhattacharya, 2008). The sweetly acidic taste of tamarind fruit pulp arises from its rich blend of tartaric acid and reducing sugars. Tamarind has been reported to have anti-diabetic (Koyagura *et al*., 2013), anti-inflammatory (Librandi *et al*., 2007), cholesterol-lowering (Chowdhury *et al*., 2005), anti-obesity (Khairunnuur *et al*., 2011), antifungal (Abubakar *et al*., 2010), antioxidant (Khairunnuur *et al*., 2009; Bhutkar and Bhise, 2011; Atawodi *et al*., 2014; Bhusari and Kumar, 2014) and antimicrobial (Doughari, 2006; Abukakar *et al*., 2008) properties. Previous research has identified the effect of tamarind seed meal (Nwanna *et al*., 2004; Kaewpoy *et al*., 2021), tamarind leaves (Olusola *et al*., 2020; Adeniyi *et al*., 2021) and tamarind pulp extract (Adeniyi *et al*., 2022) supplemented diets on the growth performance and immune system of fish. Additionally, tamarind anti-bacterial (Olusola *et al*., 2020; Adeniyi *et al*., 2021) activity was also reported. However, this present study is the first to determine the effectiveness of tamarind pulp-powder on *L. vannamei.* This study was conducted to analyze the growth performance of *L. vannamei*, immune response, immune gene expression and disease resistance to *V. harveyi* of *L. vannamei* fed a diet containing graded levels of tamarind pulp-powder.

Materials and Methods

Preparation of experimental diet

Dried tamarind pulp was purchased from M/s Natural Dehydrate Pvt. Ltd., Chennai, India. Commercial shrimp feed (crude protein 35%) was obtained from CP Aquaculture, India Pvt. Ltd., India. Graded levels of tamarind pulp-powder viz., 01, 05, and 10 g/kg feed were initially incorporated into egg white and homogenized using a blender. Subsequently, this mixture was meticulously blended with pellet feed to ensure uniform coating. The coated pellets were then subjected to an overnight drying process at a controlled temperature of 50ºC in a hot air oven, packed in air-tight bottles, and stored at 4ºC for further usage.

Feeding trial and experimental conditions

A total of 900 healthy juveniles of *L. vannamei* (mean weight 5±0.05 g) were procured from a shrimp farm located at Molluru village (1426°N 8010°E), Muthukur, Andhra Pradesh. The animals were acclimatized for 15 days in a reservoir tank (1000 L) and fed with a commercial diet containing 35% crude protein at 3% of body weight. Four dietary treatments viz., T0 (Control), T1 (1 g/kg of feed), T2 (5 g/kg of feed) and T3 (10 g/kg of feed) with four replicates per treatment were designed. After the acclimatization period, the shrimp with uniform size (initial mean weight 5.60±0.45 g) were randomly distributed into 16 HDPE rectangular tanks of 250 L capacity stocked with fifty numbers per tank. Juvenile shrimp were fed four times a day (7:00, 12:00, 16:00 and 21:00) at 3% of body weight.

During the experimental period, physicochemical conditions namely temperature (29-32°C); ammonia nitrogen (<0.05 mg L^{-1}); pH (8.0-8.5); and dissolved oxygen (>5 mg L^{-1}) were maintained at optimum levels (Chen, 1985).

Collection for serum samples

At the end of the 49-day experimental period, all experimental groups underwent a 24 h fasting period before the final sampling. The total number of surviving shrimp and their respective body weights per tank was assessed after shrimp were anesthetized with clove oil (50 µL L^{-1}). Thereafter, five shrimp from each experimental tank were collected for obtaining serum to analyze immune parameters. 50 µL of haemolymph sample was collected from each shrimp ventral sinus cavity by using anticoagulant-coated 1 ml syringe and immediately transferred to anticoagulant-free centrifuge tubes and kept undisturbed at 4°C. After a period of 6 h, the clotted heamolymph tubes were centrifuged at 6700 ×g for 30 min followed by a collection of the supernatant as serum and stored at -80°C until further use. Samples from the hepatopancreas and gills were obtained, from the respective treatment tanks. These samples were preserved at -80°C until subsequent analysis for immune parameters.

Proximate composition analysi

The experimental diets underwent analysis for crude protein, crude lipid, moisture, and ash content using the standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2006). Moisture

levels were determined by oven-drying at a constant temperature of 105°C. Crude protein was assessed via the Kjeldahl method, involving acid digestion using an Auto Kjeldahl System (Pro-Nitro M, Mexico). Crude lipid content was determined through a chloroform-methanol extraction method following Cejas et al. (2004) while ash content was determined using a muffle furnace set at 550°C for 8 h.

Growth performance and survival percentage

At the end of the experiment, shrimp samples were acquired for assessment of survival and growth [Survival Rate (%) = (final number of shrimp/initial number of shrimp) × 100. Specific growth rate (SGR) = 100 × [Ln (final body weight) – Ln (initial body weight)] / duration. Feed conversion ratio (FCR) = Total feed consumed (dry weight) / Net wet weight of shrimp].

Assessment of haematological and immune parameters activity

After the feed trial, 500 µL of haemolymph sample was obtained from three shrimps per tank. Extraction was performed from the base of the third pereiopod using a 2 ml syringe equipped with a 26-gauge needle containing 50 µL of an anticoagulant solution. This solution consisted of 100 mM glucose, 30 mM trisodium citrate, 26 mM citric acid, 510 mM NaCl, and 10 mM EDTA (Na_2), pH 7.3. Haematological parameters viz., Total haemocyte count (THC) and Differential haemocyte count (DHC) were observed using Rodrıguez and Le Moullac (2000) method. 150 µL of haemolymph collected per replicate of each treatment mixed with 1350 µL pre-cooled anticoagulant was transferred to a Neubauer haemocytometer and kept under Olympus light microscope (CX21i, LED) at ×400 magnification for observing total and differential haemocyte counts. Total haemocyte count (THC) was expressed as total haemocyte cells ml^{-1} haemolymph. Further, haemocytes were differentiated into granulocytes and agranulocytes (hyaline cells) based on the granular content of the cell were termed as total granulocyte cells ml-1 (TGC ml^{-1}) and total agranulocyte cells count (TAC ml^{-1}).

The total serum protein content was assessed by the method of Bradford (1976). Each tube containing 1 µL of serum was mixed with Bradford reagent, followed by incubation at room temperature for 20 min. Subsequently, the absorbance at 595 nm was measured using a spectrophotometer (T60, LABINDA).

Cheng and Chen (2000) method was used to assess prophenol oxidase (ProPO) activity. 25 µL of serum was added with 150 µL of substrate (5 mM L-3,4-dihydroxyphenylalanine), reaching a total volume of 2 ml using tris buffer (50 mM Tris-HCl; pH 75). The mixture was then incubated for 15 min at room temperature. The developed coloration was quantified by an ELISA plate reader (POWERWAVE XS, BIOTEK, USA) at 490 nm, and the optical density (OD) was assessed against the blank solution. Serum lysozyme activity was assessed by the method of Parry *et al.* (1965).

Challenge studies

On the 49th day of the growth trial, 40 shrimp per treatment and control were selected and placed into four separate 100 L tanks. Additionally, one more tank was maintained with 40 shrimp as a blank control. These shrimps were subjected to a challenge using a potent strain of *V. harveyi* obtained from diseased *L. vannamei.* The *V. harveyi* strain was cultivated in tryptic soy agar (Difco) supplemented with 15% NaCl (w/v) for 24 h at a temperature of 35°C. After this incubation period, bacterial colonies were transferred to 10 ml of tryptic soy broth (Difco) with 15% NaCl and allowed to incubate for an additional 24 h at 35°C. Subsequently, the bacterial culture underwent centrifugation at 6,700 ×g for 15 min at room temperature. The resulting supernatant was removed, and the bacterial pellet was suspended in a saline solution, achieving a concentration of 1×10^5 CFU/ml. Each shrimp from treatments and control were challenged with an injection volume of 20 µL/10 gm body weight of shrimp at second abdominal segment. Shrimp injected with a 2% saline solution of 25 µL using a 26-gauge needle fitted to a 2 ml syringe were used as the saline control group. Mortalities were carefully monitored and recorded for up to 96 h post-injection to evaluate the impact and response to the *V. harveyi* challenge.

Enzymatic activity biomarkers

The levels of hepatopancreatic enzymes viz., aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assessed in the haemolymph of treatments and control groups before and after challenge with *V. harveyi*. Following the completion of feed trial and challenge study, 500 µL samples of haemolymph were extracted from three shrimps within each tank. Collection was performed at the base of the third pereiopod using a 2 ml syringe equipped with a 26-gauge needle. This syringe contained 50 µL of an anticoagulant solution comprised of 100 mM glucose, 30 mM trisodium citrate, 26 mM citric acid, 510 mM NaCl, and 10 mM EDTA (Na^{2+}), adjusted to a pH of 7.3. The haemolymph samples underwent immediate centrifugation at 12000 ×g for 20 min. Following centrifugation, the supernatant was collected and preserved at -20°C until required for analysis. The ALT, AST, and ALP were analyzed by a spectrophotometric method using an ELISA reader (Bio Tek, USA). Specific kits for each parameter were utilized according to the manufacturer's instructions (Sigma). The activities of ALT, AST, and ALP were determined using the respective kits: ALT (Cat No MAK052), AST (Cat No MAK055), and ALP (Cat No MAK447).

Immune related gene expression

The hepatopancreas samples from three shrimp tanks were processed for total RNA extraction using the

NucleoSpin RNA Plus kit (Takara Bio, Inc, Japan). The quantity and quality of the isolated RNA were assessed using a Nano Drop 2000 spectrophotometer for quantification and a 1% agarose gel electrophoresis for quality analysis. Approximately 2 µg of each total RNA underwent reverse transcription into cDNA utilizing the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). After the treatment trial, the transcription patterns of immune genes encoding toll-like receptor (Angela *et al*., 2020) and lysozyme (Angela *et al*., 2020) were evaluated through quantitative real-time PCR (CFX96 Real-time PCR system Bio-Rad, USA). Beta-actin (internal reference housekeeping gene) is chosen for its consistent expression, which ensures accurate comparison of target gene expression (Lin and Redies, 2012). This assessment utilized published primers and their corresponding protocols given in Table 1.

Table 1: Primers used for qRT-PCR analysis of selected genes of *L. vannamei* fed with graded levels of Tamarind fruit pulp powder

Primer	Primer sequence
Lysozyme	F: 5´-CGA CCT CGA TCA GTA CAT GG-3´ R: 5´-GTA ACC CTG GTG ACA AGC CT-3´
Toll-like receptor	F: 5´-TGG TGC TTT CGT CAA ACT TC-3´ R: 5´-AAC CTG GCC ATA CAC AAT GA-3´
β-actin	F: 5´-CGC GAC CTC ACA GAC TAC CT-3´ R: 5´-CTC GTA GGA CTT CTC CAG CG-3´

Data analysis

The statistical analysis was conducted using IBM SPSS 20 software (SPSS Inc) to assess normality via the Shapiro-Wilk test Results were expressed as mean ± standard deviation (SD). Tukey's post hoc test was employed to compare the variations among the experimental groups at specific time intervals concerning the dependent variable, with a significance level set at 0.05 for multiple mean comparisons.

Results

Growth performance

Following a 49-days feeding trial, the study observed notable impacts on growth performance parameters, as outlined in Table 2. The Net Weight Gain (NWG), Specific Growth Rate (SGR), and Feed Conversion Ratio (FCR) were significantly influenced by varying levels of dietary *T. indica.* Significantly higher growth rate, survival and lower feed conversion ratio (FCR) were noted in the T2 diet fed group compared to the control (T0) group.

Proximate analysis of feed

In proximate analysis, the addition of *T. indica* in feed did not lead to significant (P>0.05) changes in moisture, crude protein, crude lipid, or ash contents as presented in Table 3.

Table 2: Growth performance of *L.vannamei* fed with graded levels of Tamarind fruit pulp powder

Treatments	T0 (Control)	T1 (1 g/kg of feed)	T2 (5 g/kg of feed)	T3 (10 g/kg of feed)
Net weight gain (g)	6.94±0.05 ^{a}	7.51±0.11 ^{b}	9.84±0.05 ^{d}	9.43±0.06 ^{c}
Specific growth rate (%)	2.39±0.05 ^{a}	2.53±0.05 ^{b}	2.77±0.05 ^{d}	2.67±0.04 ^{c}
Feed conversion ratio	1.49±0.05 ^{d}	1.37±0.03 ^{c}	1.24±0.03 ^{a}	1.39±0.03 ^{b}
Survival (%)	68.00±0.81 ^{a}	84.00±1.00 ^{b}	91.00±0.81 ^{d}	87.50±0.95 ^{c}

Values were expressed as mean ± SD of three replicate per treatment and values with different superscripts indicate significant differences in a row determined by Tukey's test (P<0.05)

Table 3: Proximate composition of experimental diets supplemented with graded levels of Tamarind fruit pulp powder

Experimental diet	Protein	Fat	Moisture	Fiber	Ash	NFE
T0 (0 tamarind)	34.57±0.42	5.23±0.04	9.07±0.05	5.54±0.32	5.32±0.23	49.35±0.52
T1 (1 g)	35.21±0.37	6.03±0.02	9.12±0.04	6.0±0.24	5.21±0.32	47.55±0.43
T2 (5 g)	35.16±0.43	6.10±0.03	9.10±0.05	6.1±0.23	5.27±0.34	47.37±0.48
T3 (10 g)	35.27±0.38	6.06±0.03	9.15±0.02	6.03±0.26	5.26±0.33	47.38±0.54

Table 4: Immune parameters of *L. vannamei* fed with graded levels of Tamarind fruit pulp powder

Immune parameters	T_0	T_1	T_2	T_3
Total haemocyte count THC (× 10^6 cells ml^{-1})	16.32±1.40 ^{a}	20.22±1.45 ^{b}	29.18±1.82 ^{d}	24.45±1.10 ^{c}
Total agranulocyte count (× 10^6 cells ml^{-1})	10.92±0.10 ^{a}	11.75±0.28 ^{b}	15.95±0.11 ^{d}	14.22±0.40 ^{c}
Total granulocyte count (× 10^6m cells ml^{-1})	5.40±0.10 ^{a}	8.47±1.15 ^{b}	13.23±0.99 ^{d}	10.23±0.64 ^{c}
Serum protein (mg/ml)	39.52±1.33 ^{a}	48.54±1.05 ^{b}	67.75±2.03 ^{d}	52.36±0.58 ^{c}
Phenoloxidase activity (OD at 490 nm)	0.72±0.03 ^{a}	0.78±0.04 ^{b}	0.88±0.01 ^{d}	0.83±0.02 ^{c}
Serum lysozyme activity (%)	60.25±0.54 ^{a}	63.19±0.51 ^{b}	70.35±0.58 ^{d}	64.23±0.60 ^{c}

Values were expressed as mean ± SD of three replicate per treatment and values with different superscripts indicate significant differences in a row determined by Tukey's test (P<0.05)

Immune parameters activity

The impact of different experimental dietary levels of *T. indica* on the immune system of *L. vannamei* was observed following a 49-day feeding trial, as depicted in Table 4. Among the treatment groups, T2 had significantly (P< 0.05) higher THC, TGC, TAC, serum protein, ProPO activity and lysozyme activity when compared with other treatments.

Challenge study with *V. harveyi* on survival of *L. vannamei*

Initially, mortality was noted in the challenged control (T0) group. After 72 h of challenge 63.5%, 89.7%, and 84.3% survival rates were observed in the T1, T2 and T3 groups respectively. Uninfected control did not depict any mortality (Fig. 1).

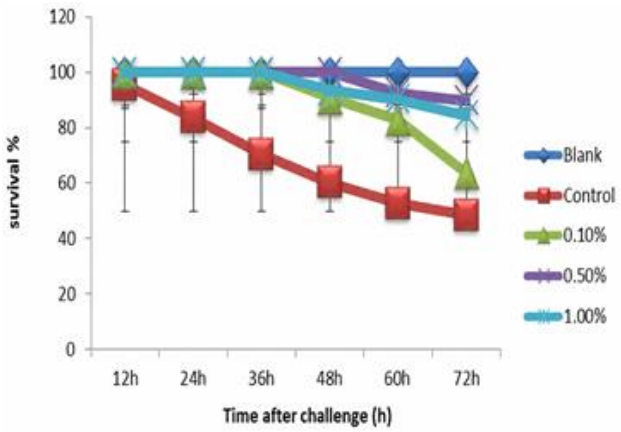

Fig. 1: Survival rate of *L. vannamei* juveniles fed on tamarind fruit pulp powder-supplemented diets upon challenged with *vibrio harveyi.* Values are given as the mean ± Standard Deviation for each treatment

Enzymatic activity biomarkers

At the end of the trial the levels of hepatopancreatic enzymes AST, ALT, and ALP were assessed in the haemolymph of uninfected controls, vibrio-infected *T. indica* treatment and control groups. Specifically, in vibrio-infected shrimps within T0, T1, T2 and T3 groups, the levels of AST, ALT, and ALP were significantly elevated (P<0.05) in the control (T0) group. However, shrimps fed with T1, T2 and T3 groups showed a significant reduction (P<005) in AST and ALT levels (Figs. 2a-c).

Immune gene expression

The gene expression of Toll-like receptor and lysozyme in *L. vannamei* gills demonstrates noteworthy changes. Specifically, in the *T. indica* supplementation treatments, there was a significant increase in the expression levels of Toll-like receptor and lysozyme compared to the control group. In T2 diet-fed shrimps the relative expression of lysozyme was 4.80-fold (P<0.05) higher than T0. The relative increase in the expression levels was 2.04 and 1.60-fold in T3 and T1 respectively. Expression of TLR showed significant (P<0.05) up-regulation with a fold change of 3.90 (P<0.05) in the T2 group during *V. harveyi* challenge, when compared to the control (Figs. 3a and b).

Discussion

Tamarindus indica leaves, fruit pulp and seeds have antioxidant, antibacterial, antifungal, antiviral and laxative properties (Bhadoriya *et al*., 2011; Gupta *et al*., 2012; Adeniyi *et al*., 2017). Tamarind pulp contains a

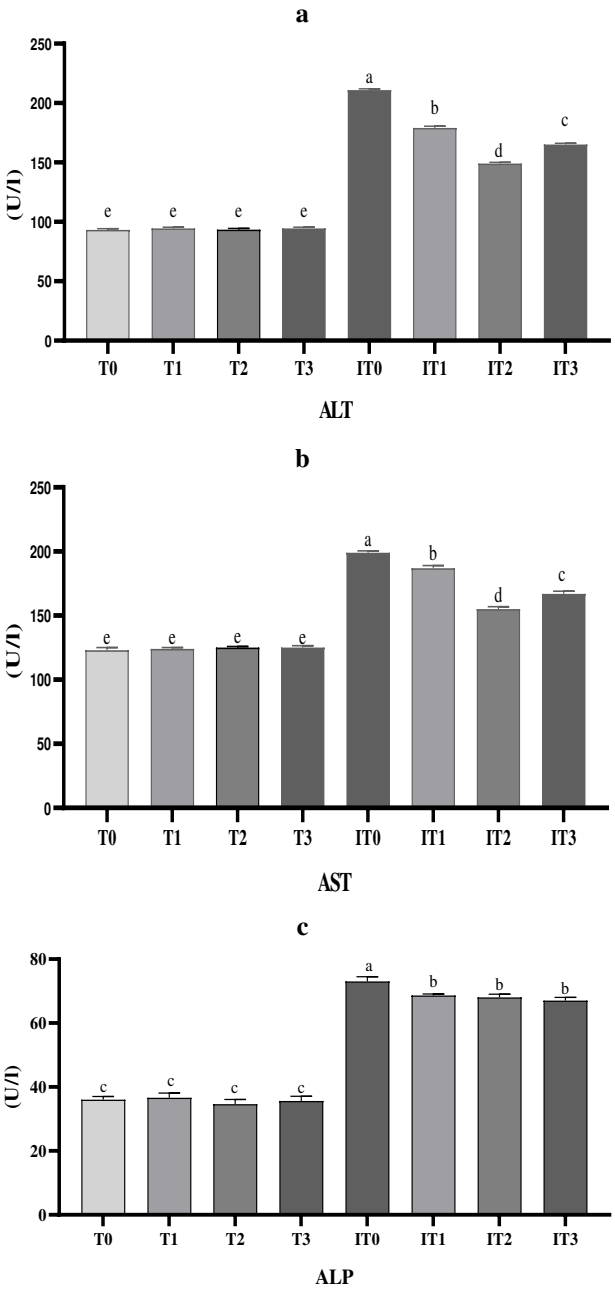

Fig. 2: (**a**) Alanine aminotransferase (ALT), (**b**) Aspartate aminotransferase (AST), and (**c**) Alkaline phosphatase (ALP) in tamarind fruit pulp powder-supplemented diets fed *L. vannamei* juveniles. Values are mean ± Standard Deviation represented by vertical error bars for each treatment. Different letters indicate significant (P<0.05) differences among treatments determined by Tukey's test

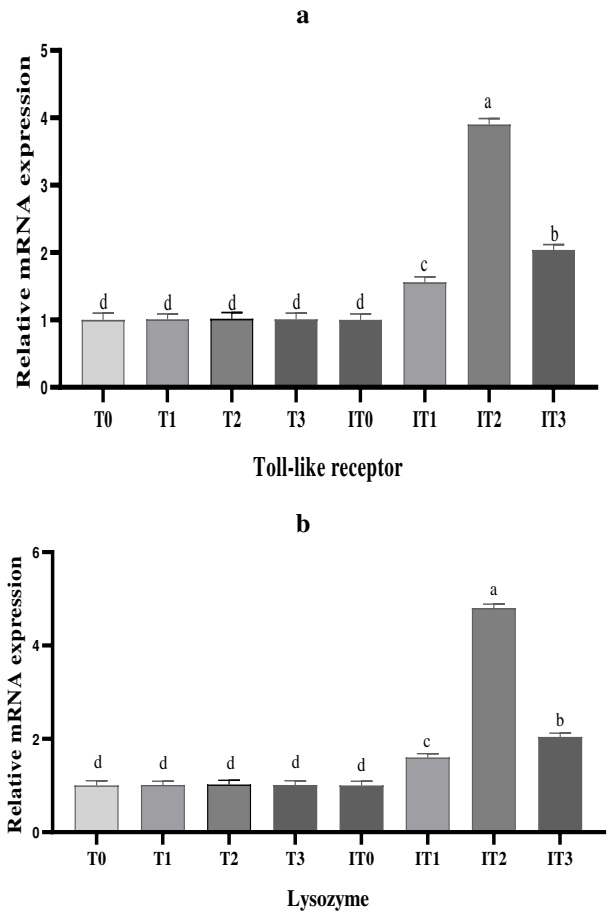

Fig. 3: (**a**) The expression ratio of Toll Like Receptor and (**b**) Lysozyme genes in tamarind fruit pulp powder-supplemented diets fed *L. vannamei* juveniles. Values are mean ± Standard Deviation represented by vertical error bars for each treatment. Different letters indicate significant (P<0.05) differences among treatments determined by Tukey's test

wide range of bioactive compounds such as polyphenols, flavonoids, organic acids, tannins, and vitamins. These compounds are believed to synergistically enhance_the growth and physiological performance of animal by modulating antioxidant capacity, digestibility, nutrient absorption, and immune function (Adeniyi *et al*., 2021). Dietary supplementation of tamarind fruit pulp and leaf meal has given promising results as antimicrobial agent against *A. hydrophila* challenged in *Clarias gariepinus* (Adeniyi *et al*., 2017). Improved growth performance, survival and reduced FCR were reported in *T. indica*-supplemented diet fed African catfish and Tilapia (Adeniyi *et al*., 2018; Adeniyi *et al*., 2021; Kaewpoy *et al*., 2021). Lin *et al*. (2015) demonstrated that the active components found in herbs, acting as growth promoters, stimulate the production of digestive enzymes. This stimulation encourages an increase in appetite, subsequently boosting both food intake and the absorption of nutrients. The phytobiotic herbs and their by-products boast a range of active components, including polysaccharides, alkaloids, flavonoids, terpenoids, and polypeptides, are crucial in bolstering aquatic animals' resistance to diseases by strengthening their natural defense mechanisms (Fuchs *et al*., 2015). Our research findings are aligning with the above studies, in enhancing growth performance, immunity and resistance against *V. harveyi* in *L. vannamei* fed tamarind pulp powder supplemented diets.

The total haemocyte counts (THC) are responsible for clotting, exoskeleton hardening, elimination of foreign materials, development of organs, reproductive status, and also responsible for molting (Song and Hsie, 1994). In the present study, shrimp fed on *T. indica* fruit pulp-powder supplemented diets showed 20-29 × 10^6 cells ml^{-1} of haemocyte as studied by AftabUddin *et al*. (2017) in *P. monodon* fed Aloe Vera, *Andro graphispariculata*, *Annona squamosa*, *Azadirachtaindica*, *Citrus aurantifolia*, *Coriandrum sativum*, *Ocimum sanctum*, *Olliumcepa*, and *Psidiumguajava*. *L. vannamei* fed *Gallachinensis*, *Terminalia chebula*, *Scutellaria baicalensis*, *Rheum officinale*, and *Phillanthus amarus* also showed increased haemocyte counts (Huang *et al*., 2020; Pan and Yan, 2020).

In crustaceans, serum proteins and enzymes confer lytic and defence properties to the shrimp. Concentrations of haemolymph metabolites such as protein, albumin, glucose, triglycerides and cholesterol reflect the nutritional status of shrimp (Gong *et al*., 2000). In the present study tamarind supplemented diets have shown significantly higher serum protein values than control as reported by Citarasu *et al*. (2006) in *P monodon* fed on *Cyanodon dactylon*, *Aegle marmelos*, *Tinospora cordifolia*, *Picrorhiza kurooa*, and *Eclipta alba* herbs.

Lysozyme level is a significant element for the nonspecific defense system whichhydrolyze β-1-4-glucosidic linkages in the mucopolysaccharide cell wall of bacterial pathogens. In the current study, the lysozyme activity of *L. vannamei* was enhanced after 49 days of feeding with tamarind fruit pulp supplemented diets. Zingerone fed *L. vannamei* and *Acalyphaindica*, *Hygrophila spinosa*, *Picrorhiza kurooa*, *Tinospora cordifolia*, and *Zingiber officinale* fed *F. indicus* showed increased lysozyme level (Chang *et al*., 2012; Rajeswari *et al*., 2012; Deng *et al*., 2015; Pan and Yan, 2020).

The ProPO system in invertebrates, acts to promote defense mechanism and it subsequently eliminates the pathogens from the animal body (Söderhäll and Cerenius, 1998). Significantly increased ProPO level was observed in the present study indicating that tamarind could enhance the non-specific immune systems in *L. vannamei.* Similar to our findings, Lawhavinit *et al*. (2011) and Huang *et al*. (2018) reported enhanced ProPO activity in turmeric powder supplemented diet and *Astragalus polysaccharides* supplemented diet fed *L. vananmei* respectively.

In the present study, *V. harveyi* challenged with *L. vannamei* revealed that the administration of tamarind incorporated diets boosted the resistance and survival in the T2 diet fed group by up to 89% as reported by Pan and Yan (2020) in *L. vannamei* fed Chinese herbs challenged with *V. harveyi* showed 90% survival rate.

The phyto-constituents of tamarind such as tamarindienal, tartaric acid (Bala, 2006; Dipali *et al*., 2010), and high flavonoids (Adeniyi *et al*., 2018; 2017) might have enhanced immunity and high level of protection against *V. harveyi* infection and higher survival rate.

AST, ALT, and ALP are crucial enzymes released by the liver to facilitate various processes such as breaking down proteins for energy, converting alanine to pyruvate for the Krebs cycle, and actively producing proteins from nucleic acid during acute energy shortage (Goltzman and Miao, 2004; Giannini *et al*., 2005). Any deviations from normal levels of these enzymes in the haemolymph could serve as a reliable indicator of potential hepatic damage and the host's energy production status (Cheng *et al*., 2001; Allameh *et al*., 2005). Therefore, the decrease in haemolymph AST and ALT levels in T2 diet-fed groups after being infected with vibrio indicated the rehabilitation of those shrimps from vibrio infection. However, there were not notable (P<005) fluctuations observed in ALP levels among the shrimps fed with *T. indica.* This suggests an enhanced energy metabolism, potentially linked to a simultaneous decrease in vibrio infection. Similarly, *L. vannamei* fed different levels of apple cider vinegar did not affect the plasma ALP activity after infection with *Vibrio* spp. (Jahromi *et al*., 2021).

Crustaceans possess an innate immune system that detects invading pathogens, initiating anadvanced defense mechanism. This defense system is intricately regulated by numerous genes (Liu *et al*., 2016). One critical aspect of this regulation involves the toll signaling pathways (Yan *et al*., 2020) which play an essential role in controlling immune-related genes. These pathways are vital for generating a variety of antibacterial peptides, effectively safeguarding against both gram-positive and gram-negative bacteria. Boosting the transcription of immune effectors might enhance the body's ability to respond to invading pathogens by improving the immune response. The study revealed that adding *T. indica* to the shrimp's diet had a significant impact on the expression of toll-like receptor mRNA in their gills. Moreover, there was a notable increase in lysozyme transcript levels in response to dietary *T. indica*, which corresponded with elevated lysozyme activities. As a result, the findings strongly suggested that incorporating *T. indica* into the diet improved the shrimp's immune response, potentially offering heightened protection against pathogen infections.

The present study concluded that *L. vannamei* that received tamarind pulp at 5 g/kg of diet showed improved growth, survival, enhanced resistance to *V. harveyi* infections, immune parameters, and immune genes expression. This might be attributed to the improving immune response of *L. vanammei*, as indicated by elevated levels of total haemocyte count, serum protein, ProPO, lysozyme activity, lysozyme and toll-like receptor gene expression. The findings suggest that tamarind pulp supplementation in the *L. vanammei* diet has the potential to act as a growth promoter and immunostimulant and enhance the resistance power against *V. harveyi*. As of our knowledge, this is the first study to investigate the effects of dietary tamarind pulp-powder supplementation on *L. vanammei*.

Acknowledgements

The authors acknowledge the gratitude to the authorities of Andhra Pradesh Fisheries University, Andhra Pradesh, India and National Surveillance Programme for Aquatic Animal Diseases (NSPAAD Phase II) Project under PMMSY scheme of Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India for financial support to carry out the research work.

Conflict of interest

The authors declare no conflict of interest.

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