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# Effects of graded dietary lipid levels on growth, feed utilization, body composition and metabolism in juvenile white leg shrimp, *Penaeus vannamei* (Boone, 1931) reared in inland saline water of 10 g/L salinity

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## Abstract

A feeding trial was performed for 60 days to determine the dietary lipid requirement of *Penaeus vannamei* juveniles reared in inland saline water (ISW) of 10 g/L salinity. Six iso-nitrogenous (360 g/kg crude protein) and heterocaloric (15.4–17.6 KJ DE/g) semi-purified diets were prepared with graded level of lipid viz., 20, 40, 60, 80, 100 and 120 g/kg (L2–L12 with 20 g/kg incremental level). Two hundred and seventy *P. vannamei* juveniles (5.51 ± 0.01 g) were randomly allocated (15 shrimps/200 L) into six treatment groups in triplicate following a completely randomized design. The shrimp of L4 and L6 groups exhibited significantly higher per cent weight gain (WG%), specific growth rate and protein efficiency ratio and the lowest feed conversion ratio. The lipid efficiency ratio and hepatopancreatic somatic index were significantly decreased and increased with increasing levels of lipid respectively. The L6, L8 and L10 groups exhibited significantly higher ($p < .05$) lipase activity. Shrimp of higher dietary lipid fed groups exhibited significantly higher ($p < .05$) aminotransferase activity than their lower dietary lipid fed counterparts. Based on WG% data, broken-line linear and second-order polynomial regression analysis showed that the optimal dietary lipid requirement of *P. vannamei* in ISW of 10 g/L salinity was 46.5 and 51.4 g/kg respectively.

**KEYWORDS**
dietary lipid, growth, inland saline water, metabolism, nutrient utilization, *Penaeus vannamei*

## 1 | INTRODUCTION

In order to achieve global food and nutrition security, sustainable aquaculture has drawn closer attention. Moreover, more than 3.3 billion people globally rely on fish as a primary source of animal protein and the demand is increasing day by day (FAO, 2020). Hence, the expansion of aquaculture production is a necessity to satisfy the surge in demand and compensate for the overexploitation of capture fisheries. The unavailability of land and water and limitation in expanding coastal aquaculture are the major constraints for the expansion of aquaculture. Hence, the best strategy for horizontal expansion of aquaculture resources is the utilization of non-productive land that cannot support further traditional agriculture activities. One such potential area is the saline-affected agricultural lands, which can wisely be used for expanding aquaculture activities (Singha, Shamna, Sahu, Sardar, Harikrishna, Thirunavukkarasar,

  

Chowdhury, et al., 2020; Singha, Shamna, Sahu, Sardar, HariKrishna, Thirunavukkarasar, Kumar, et al., 2020). Globally, the extent of the salt-affected area has reached nearly 1000 million ha (CSSRI, 2011; Sandeep et al., 2013).

Consequently, these inland salt-affected, waterlogged areas and saline groundwater resources can be potentially utilized for aquaculture, especially with euryhaline species, to support food security and livelihood. But, the ionic composition (*viz*., chloride, sulphate, bicarbonate, sodium, magnesium, calcium and potassium) of inland saline water (ISW) makes it different from the normal seawater (Aklakur, 2017). The ionic imbalance in the culture water can cause a pronounced effect on the physiology of cultivable species. In most of the groundwater, Ca:Mg ratio is more than that of the sea and ocean water, resulting in higher water hardness, which ultimately affects the performance of cultured species. Besides, potassium is an essential mineral for aquatic animals to activate $Na^+/K^+$ ATPase, mainly dealing with whole-body ion regulation and cellular water balance (Wang et al., 2002). Moreover, alteration of $Na^+$ and $K^+$ ratio in the haemolymph may disturb the growth performance and survival of the shrimp. Therefore, the ions are also crucial for the normal growth, survival and osmoregulatory function of crustaceans (Pequeux, 1995). However, $Mg^{2+}$ and $K^+$ are considered deficient minerals for shrimp culture in ISW (Roy et al., 2007).

Globally, culturing of crustaceans and fish in ISW is a positive dynamic (Roy et al., 2010; Zadereev et al., 2020). Among the crustaceans, white leg shrimp, *P. vannamei* has become one of the potential candidate species for inland saline aquaculture. Moreover, the farming of *P. vannamei* is emerging in low saline water due to its capability to tolerate a wide range of salinity from 28.3 to 0.5 g/L with uninterrupted availability of postlarvae (Smith and Lawrence, 1990; Samocha et al., 2001). However, the immunological and physiological status of the shrimp, including osmoregulation, is widely affected by the ionic imbalanced water leading to less production. Therefore, this ionic imbalance needs to be rectified for the successful and sustainable culture of finfish and shellfish in ISW. Nevertheless, the feed used for *P. vannamei* culture in the ISW is not yet standardized, and the farmers are relying on regular commercial feeds available in the market.

Growth performance and feed utilization are the key determinants in relation to nutrient requirement studies. According to NRC (2011), the polynomial regression model or broken-line linear model is employed for growth responses to determine the nutrient requirement of a species under a particular culture condition. The activities of the digestive enzyme and metabolic enzyme support the physiological efficiency and ultimate performance of the animal. Hepatopancreas is the primary site of secretion of digestive enzymes in crustaceans, including shrimp. The activities of the digestive enzyme are directly related to nutrient digestibility and its availability. Metabolic enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are considered as damage markers and principal modulators for the functionality of hepatopancreas (Cai et al., 2014). Similarly, antioxidant enzymes *viz*., superoxide dismutase (SOD) and catalase (CAT) are considered as biomarkers of oxidative stress (Gutteridge, 1995) that is activated by elevated reactive oxygen species (ROS), lipid peroxidation and nutrient alternation. Hence, these enzymes play a vital role in maintaining homeostasis for normal cell function.

Protein is the costliest nutrient in aquafeed that not only helps in growth but also supplies energy for body function. Excess dietary protein causes environmental pollution in terms of more nitrogenous wastes that induce the stress and leading to less production. Thus, to make the aquafeed cost-effective and environment-friendly, protein level needs to be spared by non-protein energy source, so that dietary protein should be used for growth. Dietary lipid being a concentrated source of energy can spare the dietary protein. Moreover, dietary lipid acts as a source of essential fatty acids (EFA), phospholipids, steroids, especially cholesterol and carotenoids required for the proper physiological functions of the animals including shrimp.

As feed cost contributes more than 60% of the recurring expenditure of aquaculture and the nutritional requirement of the fish varies due to change in environmental condition, it is essential to optimize the dietary nutritional requirements of species for the development of a cost-effective and eco-friendly feed with a standardized formula for successful and sustainable fish and shrimp culture in ISW. Therefore, the present experiment was designed to study the lipid requirement of *P. vannamei* reared in ISW of 10 g/L salinity.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental animal

*Penaeus vannamei* (Boone, 1931) juveniles were acquired from the Baniyani farm of Rohtak centre, ICAR-CIFE and transferred to the wet laboratory using polythene bags filled with oxygenated ISW of 10 g/L salinity. The shrimp were then carefully shifted to two circular tanks (1.05 $m^2 \times 0.89$ m, 1000 L capacity and 770 L water volume) containing ISW of 10 g/L salinity, covered with fibre mat to prevent shrimp escape and acclimatized for 15 days with round the clock aeration facility. During the acclimatization period, the shrimps were fed with commercial feed (380 g/kg crude protein and 40 g/kg lipid) thrice daily to satiation level. The ground saline water of 10 g/L salinity was drawn from a bore well using a pump, filtered through a 100 μm filter bag to remove any redundant debris and then drained into six cemented tanks (3 m × 2 m × 1.5 m and 8000 L capacity). After allowing it to settle for a week, ISW was transferred to six circular storage tanks (1.05 $m^2 \times 0.89$ m and 935 L capacity). The ISW (10 g/L salinity) in the storage tank was routinely fortified with $K^+$ ion using muriate of potash (KCl). According to Davis et al. (2005), the requirement of $K^+$ for ISW fortification = (10.7 × desired salinity)—available $K^+$ in ISW. $K^+$ fortified ISW in the storage tank was used for filling the experimental tanks, when necessary, during the entire experimental period.

## 2.2 | Formulation and preparation of the experimental diets

Six iso-nitrogenous (around 360 g/kg crude protein) and heterocaloric (15.4–17.6 KJ DE/g) semi-purified diets containing graded levels of lipid *viz*., 20, 40, 60, 80, 100 and 120 g/kg were formulated and prepared (Table 1). All the dry ingredients were finely mashed and weighed as per the feed formula. Meanwhile, the gelatin crystals were dissolved to form a jelly mass using lukewarm water and mixed uniformly with other ingredients except oils and additives to prepare the dough. The dough was then steam cooked using a pressure cooker for 20 min. The remaining ingredients *viz*. oil, lecithin, vitamin-mineral mixture and Stay C were added after cooling the dough and mixed uniformly. The dough was pressed through the mechanical pelletizer fitted with a 1 mm diameter die and kept for drying at room temperature with subsequent oven drying at 40°C until acquiring a moisture level below 100 g/kg feed. Eventually, the dried pellets were then broken into small pieces with a length of

**TABLE 1** Formulation and proximate composition of different experimental diets

| | Diets[a] (Treatments) | | | | | |
|---|---|---|---|---|---|---|
| | L2 | L4 | L6 | L8 | L10 | L12 |
| Ingredients composition (g/kg) | | | | | | |
| Fish meal[b] | 150 | 150 | 150 | 150 | 150 | 150 |
| Defatted soybean meal | 150 | 150 | 150 | 150 | 150 | 150 |
| Casein[c] | 174 | 174 | 174 | 174 | 174 | 174 |
| Gelatin[c] | 43.5 | 43.5 | 43.5 | 43.5 | 43.5 | 43.5 |
| Starch[c] | 305 | 285 | 265 | 245 | 225 | 205 |
| Dextrin[c] | 100 | 100 | 100 | 100 | 100 | 100 |
| Cellulose | 24.8 | 24.8 | 24.8 | 24.8 | 24.8 | 24.8 |
| Cod liver oil[d] | 0.0 | 10 | 20 | 30 | 40 | 50 |
| Sunflower oil[e] | 0.0 | 10 | 20 | 30 | 40 | 50 |
| Vit-Min mix[f] | 15 | 15 | 15 | 15 | 15 | 15 |
| Lecithin | 5 | 5 | 5 | 5 | 5 | 5 |
| Cholesterol | 2 | 2 | 2 | 2 | 2 | 2 |
| CMC | 25 | 25 | 25 | 25 | 25 | 25 |
| BHT | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Betaine | 5 | 5 | 5 | 5 | 5 | 5 |
| Choline chloride | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Stay C | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Vit-E | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Proximate composition (g/kg; on dry matter basis) | | | | | | |
| Dry matter | 911.6 | 918.1 | 922.9 | 926.8 | 938.0 | 939.8 |
| Crude protein | 356.2 | 363.3 | 356.3 | 361.0 | 360.2 | 365.3 |
| Ether extract | 19.5 | 41.8 | 60.1 | 79.1 | 100.3 | 120.7 |
| Crude fibre | 45.5 | 46.6 | 46.4 | 44.1 | 44.7 | 40.6 |
| Nitrogen free extract | 519.4 | 490.0 | 478.5 | 456.9 | 435.4 | 412.9 |
| Total ash | 59.4 | 58.3 | 58.7 | 58.9 | 59.4 | 60.5 |
| DE (KJ/g) | 15.4 | 15.9 | 16.2 | 16.7 | 17.1 | 17.6 |

Abbreviations: BHT, butylated hydroxytoluene; CMC, carboxymethyl cellulose; DE, digestible energy; Stay C, ROVIMIX® STAY-C®35 (DSM in Animal Nutrition & Health).

[a]L2-L12, 20–120 g/kg dietary lipid with 20 g/kg incremental level (hetero-lipidic diets) with 360 g/kg crude protein in each (iso-nitrogenous diets).

[b]Purchased from local dealers, Mumbai, India.

[c]Purified ingredients procured from HiMedia Ltd., India.

[d]Procured from Seacod Oil by Sanofi India Ltd., India.

[e]Fortune Refined Sunflower Oil procured from DMart, Mumbai, India

[f]Composition of the vitamin–mineral mixture (quantity/kg): vitamin A, 55,00,000 IU; vitamin $D_3$, 11,00,000 IU; vitamin E, 750 mg; vitamin K, 1000 mg; ascorbic acid, 2500 mg; vitamin $B_2$, 2000 mg; vitamin $B_6$, 1000 mg; vitamin $B_{12}$, 6 mg; calcium pantothenate, 2500 mg; nicotinamide, 10 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; selenium, 125 mg.

approximately 4–6 mm, packed in the airtight zipper bags and stored at 4°C until used for feeding.

## 2.3 | Proximate analysis of the diets and the whole body of shrimp

The proximate composition of the experimental diets and the whole body of shrimp was determined on a dry matter basis using standard methods (AOAC, 1995) at Fish Nutrition Laboratory of FNBP division, ICAR-Central Institute of Fisheries Education, Mumbai, India. However, nitrogen free extract (NFE) in the case of diets and total carbohydrate in the case of the whole body of shrimp were calculated by the subtraction method. The crude fibre content of the shrimp sample was not estimated. However, the proximate composition of the whole body of shrimp was designated on a wet weight basis except moisture. The digestible energy (DE) of the diet was calculated according to Halver (1976).

Nitrogen free extract (NFE) of the diets was calculated as follows:

$$\text{NFE(g/kg)} = 1000 - \{\text{crude protein (g/kg)} + \text{ether extract (g/kg)} + \text{crude fibre (g/kg)} + \text{total ash (g/kg)}\}$$

The total carbohydrate (TC) of the whole body of shrimp sample was calculated (Hasting, 1969) as follows:

$$\text{Total carbohydrate (g/kg)} = 1000 - \{\text{Crude protein (g/kg)} + \text{Ether extract (g/kg)} + \text{Total ash (g/kg)}\}$$

## 2.4 | Experimental design, setup and feeding trial

The present experiment was planned to find out the effects of six graded levels of dietary lipid on growth, nutrient utilization and physio-metabolic responses of *P. vannamei* juveniles grown in ISW of 10 g/L salinity. For feeding trial purpose, 18 circular fibre reinforced plastic (FRP) tanks (1 m × 0.8 m, 300 L capacity, 200 L water volume) were used. The tanks were initially acid-washed, followed by filling with potassium permanganate solution (4 mg/L) and left overnight. The tanks were flushed out the next day morning, thoroughly washed with clean water and further disinfected with bleaching powder and then washed well with the fresh water to remove chlorine residue and dried properly under bright sunlight for the whole day. The tanks were then filled with $K^+$ fortified ISW collected from the storage tanks, provided with round the clock aeration using a 2 HP air blower and kept ready for stocking.

Two hundred seventy acclimated healthy *P. vannamei* juveniles (avg. b. wt. 5.51 ± 0.01 g) were randomly distributed to six experimental groups in triplicates following a completely randomized design with a stocking density of 15 shrimps per tank. The water salinity was maintained at 10 g/L during the experimental period. The *P. vannamei* juveniles belong to different experimental groups were fed to satiation level thrice every day, following a timeframe of 10 am, 5 pm and 10 pm during the experimental period of 60 days. About 30% of water was exchanged from each experimental tank with equal volume ISW of the same salinity at an interval of every three days to maintain the water quality. The ethical procedures for the Animal Care of ICAR-CIFE, Mumbai, India, were strictly adhered to conduct the current study.

## 2.5 | Physico-chemical parameters of water

The water temperature, pH and dissolved oxygen (DO) of all the experimental tanks were examined within the time frame of 8 am, 4 pm and 10 pm daily by using a water thermometer (MERCK, Germany), a pH probe (HI11310, HANNA Instruments, Singapore) and the DO probe (HI764080, HANNA Instruments) respectively. The refractometer (Z741839, Merck Instruments) was used to monitor the water salinity of the experimental tanks every day. The total hardness of water of all the experimental tanks was determined at three days intervals following the titrimetric method using EDTA (ethylenediaminetetraacetic acid) and eriochrome black-T (APHA, 2005). The free carbon dioxide and total alkalinity were estimated at three days intervals by titrimetric method (APHA, 2005) using standard $H_2SO_4$ and phenolphthalein and methyl orange as indicators. An ammonia-nitrite test kit (Spectro quant NOVA-MERCK, Germany) was used to estimate the total ammonia-N and nitrite-N concentration of experimental water in an interval of three days. The water ions like potassium ($K^+$) were determined using a flame photometer (Electronics India, India), and calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) were measured using the titrimetric method (APHA, 2005) at an interval of three days. The water osmolality was estimated by using a vapour pressure osmometer (VAPRO®). Ten microliters of sample and 1000 mole/Kg osmolality standard (OPTI-MOLE™) were used to estimate the osmolality of different samples.

## 2.6 | Sampling

At the beginning of the growth experiment, the initial weight was calculated by measuring the total biomass. At the end of the 60 days experiment, the number of the final surviving shrimps in each replicate was recorded, and the final body weight (FBW) of shrimps was measured after 24-h starvation. After that, shrimps from all the experimental tanks were collected and anaesthetized with clove oil (50 μl/L). Five shrimps were randomly selected from each replicate for the whole-body composition analysis. Another four shrimps were dissected to obtain the gill, hepatopancreas and muscle tissue sample.

## 2.7 | Growth, nutrient utilization and body indices parameters

Growth parameters such as per cent weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency

ratio (PER) and lipid efficiency ratio (LER) and body indices like hepatopancreatic somatic index (HPSI) were calculated using standard formula as following:

$$\text{WG}\,\% = \frac{\text{Final wet weight (g)} - \text{Initial wet weight (g)}}{\text{Initial wet weight (g)}} \times 100$$

$$\text{SGR}\,\% = \frac{\text{lnoffinal wet weight (g)} - \text{lnofinitial wet weight (g)}}{\text{Experimental period (days)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed intake (dry weight in g)}}{\text{Body weight gain (wet weight in g)}} \times 100$$

$$\text{PER} = \frac{\text{Body weight gain (wet weight in g)}}{\text{Protein intake (dry weight in g)}} \times 100$$

$$\text{LER} = \frac{\text{Body weight gain (wet weight in g)}}{\text{lipid intake (dry weight in g)}} \times 100$$

$$\text{HPSI}\,(\%) = \frac{\text{Wet weight of the hepatopancreas (g)}}{\text{Wet weight of the shrimp (g)}} \times 100$$

## 2.8 | Survival rate

After completion of the experiment, the number of the experimental shrimp remaining in each experimental tank was recorded, and the survival percentage was evaluated by using the formula:

$$\text{Survival}\,(\%) = \frac{\text{Total number of experimental shrimp collected at the end of experiment}}{\text{Number of experimental shrimp stocked}} \times 100$$

## 2.9 | Enzyme analysis

### 2.9.1 | Tissue homogenate preparation

The Teflon coated mechanical homogenizer (REMI Equipment) was used to prepare a 5% tissue homogenate with chilled 0.25 M sucrose solution under the ice-cold condition for sustaining the enzyme activity. The prepared tissue homogenates were then kept for centrifugation at 2800 *g* for 10 min at 4°C with the help of a refrigerated centrifuge machine (Heraeus Megafuge 8R Centrifuge, Thermo Fisher Scientific). The obtained supernatant was then collected in 2 ml Eppendorf tubes and stored in a deep freezer at −20°C until enzyme assays were done. Suitable dilution of the samples was done if required during assays of different enzymes.

### 2.9.2 | Estimation of tissue protein

Quantification of protein in the different tissue homogenate was carried out by Lowry's method (Lowry et al., 1951). The resultant tissue protein value was used for the calculation of enzyme activity. Tissue homogenates (0.1 ml) were precipitated using 1 ml of 100 g/L trichloroacetic acid (TCA) in 1.5 ml Eppendorf tubes. Eventually, the protein precipitate was obtained by discarding the supernatant after centrifugation at 2800 *g* for 20 min. The residue was then dissolved in 0.5 ml of 0.1 N NaOH by vortexing, and 0.1 ml of the sample containing tissue protein was used for further analysis. Alkaline copper sulphate (5 ml) was added to dissolve protein residue and left for 10 min. Subsequently, 0.5 ml of 1 N Folin's reagent was added and incubated for 30 min in the dark. The optical density was read at 660 nm against the blank. The standard curve is created by using standard (Bovine serum albumin), and the unknown concentration of protein in the tissue samples was calculated.

### 2.9.3 | Digestive enzymes activity analysis

Protease activity in the hepatopancreas tissue homogenate was quantified by the casein digestion method, as described by Drapeau (1976). One unit of enzyme activity was expressed as the amount of enzymes needed to release acid-soluble fragments equivalent to $\Delta 0.001 A_{280}$ per minute at 37°C and pH 7.8. Finally, the protease activity was expressed as micromole of tyrosine released/min/mg protein. DNS (3,5-di-nitrosalicylic acid) method, described by Rick and Stegbauer (1974), was employed to estimate the amylase activity of hepatopancreas tissue homogenate. The activity was evaluated based on the production of the reducing sugars because of the action of gluco-amylase and α-amylase on carbohydrates. Maltose was used as the standard. Amylase activity was expressed as micromole of maltose released/min/mg protein. Lipase activity in hepatopancreas tissue homogenate was assayed by using the titrimetric method of Cherry and Crandell (1932) based on the measurement of fatty acids released by the enzymatic hydrolysis of triglycerides present in a stabilized emulsion of olive oil. The lipase activity was expressed as units/h/mg protein.

### 2.9.4 | Estimation of protein metabolic enzymes activity

The AST and ALT activity in hepatopancreas and muscle tissue homogenates was estimated by the method described by Wooten (1964). A substrate comprised of 0.2 M D, L-aspartic acid and 2 mM α-ketoglutarate in 0.05 M phosphate buffer (pH 7.4) was used. In case of ALT, substrate was comprised of 0.2 M D, L-alanine instead of D, L-aspartic acid. In the experimental and control tubes, 0.5 ml of the substrate was added. The reaction was started after adding 0.1 ml of tissue homogenate. The assay mixture was then incubated at 37°C for 60 min. Then, the reaction was terminated by adding 0.5 ml of 1 mM 2,4 dinitrophenyl hydrazine (DNPH) solution. Afterwards, the tubes were kept at room temperature for 20 min with occasional shaking. Then, 5 ml of 0.4 N NaOH solution was

**TABLE 2** Ranges of physico-chemical parameters of inland saline water in different experimental units during 60 days

| Treatments[a] | Temp | pH | Salinity[b] | DO | $CO_2$ | TA | TH |
|---|---|---|---|---|---|---|---|
| L2 | 27.23 ± 0.65 | 8.23 ± 0.22 | 9.63 ± 0.24 | 7.07 ± 0.32 | ND | 263.33 ± 11.57 | 2900 ± 60.83 |
| L4 | 27.17 ± 0.44 | 8.27 ± 0.18 | 9.9 ± 0.1 | 6.9 ± 0.32 | ND | 270.67 ± 11.1 | 2963.33 ± 24.04 |
| L6 | 27.00 ± 0.76 | 8.27 ± 0.09 | 9.93 ± 0.22 | 7.1 ± 0.35 | ND | 261.33 ± 10.91 | 2913.33 ± 38.44 |
| L8 | 27.47 ± 0.49 | 8.13 ± 0.15 | 9.53 ± 0.24 | 6.97 ± 0.26 | ND | 272 ± 9.64 | 2936.67 ± 34.8 |
| L10 | 27.10 ± 0.90 | 8.13 ± 0.18 | 9.8 ± 0.25 | 7.2 ± 0.31 | ND | 258 ± 11.02 | 2940 ± 15.28 |
| L12 | 27.17 ± 0.60 | 8.3 ± 0.12 | 10.03 ± 0.29 | 7.03 ± 0.35 | ND | 266.67 ± 9.56 | 2940 ± 26.46 |

Abbreviations: $Ca^{2+}$, calcium ion, is expressed as mg/L; $CO_2$, free carbon dioxide, is expressed as mg/L (ND, not detected);DO, dissolved oxygen, is expressed as mg/L; $K^+$, potassium ion, is expressed as mg/L; $Mg^{2+}$, magnesium ion, is expressed as mg/L; $NH_3$-N, ammonia nitrogen, is expressed as mg/L; $NO_2$-N, nitrite-nitrogen, is expressed as mg/L; $NO_3$-N, nitrate-nitrogen, is expressed as mg/L; TA, total alkalinity, is expressed as mg/L; Temp, temperature in °C; TH, total hardness, is expressed as mg/L.

[a]L2-L12, 20–120 g/kg dietary lipid with 20 g/kg incremental level (hetero-lipidic diets) with 360 g/kg crude protein in each (iso-nitrogenous diets).

[b]Salinity is expressed as g/L.

[c]Water osmolality is expressed in mOsmole/kg.

**TABLE 3** Nutrient utilization, growth and body indices of *P. vannamei* juveniles reared in ISW of 10 ppt salinity and fed with graded levels of dietary lipid for the period of 60 days

| | Experimental groups[1] | | | | | | | Contrast analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *p* values | | |
| Parameters | L2 | L4 | L6 | L8 | L10 | L12 | SEM | Overall | Linear | Quadratic |
| FBW | 13.43$^{b}$ | 14.89$^{c}$ | 14.95$^{c}$ | 13.42$^{b}$ | 12.63$^{ab}$ | 11.60$^{a}$ | 0.31 | .000 | .000 | .000 |
| WG% | 144.68$^{b}$ | 169.07$^{c}$ | 172.32$^{c}$ | 141.57$^{b}$ | 128.99$^{ab}$ | 111.25$^{a}$ | 5.56 | .000 | .000 | .000 |
| SGR | 1.49$^{b}$ | 1.65$^{c}$ | 1.67$^{c}$ | 1.47$^{b}$ | 1.38$^{b}$ | 1.24$^{a}$ | 0.04 | .000 | .000 | .000 |
| FCR | 1.62$^{bc}$ | 1.44$^{a}$ | 1.46$^{ab}$ | 1.64$^{c}$ | 1.73$^{c}$ | 1.92$^{d}$ | 0.04 | .000 | .000 | .001 |
| PER | 1.73$^{b}$ | 1.92$^{c}$ | 1.92$^{c}$ | 1.69$^{b}$ | 1.61$^{b}$ | 1.43$^{a}$ | 0.04 | .000 | .000 | .000 |
| LER | 31.71$^{f}$ | 16.74$^{e}$ | 11.37$^{d}$ | 7.71$^{c}$ | 5.78$^{b}$ | 4.34$^{a}$ | 2.27 | .000 | .000 | .000 |
| HPSI | 2.28$^{a}$ | 2.42$^{ab}$ | 2.59$^{b}$ | 3.11$^{c}$ | 3.25$^{c}$ | 3.59$^{d}$ | 0.12 | .000 | .000 | .306 |
| Survival% | 95.56$^{b}$ | 100.0$^{b}$ | 93.33$^{b}$ | 95.56$^{b}$ | 84.44$^{a}$ | 84.44$^{a}$ | 1.64 | .003 | .000 | .094 |

*Note:* All values are expressed as mean ($n = 3$); mean values in the same row with different superscripts differ significantly at 5% probability level ($p < .05$).

Abbreviations: FBW, final body weight; FCR, feed conversion ratio; HPSI, hepatopancreatic somatic index; LER, lipid efficiency ratio; PER, protein efficiency ratio; SEM, average standard error of means; SGR, specific growth rate; WG, weight gain.

[1]L2-L12, 20–120 g/kg dietary lipid with 20 g/kg incremental level (hetero-lipidic diets) with 360 g/kg crude protein in each (iso-nitrogenous diets).

added and the contents were mixed thoroughly. After 10 min, the optical density (OD) of the reaction solution was recorded at 540 nm against the blank. The AST activity was expressed as nanomoles of oxaloacetate released/min/mg protein at 37°C.

### 2.9.5 | Estimation of oxidative stress enzymes activity

Superoxide dismutase activity of gill and hepatopancreas tissue homogenates was estimated according to the method described by Mishra and Fridovich (1972) based on the oxidation of epinephrine–adrenochrome transition by the enzyme. A volume of 50 µl sample was taken in the cuvette, and 1.5 ml of 0.1 M carbonate–bicarbonate buffer containing 57 mg/dl EDTA (10.2), and 0.5 ml epinephrine (3 mM) was added and mixed properly. Change in OD at 480 nm was recorded immediately for 3 min in a UV spectrophotometer (Thermoscientific). One unit of SOD activity was the amount of the protein required to give 50% inhibition of epinephrine auto-oxidation. Thus, SOD activity was expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min.

Catalase activity of gill and hepatopancreas tissue homogenates was determined by the method described by Takahara et al. (1960). Accordingly, 50 µl of the tissue homogenate was added to 2.45 ml phosphate buffer (50 mM and pH 7.0), and the reaction was initiated by the addition of 1.0 ml of $H_2O_2$ solution. The decrement in absorbance was measured at 240 nm at 15 s intervals for 3 min. The CAT activity was expressed as nanomoles $H_2O_2$ decomposed/min/mg protein.

| $NH_3$-N | $NO_2$-N | $NO_3$-N | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ | Osmolality[c] |
|---|---|---|---|---|---|---|
| 0.04 ± 0.00 | 0.006 ± 0.001 | 0.05 ± 0.01 | 336 ± 7.23 | 500.67 ± 13.32 | 97.24 ± 1.99 | 286.33 ± 5.78 |
| 0.05 ± 0.00 | 0.004 ± 0.001 | 0.07 ± 0.01 | 340 ± 6.66 | 513.67 ± 9.7 | 97.02 ± 1.78 | 282.33 ± 2.33 |
| 0.04 ± 0.00 | 0.004 ± 0.001 | 0.05 ± 0.02 | 335.33 ± 8.37 | 504 ± 14.57 | 98.97 ± 2.52 | 282.67 ± 3.76 |
| 0.05 ± 0.01 | 0.006 ± 0.001 | 0.05 ± 0.01 | 335 ± 5.69 | 510.33 ± 6.84 | 98.62 ± 2.44 | 283.33 ± 2.73 |
| 0.04 ± 0.01 | 0.005 ± 0.001 | 0.04 ± 0.01 | 337.33 ± 8.19 | 509.33 ± 3.76 | 98.57 ± 1.81 | 286.67 ± 4.41 |
| 0.04 ± 0.01 | 0.004 ± 0.001 | 0.04 ± 0.01 | 335 ± 8.39 | 511 ± 9.71 | 99.13 ± 2.02 | 289.67 ± 1.86 |

### 2.10 | Statistical analysis

The experimental data were subjected to one-way ANOVA (analysis of the variance) to calculate the means and standard error values using SPSS 22.0 software. The overall treatment effects were assessed followed by polynomial contrast analysis to measure the linear and quadratic effects of graded levels of lipid. At a 5% probability level ($p < .05$), Duncan's multiple range test was performed for post hoc comparison of means. The analysed data were represented as means ± standard error. Moreover, based on the WG% values, broken-line linear regression (Robbins et al., 2006) and second-order polynomial regression analysis were performed for optimizing the dietary lipid level (Jobling, 1994).

## 3 | RESULTS

### 3.1 | Physico-chemical parameters of water

In the present study, the physico-chemical parameters of water such as temperature (°C), pH, dissolved oxygen (mg/L), free carbon dioxide (mg/L), total hardness (mg/L), alkalinity (mg/L), Ca concentration (mg/L), Mg concentration (mg/L), K content (mg/L), ammonia-N (mg/L), nitrite-N (mg/L), nitrate-N (mg/L) and osmolality (mOsmole) were found to be under optimum ranges (Table 2). The water salinity level was maintained within the range of 9.53 ± 0.24 to 10.03 ± 0.29 g/L throughout the experimental period of 60 days.

### 3.2 | Growth, nutrient utilization, body indices and survival

In the present study, dietary lipid levels significantly affected the growth, nutrient utilization, body indices and survival of the juvenile *P. vannamei* (Table 3). Growth indices such as FBW, WG%, SGR and PER showed an overall, linear and quadratic trend as well. The trends of FBW, WG%, SGR and PER of shrimp were significantly ($p < .05$) increased with the graded level of dietary lipid up to 60 g/kg after which it decreased. In contrast, FCR was found to be the lowest in 40 and 60 g/kg lipid fed groups. The overall, linear and quadratic trends of LER value in shrimp decreased significantly ($p < .05$) with the increasing dietary lipid levels (Table 3). Body indices such as HPSI followed an overall and linear effect and showed an increasing trend in relation to increasing dietary lipid with the highest ($p < .05$) value in the L12 group. The survival of L10 and L12 groups was significantly lower than the other groups, and the highest value recorded in the L4 group, but there was no significant ($p > .05$) difference among L2-L8 groups.

### 3.3 | Proximate composition of the whole body of shrimp

Dietary lipid levels did not influence the whole-body moisture, crude protein and total ash contents of shrimp significantly ($p > .05$). However, the overall and linear trend of whole-body lipid and total carbohydrate were significantly ($p < .05$) affected due to variation of dietary lipid level. The highest ($p < .05$) lipid level was found in the L12 group. However, there was no significant difference ($p > .05$) of whole-body lipid among L4-L10 groups. The lipid level was significantly lower ($p < .05$) in the L2 group than the L8-L12 groups but similar to L4 and L6 groups (Table 4). On the other hand, the whole-body total carbohydrate content of the L4 group was significantly higher ($p < .05$) than the L6, L10 and L12 groups, but similar ($p > .05$) to L2 and L8 groups.

### 3.4 | Digestive enzymes activity

The hepatopancreatic protease activity did not vary significantly ($p > .05$) among the experimental groups fed with different dietary lipid levels. Moreover, hepatopancreatic amylase followed the overall and linear trend in relation to dietary lipid levels. The lipase activities were significantly affected ($p < .05$) with dietary lipid. Shrimps

**TABLE 4** Whole-body proximate composition (on g $kg^{-1}$ wet weight basis) of *P. vannamei* juveniles reared in ISW of 10 ppt salinity and fed with graded levels of dietary lipid for the period of 60 days

| Proximate composition | Experimental groups[1] | | | | | | | Contrast analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *p* values | | |
| | L2 | L4 | L6 | L8 | L10 | L12 | SEM | Overall | Linear | Quadratic |
| Moisture | 752.8 | 746.2 | 757.3 | 745.4 | 754.1 | 744.0 | 2.74 | .715 | .600 | .688 |
| Crude protein | 162.9 | 162.0 | 162.4 | 169.9 | 163.3 | 169.8 | 2.24 | .851 | .382 | .866 |
| Crude lipid | $17.2^{a}$ | $19.3^{ab}$ | $18.8^{ab}$ | $19.9^{b}$ | $21.1^{b}$ | $24.3^{c}$ | 0.60 | .001 | .000 | .097 |
| Total carbohydrate | $35.1^{ab}$ | $40.4^{b}$ | $31.5^{a}$ | $33.6^{ab}$ | $30.2^{a}$ | $29.3^{a}$ | 1.15 | .029 | .007 | .658 |
| Total ash | 31.9 | 32.2 | 30.0 | 31.2 | 31.3 | 32.5 | 0.37 | .486 | .840 | .130 |

*Note:* All values are expressed as mean ($n$=3); mean values in the same row with different superscripts differ significantly at 5% probability level ($p < .05$).

Abbreviation: SEM, average standard error of means.

[1]L2-L12, 20–120 g/kg dietary lipid with 20 g/kg incremental level (hetero-lipidic diets) with 360 g/kg crude protein in each (iso-nitrogenous diets).

received 60 g/kg dietary lipid showed significantly ($p < .05$) higher amylase activity than the higher lipid fed groups (L8, L10 and L12) but did not differ statistically with the lower lipid fed groups (L2 and L4). Alternatively, the L12 group registered significantly lower amylase activity than the L2, L4 and L6 groups without showing a significant difference with the L8 and L10 groups. The overall, linear and quadratic trends of hepatopancreatic lipase activity were significantly increased ($p < .05$) with the increasing dietary lipid level up to 100 g/kg. Further increase in dietary lipid caused a significant reduction ($p < .05$) in this enzyme activity (Table 5). However, no significant difference ($p > .05$) of the lipase activity was observed among L2, L6 and L12 groups.

## 3.5 | Protein metabolic enzymes activity

The overall, linear and quadratic trends of hepatopancreatic AST and the overall and linear trend of muscle AST activities of *P. vannamei* juveniles were found to be varied significantly due to feeding graded levels of lipid (Table 5). The hepatopancreatic AST activity of the L12 group was significantly ($p < .05$) higher than all other groups except the L10 group. However, the L4 group showed significantly ($p < .05$) lower hepatopancreatic AST activity than L10 and L12 groups, without showing any variation ($p > .05$) with L2, L6 and L8 groups. Muscle AST activity of the L12 group was significantly higher ($p < .05$) than L4 and L6 groups but similar to L2, L8 and L10 groups. Graded level of dietary lipid significantly affected ($p < .05$) the overall and linear trends of hepatopancreatic ALT and the overall, linear and quadratic trends of muscle ALT activities of *P. vannamei* juveniles (Table 5). The hepatopancreatic ALT activities of L2 and L8 did not show a significant difference ($p > .05$) with all other experimental groups. However, the L4 and L6 groups showed significantly ($p < .05$) lower ALT activity in the hepatopancreas than L10 and L12 groups. On the other hand, the shrimp of the L10 and L12 groups exhibited significantly higher ($p < .05$) muscle ALT activity than L4 and L6 groups and similar ($p > .05$) activity to L2 and L8 groups. However, muscle ALT activity was found significantly ($p > .05$) lower in the L6 group than higher lipid fed groups (L8, L10 and L12) with no significant difference with L2 and L4 groups.

## 3.6 | Oxidative stress enzymes activity

Hepatopancreatic SOD activity of shrimp showed a linear significant increasing trend ($p < .05$) with the increasing dietary lipid level up to 60 g/kg, thereafter no significant change in enzyme activity with further increase in dietary lipid level (Table 5). On the other hand, gill SOD activity of shrimp followed an overall and linear increasing pattern ($p < .05$) with the increasing dietary lipid level up to 100 g/kg and no further change was noticed beyond this level. The overall, linear and quadratic trends of gill CAT and only linear trend of hepatopancreatic CAT activities of *P. vannamei* juveniles were significantly affected ($p < .05$) by the dietary lipid levels (Table 5). Increasing dietary lipid exhibited an increasing trend of gill CAT activity in shrimp. Gill CAT activity of the L10 group was significantly higher ($p < .05$) than L2, L4 and L6 groups and similar ($p > .05$) to L8 and L12 groups, although no significant difference ($p > .05$) of gill CAT activity was found among L2, L4 and L6 groups.

## 3.7 | Optimum dietary lipid requirement

Based on the weight gain percentage, optimum dietary lipid requirement of *P. vannamei* juveniles was determined by using broken-line linear and second-order polynomial regression analysis, and accordingly, the optimum dietary lipid requirement of *P. vannamei* juveniles reared in ISW of 10 ppt salinity was found to be 46.5 and 51.4 g/kg respectively (Figure 1).

**TABLE 5** Digestive, metabolic and oxidative stress enzyme activities of *P. vannamei* juveniles reared in ISW of 10 ppt salinity and fed with graded levels of dietary lipid for the period of 60 days

| Enzyme activities | Experimental groups[1] | | | | | | | Contrast analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *p* values | | |
| | L2 | L4 | L6 | L8 | L10 | L12 | SEM | Overall | Linear | Quadratic |
| Protease[2] | 1.32 | 1.39 | 1.26 | 1.21 | 0.97 | 0.95 | 0.09 | .626 | .107 | .655 |
| Amylase[3] | $3.88^{bcd}$ | 3.96 cd | $4.16^{d}$ | $3.40^{abc}$ | $3.30^{ab}$ | $3.13^{a}$ | 0.11 | .014 | .02 | .189 |
| Lipase[4] | $0.16^{a}$ | $0.21^{b}$ | $0.23^{bc}$ | $0.29^{c}$ | $0.28^{c}$ | $0.19^{ab}$ | 0.01 | .001 | .014 | .000 |
| AST[5] | | | | | | | | | | |
| HP | $6.32^{ab}$ | $5.92^{a}$ | $6.27^{ab}$ | $6.53^{ab}$ | $7.36^{bc}$ | $8.21^{c}$ | 0.23 | .009 | .001 | .035 |
| Muscle | $6.29^{abc}$ | $5.4^{ab}$ | $4.95^{a}$ | $6.73^{bc}$ | $6.88^{bc}$ | $7.46^{c}$ | 0.27 | .029 | .013 | .051 |
| ALT[6] | | | | | | | | | | |
| HP | $5.5^{ab}$ | $4.87^{a}$ | $4.89^{a}$ | $5.3^{ab}$ | $6.31^{b}$ | $6.37^{b}$ | 0.20 | .047 | .013 | .051 |
| Muscle | $4.86^{abc}$ | $4.13^{ab}$ | $3.54^{a}$ | $5.00^{bc}$ | $5.58^{c}$ | $5.65^{c}$ | 0.23 | .024 | .016 | .041 |
| SOD[7] | | | | | | | | | | |
| HP | $13.44^{a}$ | $13.72^{a}$ | $16.87^{b}$ | $16.81^{b}$ | $16.82^{b}$ | $16.21^{ab}$ | 0.47 | .040 | .009 | .058 |
| Gill | $13.29^{a}$ | $14.55^{ab}$ | $15.24^{b}$ | $15.93^{bc}$ | $17.86^{d}$ | $17.04^{cd}$ | 0.41 | .001 | .000 | .280 |
| CAT[8] | | | | | | | | | | |
| HP | 5.96 | 5.23 | 6.41 | 7.24 | 7.39 | 7.95 | 0.30 | .058 | .004 | .677 |
| Gill | $5.89^{a}$ | $6.96^{ab}$ | $7.04^{ab}$ | $8.13^{bc}$ | $8.72^{c}$ | $7.39^{abc}$ | 0.28 | .023 | .006 | .048 |

*Note:* All values are expressed as mean ($n = 3$); mean values in the same row with different superscripts differ significantly at 5% probability level ($p < .05$).

SEM, average standard error of means.

[1]L2-L12, 20–120 g/kg dietary lipid with 20 g/kg incremental level (hetero-lipidic diets) with 360 g/kg crude protein in each (iso-nitrogenous diets).

[2]Protease activity is expressed as millimole of tyrosine released/min/mg protein (equivalent to $1.67^{-05}$ katal/mg protein or, $10^{3}$ U/mg protein).

[3]Amylase activity is expressed as micromole of maltose released/min/mg protein (equivalent to $1.67^{-08}$ katal/mg protein or, 1 U/mg protein).

[4]Lipase activity is expressed as unit/h/mg protein (equivalent to $2.78^{-4}$ katal/mg protein or, $1.67^{4}$ U/mg protein).

[5]AST, aspartate aminotransferase, activity is expressed as nanomoles of oxaloacetate released/min/mg protein (equivalent to $1.67^{-11}$ katal/mg protein or, $1.00^{-03}$ U/mg protein).

[6]ALT, alanine aminotransferase, activity is expressed as nanomoles of sodium pyruvate released/min/mg protein (equivalent to $1.67^{-11}$ katal/mg protein or, $1.00^{-03}$ U/mg protein).

[7]SOD, superoxide dismutase, activity is expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min (equivalent to $1.67^{-05}$ katal/mg protein or, $10^{3}$ U/mg protein).

[8]CAT, catalase, activity is expressed as nanomoles $H_2O_2$ decomposed/min/mg protein (equivalent to $1.67^{-11}$ katal/mg protein Or, $1.00^{-03}$ U/mg protein).

## 4 | DISCUSSION

The current study was carried out to optimize the dietary lipid requirement of *P. vannamei* juveniles under the ISW condition of 10 g/L salinity. Inland saline water is a new diversified aquaculture resource where euryhaline species like *P. vannamei* can be suitably cultured. In this study, performances of the shrimp such as growth, nutrient utilization and physio-metabolic responses were evaluated in relation to graded levels of dietary lipid under the rearing condition of ISW at 10 g/L salinity.

Water quality plays a pivotal role as it directly or indirectly governs the survival of organisms in the aquatic ecosystem. In the present study, the water temperature, pH and dissolved oxygen of the culture system were reported within the favourable range of *P. vannamei* cultivation (Bett & Vinatea, 2009; Li et al., 2006; Ponce-Palafox et al., 1997; Wang et al., 2004). *P. vannamei* is capable of tolerating a wide range of salinities ranging from 0.5 to 40 g/L because of its capability to maintain osmotic regulation (Saoud et al., 2003). However, the isosmotic point of *P. vannamei* lies within the range of 21.1 to 26.1 g/L (Castille & Lawrence, 1981; Gong et al., 2004 and Jaffer et al., 2020). Moreover, Salinity fluctuation can affect the growth and nutrient utilization of the shrimp (Maicá et al., 2014). The water salinity of the present study was maintained at around 10 g/L in experimental ISW. According to Boyd and Tucker (1998), total alkalinity of higher than 75 mg/L could be favourable for aquaculture. However, the total water alkalinity of less than 100 mg/L for a long period might adversely affect the growth performance of *P. vannamei* (Furtado et al., 2011). In the present study, the total alkalinity, total hardness, ammonium-nitrogen, nitrite-nitrogen, nitrate-nitrogen, Ca, Mg and K ion concentration were found to be within the recommended range of ISW (Allan et al., 2009 and Talukdar et al., 2020). The osmolality of water in the present experiment

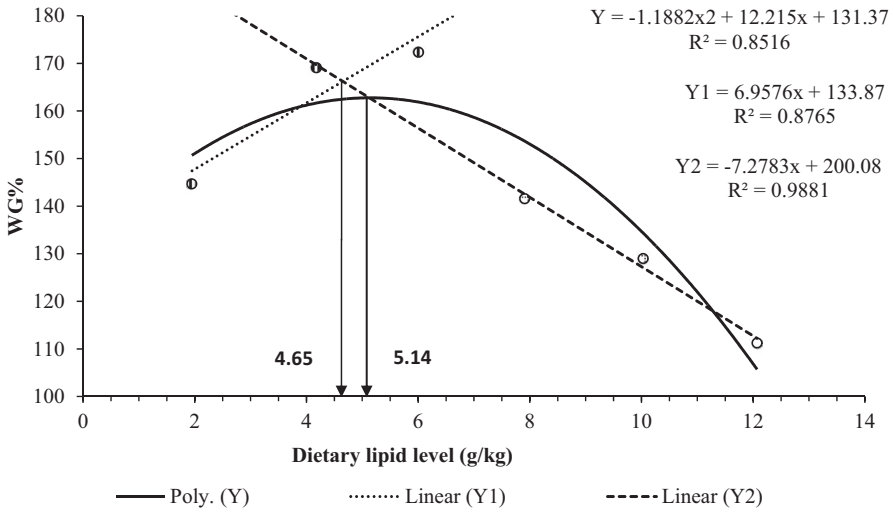


**FIGURE 1** The broken-line linear and second-order polynomial regression analysis to optimize the die tary lipid requirement based on weight gain percentage of *Penaeus vannamei* juveniles reared in inland saline water at 10 g/L salinity

was noticed within the range of 275–294 mOsmole/Kg to support the observation of Singha, Shamna, Sahu, Sardar, HariKrishna, Thirunavukkarasar, Kumar, et al. (2020). However, there was no significant difference in water osmolality for different treatments.

In the current study, the whole-body lipid content increased with the increasing dietary lipid levels. The highest body lipid content was found in shrimp fed with 120 g/kg dietary lipid might be due to the excess dietary lipid beyond optimum level which could have been deposited in the viscera, but lower dietary lipid could have optimally been utilized for energy production required for osmoregulation of shrimp in ISW leading to less deposition of lipid in the body. The present finding was consistent with the Xu et al., 2018, which has shown that excess intake of dietary lipid is deposited in hepatopancreas corresponding to the highest HPSI at high dietary lipid levels. In addition, a study indicated that increasing the dietary lipid level influenced the total lipid composition of shrimp, by increasing lipid deposition in hepatopancreas and muscle tissue (González-Félix et al., 2002). Likewise, result was also supported by observations of many other previous studies in shrimps (Jannathulla et al., 2019; Xie et al., 2019; Zhu et al., 2010). Whole-body moisture, protein and ash contents were not significantly affected by the dietary lipid level in the present study. Similarly, in agreement with the present finding, Zhu et al. (2010) found no significant effect of dietary lipid on moisture, protein and ash content of body. Moreover, Zhang et al. (2013), Xu et al. (2018) and Hamidoghli et al. (2020) reported that dietary lipid level could not significantly affect the body moisture and ash contents of shrimp. Moreover, in corroboration with the present finding, Wang et al. (2014) found that dietary lipid level did not significantly influence the body protein content of shrimp. On the other hand, shrimp fed with 40 g/kg dietary lipid exhibited significantly higher whole-body total carbohydrate content than the higher dietary lipid fed groups, indicating at lower dietary lipid level, shrimp could utilize lower dietary lipid for energy production with less share from dietary carbohydrate leading to storage of carbohydrate as glycogen, but shrimp could not utilize higher dietary lipid for energy production (Chuntapa et al., 1999; González-Félix et al., 2002). Therefore, maximum dietary carbohydrate might be used for this purpose leading to higher and less deposition of body lipid and carbohydrate respectively. Moreover, this explanation was in corroboration with the study of Hu et al. (2008).

Growth is the result of dietary nutrients accumulation in the body of animals, including finfish and shellfish. However, the accumulation of protein, especially in muscle, is considered as lean growth, which is the most desirable criterion for quality fish production. On the other hand, the growth of fish due to more fat deposition is not a desirable characteristic. Protein is the most important nutrient for fish as it not only supports growth but also endows energy through amino acid oxidation. Therefore, dietary lipid, as a non-protein energy source, can spare the protein in relation to energy production. In the present study, feeding of 40 and 60 g/kg dietary lipid significantly increased final body weight, WG%, SGR and PER and decreased FCR and a further increase in dietary lipid negatively affected the growth performance of the shrimps with significantly decreased final body weight, WG (g), WG%, SGR and PER and increased FCR. Moreover, LER showed a declining trend with the increasing dietary lipid. These findings indicated that dietary lipid level of 40–60 g/kg may provide optimum P:E value for maximum utilization of dietary protein-derived amino acids in synthesis and accretion of body protein and dietary carbohydrate and lipid for energy production leading to improved growth performance of shrimp. In agreement with the present finding, Wang et al. (2014) stated that 40.2 – 59.8 g/kg dietary lipid could be the optimum range for maximum growth of *P. vannamei* juveniles at 20 g/L salinity. Similarly, González-Félix et al. (2002) reported that 60 g/kg dietary lipid could be optimum for *P. vannamei* culture at low salinity. In contrast, Zhang et al. (2013) found that 100–120 g/kg dietary lipid could be optimum for *P. vannamei* culture in low saline water (3 g/L), probably due to high-energy demand for osmoregulation. However, higher dietary lipid might have reduced the protein intake with subsequent increased consumption of lipid and carbohydrate, leading to decreased availability of amino acids to support the optimum growth of shrimp with the utilization of lipid and carbohydrate for energy production and storage of excess lipid

in the viscera of shrimp (Sheen, 1997). Similarly, a declining trend of growth performance was found in juvenile penaeids (Andrews et al., 1972) and other crustaceans (Davis & Robinson, 1986; Glencross et al., 2001; Ward et al., 2003) with the increasing dietary lipid and 90–100 g/kg dietary lipid reduced the growth performance.

The survival rate of *P. vannamei* juveniles in the current study was significantly lower in higher lipid (100 and 120 g/kg) fed groups compared with their lower lipid fed counterparts. In accordance with this finding, it was reported that excess dietary lipid probably could be detrimental to the shrimp in relation to growth and survival (Sheen & D'Abramo, 1991; Toledo et al., 2016), although a survival percentage of more than 80% was reported to be good for crustacean culture (Cuzon & Guillaume, 1997).

The body indices (*e.g.* HPSI) render an indication about the physiological and metabolic status of shrimp, including digestive enzyme activities for nutrient digestion and absorption, condition of the liver, etc. In the present study, increasing dietary lipid level significantly increased the HPSI of shrimp might be due to the reason that higher dietary lipid could have enhanced lipid intake, causing more deposition of lipid in hepatopancreas to increase its weight leading to increased HPSI. Past studies revealed that excessive lipid intake stimulated more lipid deposition in the hepatopancreas of shrimp (González-Félix et al., 2002). Similarly, the observation of Xu et al. (2018) is in agreement with the present findings.

The hepatopancreas is the central organ of the crustacean's digestive system where the entire digestive processes, including secretion of the digestive enzymes, nutrient digestion and absorption, take place. The digestive enzyme activities are positively correlated with nutrient digestibility and growth of animals, including shrimp. Moreover, the secretion of these enzymes is positively correlated with the availability of the respective substrate in the digestive system of shrimp. Accordingly, in the present study, the dietary lipid did not influence the protease activity since the dietary protein was constant for all the experimental groups. The lipase activity was significantly higher in 80 and 100 g/kg lipid fed groups than the lower lipid fed groups might be related to the availability of lipid in the digestive system. However, feeding of 120 g/kg dietary lipid caused a significant reduction in lipase activity in shrimp, which is in agreement with Hamidoghli et al. (2020) and Lista and Velásquez (2003). On the other hand, dietary lipid up to 60 g/kg did not cause a significant change in the amylase activity in shrimp. However, a further increment of dietary lipid significantly decreased the activity of this enzyme with the lowest value in the 120 g/kg lipid fed group. Reduced activity of amylase in higher dietary lipid fed group might be a consequence of less secretion of amylase in relation to lower availability of dietary carbohydrates in the digestive system of shrimp. In corroboration with the present finding, Hamidoghli et al. (2020) reported the lower amylase activity in *P. vannamei* due to feeding of the lowest and the highest level of dietary lipid.

Protein metabolic enzymes such as AST and ALT are associated with transamination reactions for the synthesis of new amino acids from existing ones that being utilized for synthesis and accretion of body protein leading to growth and sustain oxidative degradation under energy deprivation (De Silva & Anderson, 1995; Jiang et al., 2015). In this study, hepatopancreatic and muscle AST and ALT activities of *P. vannamei* were significantly higher in higher lipid (100 and 120 g/kg) fed groups in comparison with lower lipid (up to 80 g/kg) fed groups. Moreover, the lower growth trend in higher lipid fed groups indicated that higher dietary lipid could induce enhanced AST and ALT activities for the synthesis of more non-essential amino acids, which might be oxidized instead of body protein synthesis for fulfilling enhanced energy demand of shrimp at the cost of growth.

Oxidative stress arises when the organism has elevated levels of ROS (Schieber & Chandel, 2014), which may be induced by dietary, environmental and biological factors. ROS are by-products of aerobic metabolism that can damage different biomolecules in cells as well as tissues of organisms. Fish can protect themselves from ROS by antioxidant defence mechanisms. In antioxidant systems, SOD is the first and most crucial defence line for catalysing the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide (Matozzo et al., 2011). Subsequently, catalase catalyses the harmful $H_2O_2$ into water and molecular oxygen. Thus, increasing the activity of these enzymes in the tissues is the stress indicator of animals. Hence, these enzymes play an essential role in maintaining the dynamic balance of free radical generation and elimination in organisms for the prevention of ROS-mediated tissue damage and membrane lipid peroxidation (Atli & Canli, 2010). In the present study, the increased hepatopancreatic and gill SOD and CAT activities in higher lipid fed groups indicated that dietary lipid beyond optimum level caused metabolic stress with increased ROS production, which might have induced more SOD and CAT production to prevent ROS-mediated tissue damage of shrimp. Similar observations were also stated by Zhao et al. (2015) and Xu et al. (2020) in crustaceans in relation to dietary lipid levels.

In the present study, based on WG%, broken-line linear and second-order polynomial regression analysis indicated that the optimum dietary lipid requirement of *P. vannamei* juvenile reared in ISW of 10 g/L salinity was 46.5 and 51.4 g/kg respectively. Thus, the requirement could range from 46.5 to 51.4 g/kg. The current study is a first report on the optimum dietary lipid requirement of *P. vannamei* juvenile under ISW conditions of 10 g/L. Earlier studies revealed that the optimal dietary lipid level for shrimp ranged from 50 to 140 g/kg (Beseres et al., 2005; Glencross et al., 2002; Goda, 2008; Tzeng et al., 2004). Similarly, in other studies, 60 g/kg dietary lipid was reported to be optimal for *P. vannamei* (González-Félix et al., 2002). However, according to Wang et al. (2014), 40.2–59.8 g/kg dietary lipid could be optimum for *P. vannamei* juveniles at 20 g/L salinity. In contrast, Zhang et al. (2013) reported that 100–120 g/kg level dietary lipid could be optimum for *P. vannamei* culture in low saline water (3 g/L), probably to fulfil the high-energy demand for osmoregulation. In another study, Xu et al. (2018) revealed that 60–90 g/kg dietary lipid could alleviate the low salinity induced osmoregulatory pressure leading to the improved growth performance of *P. vannamei*.

## 5 | CONCLUSION

In conclusion, based on growth performance and physio-metabolic responses, the dietary lipid requirement of *P. vannamei* juveniles in ISW of 10 g/L salinity could be within the range of 40–60 g/kg at the dietary crude protein level of 360 g/kg. However, based on the weight gain percentage (WG%), broken-line linear regression and second-order polynomial regression analysis showed that the optimal dietary lipid requirement of *P. vannamei* in ISW of 10 g/L salinity was 46.5 and 51.4 g/kg respectively. Therefore, the optimum range of dietary lipid for *P. vannamei* juveniles reared in ISW of 10 g/L salinity could be 46.5–51.4 g/kg at 360 g/kg crude protein level for improving growth performance. In relation to a given environment, the knowledge of the nutritional requirement of species to be cultured is a crucial prerequisite for development of a cost-effective and environment-friendly feed to optimize sustainable production and make the aquaculture operation economically viable. Thus, these data will be helpful in the development of ISW specific feed for *P. vannamei*.

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### CONFLICT OF INTEREST

The authors do not possess any conflict of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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