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Effect of dietary lipid/essential fatty acid level on Pacific whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931) reared at three different water salinities – Emphasis on growth, hemolymph indices and body composition

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ABSTRACT

A 45-day feeding trial was conducted to evaluate the effect of dietary lipid/essential fatty acid (EFA) level on growth performance in *Litopenaeus vannamei* reared at 2, 25 and 50‰ salinity. Three iso-nitrogenous (374 g/kg) experimental diets were formulated to contain 30, 60 and 90 g/kg of lipid having 6, 12 and 24 g/kg of EFA (Diet-30/6, Diet-60/12 and Diet-90/24), respectively. A total of 540 healthy acclimatized shrimps (6.44 ± 0.31 g) were transferred into 27 experimental tanks (500 L) in triplicate (20 shrimps/tank). Results of 2 × 2 factorial design (salinity and lipid/EFA level) showed a significantly ($P < 0.05$) higher weight gain of 135.65% at 25‰. Diet-60/12 and Diet-90/24 exhibited significantly ($P < 0.05$) higher growth compared to Diet-30/6. A high correlation was observed among the salinities ($r = 1.0000$ and 0.9696). Survival was significantly high at 25‰ (84.44%) compared to 2‰ (46.66%) and 50‰ (40.55%). The hemolymph protein level enhanced significantly with increased dietary lipid/EFA level but not due to salinity. Triglycerides and cholesterol decreased significantly with increased salinity, whereas the reverse was true due to dietary change. Both salinity and lipid/EFA level had a significant ($P < 0.05$) effect on body composition. Results revealed that increasing dietary lipid/EFA level enhances the tolerance of L. *vannamei* at both hypo (2‰) and hyper (50‰) saline conditions, but would not be beneficial while rearing at 25‰.

1. Introduction

Pacific whiteleg shrimp, *Litopenaeus vannamei* is the most preferred choice among the penaeid shrimps nowadays due to its improved growth, euryhaline nature and being less susceptible to pathogens compared to other penaeid shrimps like black tiger shrimp, *Penaeus monodon*. The production of L. *vannamei* is widespread in Asian countries, in particular India, hence the shrimp production increased from 144,000 t (2006–2007) to > 400,000000 t in 2016–2017 (MPEDA, 2017). Though, *L. vannamei* effectively maintains the osmotic pressure and ionic regulation by exhibiting a pattern of hyper and hypo osmosis at low and high saline environment, respectively (Castille and Lawrence, 1981), the growth rate and survival varies according to the salinity (Ponce-Palafox et al., 1997; Jannathulla et al., 2017; Ponce-Palafox et al., 2019). This difference could partly be attributed to the variation in the utilization of energy sources. Li et al. (2015) stated that as osmoregulation is an energy depending process, providing an adequate amount of energy through the dietary manipulation is an effective way to enhance the ability of shrimp, when the ambient salinity has changed widely from the original (Chen et al., 2014; Wang et al., 2015). If not, shrimp withdraw their own energy sources from the body, leading to a rapid reduction in growth.

There are several reports with regard to protein as an energy source during salinity variation. Huang et al. (2003) reported that the dietary protein requirement of L. *vannamei* was higher at high salinity than those reared in low saline waters. Sui et al. (2015) reported a remarkable increase in growth rate with 35 to 45% of dietary protein in L. *vannamei* reared at 30 and 50‰. This positive effect is attributed to the contribution of dietary amino acids to energy requirement (Li et al., 2011). However, researchers sound a word of caution to reduce the dietary protein level, as high protein levels not only increase the cost of the feed, but also enhance the organic load and environmental pollution

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due to higher protein catabolism, which does not support sustainable aquaculture growth (Wang et al., 2015). Therefore, identifying the cheapest non-protein energy sources is an essential task to spare dietary protein. Wang et al. (2015) evaluated the protein sparing effect of carbohydrates in L. *vannamei,* and recommended a protein to carbohydrate ratio of 34:19 to meet the energy and protein requirement in L. *vannamei* at low salinity. The protein-sparing effect of carbohydrate has also been tested by Cruz-Suarez et al. (1994) in *L. vannamei* and certain fishes, like rainbow trout, *Oncorhynchus mykiss* (Pieper and Pfeffer, 1980), channel catfish, *Ictalurus punctatus* (Garling and Wilson, 1976), European eel, *Anguilla anguilla* (Hidalgo et al., 1993).

On the other hand, non-protein energy is also increased by the addition of lipids in the diet. Lipids are important in maintaining structural and physiological integrity of cellular and sub cellular membranes in addition to being the fuel source and energy reserves. Notwithstanding, lipids also provide essential fatty acids, phospholipids, sterols and certain fat-soluble vitamins, which would help to maintain the proper functioning of the physiological process of the animals (Tseng and Hwang, 2008). During salinity adaptation, the energy-demanding mechanisms for hemolymph osmotic and ionic regulation are activated. Thus, lipids play a dynamic role in the functioning of membrane-bound proteins and can modulate enzymatic activity, playing a major role for osmoregulation as reported in Atlantic salmon, *Salmo salar* (Nordgarden et al., 2002). Chen et al. (2014) reported that L. *vannamei* showed a higher activity of adipose triglycerol lipase, lipoprotein lipase and hormone sensitive lipase, that are related to lipid mobilization at hypo (3‰) and hyper (30‰) saline conditions than those were reared at an optimal salinity of 17‰. The authors also reported that the enzymes (fatty acid synthase and diacylglycerol acyltransferase) responsible for lipogenesis, increased at 3 and 30‰ compared to 17‰, which indicates that lipogenesis and lipolysis capacity of L. *vannamei* is enhanced at both high and low levels of salinity.

Commercial shrimp feed contains 6 to 7% of lipid with a maximum level of 10% (Akiyama et al., 1991). Linoleic (18:2c), linolenic (18:3), arachidonic (20:4), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids have been reported as essential fatty acids (EFA) for penaeid nutrition and physiology (Merican and Shim, 1996; Glencross et al., 2002). Zhu et al. (2010) reported that L. *vannamei* reared at 30‰ had an optimum growth with 60 g/kg of dietary lipid, while diets with 80 g/kg of lipid met the requirement of growth at 2‰. However, there was no additional effect on growth due to an increase of lipid level to 100 g/kg (Zhu et al., 2010) and 120 g/kg (Xu et al., 2018). Hurtado et al. (2006) reported that though HUFA-enriched diet enhanced fatty acid profiles in L. *vannamei* irrespective of the salinities (5, 30 and 50‰), its positive effect on growth occurred only at high salinity and not at low salinity. This is in agreement with the findings of Romano et al. (2012) in Blue swimmer crab, *Portunus pelagicus* reared at a salinity of 14, 30 and 42‰. A similar dose-response effect of dietary lipid (60, 90 and 120 g/kg) was conducted earlier by Xu et al. (2018) in L. *vannamei* at two different water salinities (3 and 25‰), whereas in the present study, three dietary lipid levels (30, 60 and 90 g/kg) having 6, 12 and 24 g/kg of essential fatty acids (EFA) at three different water salinities (2, 25 and 50‰) were evaluated.

2. Materials and methods

2.1. Experimental diets

Three iso-nitrogenous (374 g/kg of crude protein) experimental diets were prepared according to the method of Dayal et al. (2011). Briefly, all the dry ingredients listed in Table 1 were finely ground and passed through a 250 μm mesh. The additives like vitamin-mineral mix, binder and butylated hydroxytoluene (anti-oxidant) were added to the ground material. After 2–3 min of hand mixing, fish oil and soy-lecithin were included in the diets to have 60/12 and 90/24 g/kg of lipid/EFA, while no oil sources were used in the diet having 30/6 g/kg of lipid/EFA. All the ingredients in a diet were blended together for 20 min using an electric blender. The homogenized mash was hydrated with water at the rate of 500 ml/kg to make a dough. The dough was steamed at atmospheric pressure for 5 min, cooled and pelletized in a table top pelletizer having a 2 mm diameter die. The pellets were dried overnight in a hot air oven at 60 °C to bring down the moisture content to < 100 g/kg and refrigerated at 4 °C until further use. The nutrient composition of experimental diets is depicted in Table 2.

Table 1
Ingredient composition of experimental diets containing different levels of lipid/EFA (g/kg as fed basis).

Treatments	Experimental diets		
	Diet-30/6	Diet-60/12	Diet-90/24
Fishmeal[a]	200	200	200
Acetes[b]	100	100	100
Soybean meal	250	250	250
Wheat gluten	20	25	30
Sesame oil cake	50	50	50
Rapeseed meal	25	25	25
Broken rice	50	50	50
Wheat flour	274	239	204
Fish oil[a]	–	20	40
Soy-lecithin[c]	–	10	20
Vitamin mineral mix[d]	20	20	20
Binder[e]	10	10	10
Butylated hydroxytoluene[f]	1	1	1

Other feed ingredients are purchased from local markets, Chennai, India.
[a] Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India.
[b] Mantis shrimp used as a protein source.
[c] Real Soy Enterprises, Madhya Pradesh, India.
[d] Pre-mix (g/kg): Thiamine hydrochloride (25.50 g), riboflavin (25.00 g), prydoxine hydrochloride (50.00 g), cyanogobalamine (0.10 g), menadione (5.00 g), all-trans tocopherol acetate (99.00 g), retinyl acetate (10.00 g), vitamin D (50 g), nicitinic acid (101.00 g), D-Ca-pantothenate (61.00 g), biotin (25.00 g), folic acid (6.25 g), inositol (153.06 g), ferric citrate (13.70 g), $ZnSO_4.7H_2O$ (28.28 g), $MgSO_4.7H_2O$ (0.12 g), MnSO4.H2O (12.43 g), $CuSO_4.5H_2O$ (19.84 g), $CoC_{12}.6H_2O$ (4.07 g), KIO_4 (0.03 g), KCl (15.33 g), Na_2SeO_3 (0.02 g).
[e] Pegabind, Bentoli AgriNutrition Asia Pvt. Ltd., Singapore.
[f] Sigma Aldrich.

2.2. Experimental conditions

L. vannamei, including both the sexes at pre-adult stage, were procured from a local farm near Chennai, India and were acclimatized to indoor laboratory conditions for a fortnight with a diet containing 374 g/kg of crude protein and 60 g/kg of ether extract. Three salinities of 2, 25 and 50‰ were selected in the present study based on our earlier reports (Jannathulla et al., 2017). The juveniles were randomly divided into three groups and were further acclimatized to experimental salinities by either stepwise increase using natural sea salt (Jeeva Enterprises, Chennai, India) or gradual decrease using freshwater at the rate of 2‰ per day from the original salinity of 19‰, and kept in the same condition for a week. Post acclimatization, a total of 540 healthy uniform sized shrimp (6.45 ± 0.32 g) were transferred into 27 oval shape 500 l (1.31 × 0.64 × 0.73 m) fiberglass reinforced plastic (FRP) tanks with 20 shrimp per tank and covered with a fiber mat to prevent the escape of shrimp. Each diet was randomly allotted in triplicate. Shrimp were fed with the respective diet thrice a day (7.00 AM, 12.30 PM and 5.30 PM) at the rate of 6% of the body weight and the amount of diet given was adjusted according to intake in subsequent days. Shrimps were allowed to feed for an hour every time after which, the uneaten feed particles (if any) were siphoned out from the experimental tanks using a clean Falcon tube. The particles were rinsed with de-ionized water and dried at 60 °C in a hot air oven overnight to measure the feed intake on a daily basis. Ultraviolet treated water was

Table 2
Proximate, essential amino acid, major fatty acid and macro mineral composition of experimental diets containing different levels of lipid/EFA (g/kg as fed basis).

Treatments	Experimental diets			R[a]
	Diet-30/6	Diet-60/12	Diet-90/24	
Proximate composition				
Moisture	87.24	81.46	85.16	
Crude protein	374.43	374.67	374.22	
Ether extract	30.16	60.49	90.03	
Crude fiber	26.67	25.54	23.67	
Nitrogen free extract[b]	481.50	461.84	426.89	
Total ash	131.16	129.37	135.44	
Essential amino acids				
Arginine	23.34	24.12	22.87	23.21
Histidine	11.45	11.92	12.43	8.02
Isoleucine	17.16	18.43	17.84	10.11
Leucine	29.74	28.17	28.43	17.00
Lysine	16.89	18.21	17.66	16.43
Methionine	8.283	8.86	9.12	9.06
Phealanine	16.62	16.12	16.44	14.01
Threonine	15.12	14.17	14.94	15.12
Tryptophan	4.387	4.91	4.60	–
Valaine	15.96	16.31	16.83	14.08
Major fatty acids				
C14:0	0.58	2.74	7.12	
C16:0	4.24	10.28	22.54	
C18:0	1.43	3.01	6.13	
C16:1	0.12	2.81	6.27	
C18:1c	1.73	3.47	7.51	
C18:1t	0.88	2.54	6.43	
C18:2c	4.84	7.89	14.57	
C18:3	0.34	0.58	1.56	
C20:4	0.21	0.71	1.78	
C20:5	0.37	2.01	4.28	
C22:6	0.48	1.16	2.24	
Σ-EFA[c]	6.23	12.35	24.43	
Macro minerals				
Calcium	26.27	26.32	27.11	
Magnesium	4.17	4.17	4.04	
Phosphorus	14.53	13.87	14.06	
Potassium	9.18	9.35	8.98	
Sodium	2.49	3.12	3.01	

[a] Recommended level of essential amino acids for *Penaeus vannamei* (Macias-Sancho et al., 2014).
[b] Calculated by a difference.
[c] Σ-Essential fatty acids (C18:2c + C18:3 + C20:4 + C20:5 + C22:6).

used throughout the experimental period and was exchanged daily prior to the first feeding at a rate of 80% tank volume. Water quality parameters like salinity, dissolved oxygen, temperature and pH were measured on a daily basis and total ammonia nitrogen, nitrite nitrogen and nitrate nitrogen were measured once a week by standard methods of APHA (2012) given in Table 3. At the end of the experiment (45-days), weight gain (WG), specific growth rate (SGR), daily growth coefficient (DGC), survival and condition factor (CF) for each dietary treatment were determined as follows.

$$WG\ (\%) = \text{Final weight (g)} - \text{Initial weight (g)} / \text{Initial weight (g)} \times 100.$$

$$SGR = [\ln(\text{Final weight}) - \ln(\text{Initial weight})] / \text{Days of experiment} \times 100.$$

$$DGC = [\text{Final weight}^{1/3} - \text{Initial weight}^{1/3}] / \text{Days of experiment} \times 100.$$

$$\text{Survival}\ (\%) = \text{Final number of animals} / \text{Initial number of animals} \times 100.$$

$$CF = \text{Wet body weight (g)} / [\text{Whole body length (cm)}]^3.$$

Hemolymph samples were collected from five shrimp in each replicate of a treatment (fifteen shrimp per treatment) through the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle on a 1 ml syringe containing 0.3 ml of anticoagulant solution.

Table 3
Ionic composition and water quality parameters of experimental water with varied salinity.

Treatments	Experimental water salinity		
	2‰	25‰	50‰
Ionic composition (mg/l)			
Calcium	98.64	262.24	409.32
Magnesium	137.66	718.82	1387.33
Phosphorus	3.91	3.77	4.17
Potassium	23.58	149.32	337.12
Sodium	653.43	5448.45	12,619.14
Water quality parameters			
Salinity (g/l)	2.0	25.0	50.0
DO (mg/l)[a]	7.4	6.9	7.2
Temperature (°C)	27.4	28.1	27.7
pH	8.3	8.2	8.2
TAN (mg/l)[b]	0.14	0.09	0.12
NO_2-N (mg/l)[c]	0.48	0.44	0.51
NO_3-N (mg/l)[d]	2.61	2.64	2.57

[a] Dissolved oxygen.
[b] Total ammonia nitrogen.
[c] Nitrite nitrogen.
[d] itrate nitrogen.

The anticoagulant used in our study was the formulation of Söderhäll and Smith (1983), which contains a mix of sodium chloride (0.45 M), glucose (0.1 M), sodium citrate tribasic dihydrate (30 mM), citric acid monohydrate (26 mM), EDTA disodium salt (10 mM). The remaining whole shrimps were utilized for the analysis of body composition of proximate, fatty acids and minerals.

2.3. Biochemical analysis

Proximate composition of ingredients, experimental diets and shrimp body composition was analysed as per the method of AOAC (1997). Amino acid profiles were analysed using a pre-column derivatization HPLC gradient system (Shimadzu Corp, LC-30 AD) after hydrolyzing the samples with 6 N hydrochloric acid (Finlayson, 1964). The YMC-Triart C18, RRH (1.8 µm, 2.1 × 100 mm) column was used to separate the amino acids after derivatization with mercaptopropionic acid, O-pthaladehyde and fluorenylmethoxycarbonyl chloride under gradient elution using phosphate buffer (20 mmol as mobile phase A) and combination of acetonitrile: methanol: water (45:40:15 as mobile phase B) at the flow rate of 0.3 ml/min. Amino acids were quantified by a fluorescent detector (RF-20AXS) using the amino acid mixer as an external standard (Sigma Aldrich) and norleucine as an internal standard. Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis by the spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). The partial oxidation of sulphur containing amino acids like methionine during acid digestion was prevented by adding 0.1% of phenol (Jajic et al., 2013).

Lipid was extracted by using chloroform and methanol (2:1) (Folch et al., 1957) and the respective fatty acid methyl esters (FAMEs) were prepared by Metcalfe et al. (1966) method. Finally, the FAMEs were extracted into petroleum ether. Routine analysis of methyl esters was performed by a gas chromatograph (GC-2014 Shimadzu) on a RTX wax capillary column (100 m lengthx0.25 mm I.Dx0.2 µm film thickness). Nitrogen was used as a carrier gas at a linear velocity of 20.9 cm/s with 3 ml/min of purge flow. Fatty acids were identified by comparing the retention times of the 37 component FAME mix (Supelco-Sigma) as an external standard and trideconoic acid (C13:0) was used as an internal standard. The quantity of fatty acids (mg/kg) was calculated following the method of Aziz et al. (2012). The sample was digested using microwave digestion method (Anton Par microwave system) for mineral analysis with 6 ml of nitric acid and 2 ml of hydrogen peroxide in inert polymeric microwave vessels. The minerals were determined by

inductively coupled plasma-optical emission spectrometry (ICP-OES; Agilent 5100 SUV) using the 5.2 software (Jannathulla et al., 2017). The certified reference material of ICP multi-element standard solution (10 mg/l; Merck) was used for calibration. Hemolymph indices such as total protein, triglycerides and cholesterol were estimated using the respective commercial kits obtained from Sigma-Aldrich (Code No: TP0100, TR0100 and MAK043, respectively) in a UV-spectrophotometer (Shimadzu, UV-1800) at 595, 540 and 570 nm, respectively, according to the accredited methodologies given by Sigma-Aldrich (http://www.sigmaaldrich. com/content/dam/ sigma-aldrich/docs/Sigma/Bulletin/(Code No)bul.pdf).

2.4. Statistics analysis

Experimental data were subjected to two-way ANOVA for assessing the effect of lipid/EFS level on shrimp reared at 2, 25 and 50‰. Regression analysis was performed to assess the effect of salinity and lipid/EFA levels on WG. Prior to statistical analyses, data were checked for determining the homogeneity of variance after ascertaining the normal distribution. The entire data were analysed using SPSS version 16.0 at 5% significance level.

3. Results

3.1. Water quality parameters

Of all the minerals analysed in experimental water, sodium was abundant followed by magnesium, calcium and potassium. They gradually increased with increasing water salinity (Table 3), whereas phosphorus content (3.77–4.17 mg/l) was not affected due to salinity change. Other water quality parameters such as dissolved oxygen (6.9–7.4 mg/l), temperature (27.4–28.1 °C), pH (8.2–8.3), total ammonia nitrogen (0.09–0.14 mg/l), nitrite nitrogen (0.44–0.51 mg/l) and nitrate nitrogen (2.57–2.64 mg/l) were in optimal ranges required for the culture of L. *vannamei* (Jannathulla et al., 2018).

3.2. Growth performance

The effect of dietary lipid/EFA level on growth performance of L. *vannamei* reared at three different salinities is given in Table 4. Results revealed that the growth in terms of WG, SGR and DGC were higher in shrimp reared at 25‰ (135.65%, 1.90 and 1.38, respectively) than those reared at 2‰ (119.08%, 1.74 and 1.23) and 50‰ (105.09%, 1.58. and 1.10) regardless of the dietary lipid/EFA levels ($P \leq 0.001$). Similarly, diets containing 60/12 and 90/24 lipid/EFA showed significantly ($P < 0.05$) higher growth than that containing low lipid/EFA (Diet-30/12). However, the growth of shrimp reared at 25‰ was found to be significantly ($P < 0.05$) higher than those reared at 2 and 50‰ for the same diet except the Diet-90/24 at 2‰. Salinity had a significant ($P < 0.05$) influence on survival and was found to be high in shrimp reared at 25‰ (84.44%) compared to 2‰ (46.66%) and 50‰ (40.55%), whereas diet with high lipid/EFA (Diet-90/24) had a better survival in all three salinities tested compared to other two diets. Though condition factor was affected by both the factors (salinity and lipid/EFA level), the result of their interactions showed no significant difference among the treatments, but numerically lower condition factor was found at 60‰ (0.58–0.69) irrespective of the lipid/EFA levels. A higher correlation (Fig. 1) was observed when the growth was compared between the shrimp reared at 25 and 2‰ ($r = 1.0000$) as well as 25 and 50‰ ($r = 0.9696$). A similar tendency was noticed between the diet groups of 60/12 and 90/24 lipid/EFA level ($r = 0.9271$), whereas the correlation was found to be low ($r = 0.3356$) between Diet-60/12 and Diet-30/6 (Fig. 2).

Table 4
Effect of dietary lipid/EFA level on growth performance of *Litopenaeus vannamei* reared at different saline water.

Treatments	Growth performance				
	WG (%)[1]	SGR[2]	DGC[3]	Survival (%)	CF[4]
Salinity					
2‰	119.08^{b}	1.74^{b}	1.23^{b}	46.66^{b}	0.74^{a}
25‰	135.65^{a}	1.90^{a}	1.38^{a}	84.44^{a}	0.74^{a}
50‰	105.09^{c}	1.58^{c}	1.10^{c}	40.55^{c}	0.63^{b}
Lipid/EFA level					
30/6	106.05^{b}	1.59^{b}	1.11^{b}	42.77^{c}	0.68^{b}
60/12	125.31^{a}	1.80^{a}	1.28^{a}	60.00^{b}	0.77^{a}
90/24	128.45^{a}	1.83^{a}	1.31^{a}	68.88^{a}	0.66^{b}
Interactions					
2 × 30/6	104.97^{e}	1.59^{e}	1.11^{d}	31.66^{a}	0.70^{a}
2 × 60/12	119.89^{d}	1.75^{d}	1.23^{c}	48.33^{a}	0.81^{a}
2 × 90/24	132.37^{b}	1.87^{b}	1.34^{b}	60.00^{a}	0.72^{a}
25 × 30/6	129.44^{bc}	1.84^{bc}	1.33^{b}	75.00^{a}	0.71^{a}
25 × 60/12	145.98^{a}	2.00^{a}	1.47^{a}	86.66^{a}	0.82^{a}
25 × 90/24	131.52^{b}	1.86^{b}	1.33^{b}	91.66^{a}	0.70^{a}
50 × 30/6	83.75^{f}	1.35^{f}	0.89^{e}	21.66^{a}	0.63^{a}
50 × 60/12	110.06^{e}	1.64^{e}	1.15^{d}	45.00^{a}	0.69^{a}
50 × 90/24	121.46^{cd}	1.76^{cd}	1.25^{c}	55.00^{a}	0.58^{a}
P-value					
Salinity (A)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lipid/EFA level (B)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
A x B	< 0.001	< 0.001	< 0.001	0.101	0.096
Pooled SEM (+)	12.517	0.001	0.001	14.835	0.001

Mean bearing same superscript in a column within main effects and interactions between the categories do not differ significant ($P > 0.05$).
[1] Weight gain.
[2] Specific growth rate.
[3] Daily growth coefficient.
[4] Condition factor.

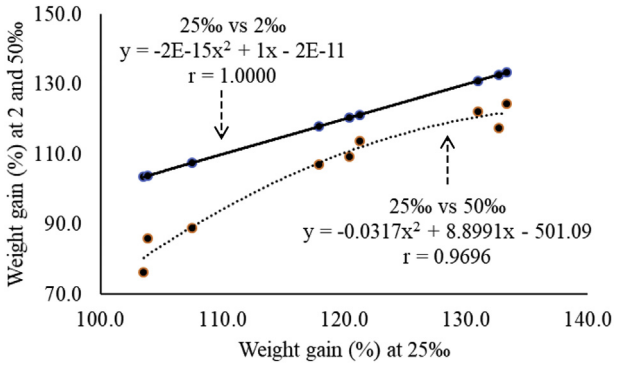

Fig. 1. Regression analysis on weight gain (%) of *Litopenaeus vannamei* reared at 25‰ with 2‰ and 50‰.

3.3. Hemolymph indices

Salinity had no effect on hemolymph total protein, whereas its level significantly ($P < 0.05$) increased with an increase in dietary lipid/EFA level (Table 5). A diet containing 60/12 lipid/EFA showed a higher ($P < 0.05$) total protein content in shrimp reared at 25‰ (9.34 g/dl), whereas high lipid diet (Diet-90/24) had significantly ($P < 0.05$) higher values in shrimp reared at hypo (2‰) and hyper (50‰) saline conditions. Both triglycerides and cholesterol significantly ($P < 0.05$) decreased with increase in salinity, whereas the reverse was true with increase in dietary lipid/EFA level. However, the interaction effects of salinity and lipid/EFA levels were non-significant.

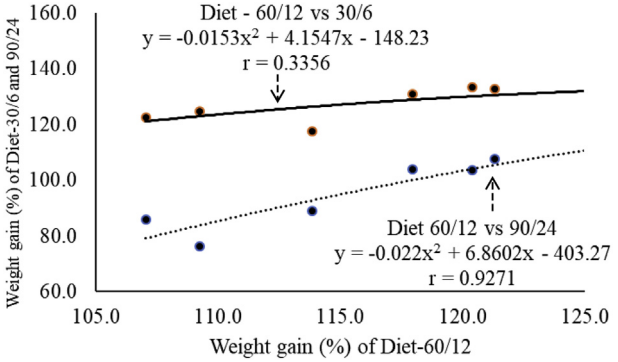

Fig. 2. Regression analysis on weight gain (%) of *Litopenaeus vannamei* fed diet containing 60/12 of lipid/essential fatty acid with those fed 30/6 and 90/24 diets.

Table 5
Effect of dietary lipid/EFA level on hemolymph indices of *Litopenaeus vannamei* reared at different saline water.

Treatments	Hemolymph indices		
	Total protein (g/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Salinity			
2‰	8.97^{a}	64.51^{a}	23.61^{a}
25‰	8.79^{a}	52.71^{b}	19.29^{b}
50‰	8.94^{a}	48.94^{c}	17.91^{c}
Lipid/EFA level			
30/6	8.48^{c}	48.87^{c}	17.88^{c}
60/12	8.84^{b}	53.77^{b}	19.68^{b}
90/24	9.38^{a}	63.52^{a}	23.24^{a}
Interactions			
2 × 30/6	8.54^{c}	56.17^{a}	20.56^{a}
2 × 60/12	8.49^{c}	63.56^{a}	23.26^{a}
2 × 90/24	9.90^{a}	73.81^{a}	27.01^{a}
25 × 30/6	8.46^{c}	46.74^{a}	17.10^{a}
25 × 60/12	9.34^{b}	51.06^{a}	18.69^{a}
25 × 90/24	8.56^{c}	60.34^{a}	22.08^{a}
50 × 30/6	8.43^{c}	43.70^{a}	15.99^{a}
50 × 60/12	8.69^{c}	46.70^{a}	17.09^{a}
50 × 90/24	9.69^{a}	56.41^{a}	20.64^{a}
P-value			
Salinity (A)	0.058	< 0.001	< 0.001
Lipid/EFA level (B)	< 0.001	< 0.001	< 0.001
A x B	< 0.001	0.269	0.269
SEM (±)	0.015	2.466	0.330

Mean bearing same superscript in a column within main effects and interactions between the categories do not differ significant ($P > 0.05$).

3.4. Body composition

Both salinity and lipid/EFA levels significantly ($P < 0.05$) decreased body moisture content from 821.36–772.56 and 795.33–786.56 g/kg, respectively. The highest moisture content of shrimp was found with 30/6 lipid/EFA diet at 2‰. Significantly higher crude protein content was observed in shrimp at 25‰ (161.58 g/kg) compared to other salinities (128.34 and 152.38 g/kg at 2 and 55‰, respectively). There was not much variation in crude protein due to dietary change (144.58–148.92 g/kg). However, shrimp reared at 25‰ had a higher value of crude protein irrespective of the lipid/EFA levels. Of all the treatments, shrimp fed with diets containing high lipid/EFA (Diet-90/24) showed higher body lipid as well those reared at high salinity (50‰) had higher ash content (Table 6). A clear trend was noticed for body fatty acid composition in shrimp fed varied lipid/EFA level (30/6, 60/12 and 90/24) at 2, 25 and 50‰. The fatty acid content was significantly ($P < 0.05$) abundant in the groups fed with high lipid

Table 6
Effect of dietary lipid level on carcass proximate composition of *Litopenaeus vannamei* reared at different saline water (g/kg wet basis).

Treatments	Carcass proximate composition			
	Moisture	Crude protein	Ether extract	Total ash
Salinity				
2‰	821.36^{a}	128.34^{c}	6.43^{a}	25.74^{c}
25‰	778.23^{b}	161.58^{a}	5.66^{c}	33.52^{b}
50‰	772.56^{c}	152.38^{b}	6.08^{b}	41.89^{a}
Lipid/EFA level				
30/6	795.33^{a}	144.58^{b}	5.66^{c}	33.53^{a}
60/12	790.26^{b}	148.80^{a}	6.04^{b}	33.85^{a}
90/24	786.56^{c}	148.92^{a}	6.48^{a}	33.78^{a}
Interactions				
2 × 30/6	831.64^{a}	120.99^{i}	5.81^{de}	25.74^{h}
2 × 60/12	823.21^{b}	125.38^{h}	6.64^{ab}	27.53^{g}
2 × 90/24	809.36^{c}	138.66^{g}	6.85^{a}	23.96^{i}
25 × 30/6	782.14^{d}	157.12^{c}	5.67^{ef}	35.12^{d}
25 × 60/12	774.81^{ef}	167.13^{a}	5.28^{g}	31.90^{f}
25 × 90/24	777.88^{de}	160.48^{b}	6.05^{cd}	33.56^{e}
50 × 30/6	772.36^{f}	155.63^{d}	5.49^{fg}	39.73^{c}
50 × 60/12	772.86^{f}	153.89^{e}	6.19^{c}	42.12^{b}
50 × 90/24	772.65^{f}	147.62^{f}	6.54^{b}	43.82^{a}
P-value				
Salinity (A)	< 0.001	< 0.001	< 0.001	< 0.001
Lipid/EFA level (B)	< 0.001	< 0.001	< 0.001	0.115
A x B	< 0.001	< 0.001	< 0.001	< 0.001
SEM (±)	4.156	0.254	0.017	0.058

Mean bearing same superscript in a column within main effects and interactions between the categories do not differ significant ($P > 0.05$).

diet (Diet-90/24) followed by Diet-60/12 and Diet-30/6 irrespective of the water salinity. Similarly, shrimp at 25‰ had a higher fatty acid composition than those reared at hyper (50‰) and hypo (2‰) salinity irrespective of the dietary lipid/EFA level (Table 7). Of all the analysed body minerals, salinity had a major influence on sodium content (1.68, 2.58 and 8.66 g/kg in shrimp reared at 2, 25 and 50‰, respectively). Calcium, phosphorus and potassium were found to be high in shrimp at 25‰ and shrimp reared at 50‰ had a higher value for magnesium compared to the rest of the treatments. However, dietary lipid/EFA levels did not significantly influence the body minerals (Table 8).

4. Discussion

In this study, *L. vannamei* reared at 25‰ showed better growth compared to other salinities (2 and 50‰). This is in agreement with the findings of Walker et al. (2009) and Xu et al. (2018) in L. *vannamei*, who reported that shrimp reared at high salinity (28 and 25‰, respectively) had a higher growth rate than those at lower salinities (2 and 3‰). Diaz et al. (2001) reported that water salinity should be near the isosmotic point of 25–26.7‰ (712–764 mM/kg) for better growth because less energy would be expended for osmoregulation. *L. vannamei* had hypo osmolality, when it was exposed to an environmental condition contain high isosmotic point and the reverse was true for low isosmotic point. Shrimp fed diets with 60/12 and 90/24 lipid/EFA showed significantly higher WG (125.31 and 128.45%, respectively) than those fed a low lipid/EFA diet (Diet-30/6) regardless of the rearing salinity. Shrimp fed diet with high content of lipid/EFA would be beneficial in enhancing the growth rate as reflected by the increased WG from 104.97% (30/6 lipid/EFA) to 132.37% (90/24% lipid/EFA) at 2‰ and as well from 83.75% to 121.46% at 50‰. However, shrimp fed diet with high lipid/EFA (Diet-90/24) had significantly lower growth compared to a Diet-60/12 (145.98–131.52%) at 25‰. The result is in consonance with the findings of Xu et al. (2018). Xu et al. (2018) stated that the excessive dietary lipid causes a metabolic burden in the cultured species, since lipid is an important cellular component and more susceptible to the

Table 7
Effect of dietary lipid level on carcass major fatty acid composition of *Litopenaeus vannamei* reared at different saline water (mg/kg wet basis).

Treatments	Carcass fatty acids									
	C16:0	C18:0	C16:1	C18:1c	C18:1 t	C18:2	C18:3	C20:4	C20:5	C22:6
Salinity										
2‰	687.74^{c}	541.28^{c}	62.12^{c}	238.64^{c}	202.20^{c}	395.90^{c}	58.89^{c}	218.73^{c}	385.67^{c}	231.23^{c}
25‰	1041.96^{a}	755.75^{a}	91.72^{a}	381.57^{a}	344.06^{a}	713.89^{a}	77.73^{a}	293.40^{a}	523.05^{a}	369.78^{a}
50‰	982.82^{b}	649.48^{b}	81.00^{b}	312.69^{b}	322.54^{b}	564.06^{b}	74.71^{b}	262.51^{b}	441.34^{b}	338.23^{b}
Lipid/EFA level										
30/6	786.31^{c}	566.60^{c}	65.32^{c}	278.30^{c}	249.04^{c}	509.88^{c}	63.20^{c}	235.42^{c}	401.08^{c}	277.26^{c}
60/12	910.80^{b}	609.70^{b}	78.07^{b}	298.08^{b}	282.64^{b}	553.12^{b}	68.67^{b}	243.90^{b}	433.60^{b}	306.79^{b}
90/24	1015.40^{a}	770.21^{a}	91.44^{a}	356.51^{a}	337.12^{a}	610.84^{a}	79.46^{a}	295.31^{a}	515.37^{a}	355.20^{a}
Interactions										
2 × 30/6	575.40^{g}	446.35^{g}	53.07^{f}	204.40^{h}	173.18^{f}	309.55^{h}	47.93^{h}	202.45^{g}	348.12^{g}	195.51^{i}
2 × 60/12	738.89^{f}	515.71^{f}	60.24^{ef}	239.74^{g}	207.68^{e}	451.57^{f}	62.12^{fg}	200.47^{g}	341.52^{g}	243.28^{h}
2 × 90/24	748.94^{f}	661.80^{d}	73.05^{d}	271.80^{e}	225.74^{d}	426.60^{g}	66.64^{ef}	253.28^{e}	467.37^{d}	254.92^{g}
25 × 30/6	979.24^{d}	735.27^{c}	79.92^{cd}	371.00^{b}	319.23^{b}	758.13^{a}	82.79^{b}	289.22^{c}	489.70^{c}	369.52^{c}
25 × 60/12	1010.06^{c}	724.60^{c}	85.81^{bc}	354.58^{c}	321.55^{b}	663.36^{d}	73.28^{cd}	276.95^{d}	516.46^{b}	359.34^{d}
25 × 90/24	1136.58^{b}	807.38^{b}	109.40^{a}	419.13^{a}	391.41^{a}	720.18^{b}	77.12^{c}	314.03^{b}	562.99^{a}	380.50^{b}
50 × 30/6	804.31^{e}	518.20^{f}	62.99^{e}	259.52^{f}	254.72^{c}	461.98^{f}	58.90^{g}	214.61^{f}	365.44^{f}	266.75^{f}
50 × 60/12	983.47^{d}	588.79^{e}	88.17^{b}	299.93^{d}	318.69^{b}	544.45^{e}	70.62^{de}	254.29^{e}	442.84^{e}	317.76^{e}
50 × 90/24	1160.68^{a}	841.47^{a}	91.84^{b}	378.62^{b}	394.23^{a}	685.76^{c}	94.63^{a}	318.64^{a}	515.75^{b}	430.19^{a}
P-value										
Salinity (A)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lipid/EFA level (B)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
A × B	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SEM (±)	22.318	25.277	10.148	24.318	8.021	68.864	5.052	1.281	20.318	12.712

Mean bearing same superscript in a column within main effects and interactions between the categories do not differ significant ($P > 0.05$).

damage by free radicals, which causes the failure of the anti-oxidant system. This would probably be a possible reason for obtaining a lower growth in shrimp reared with high lipid diet as observed in the present study. In contrast, at both hypo- and hyper-osmotic conditions, an increased dietary lipid/EFA content improved the growth of the shrimp.

In the present investigation, an increased lipid/EFA level of 90/24 g/kg has shown an improvement in the growth of shrimp at both hypo (2‰) and hyper salinity (50‰) compared to an optimal salinity of 25‰. This confirmed that the excess lipid provides energy to fulfil the energy demand at both hypo and hyper osmotic stress conditions. In earlier studies, it was documented that the supplementation of EFA enhanced the tolerance of shrimp post larvae (Rees et al., 1994) and juveniles (Chim et al., 2001) when they were suddenly exposed to hypo-osmotic conditions. Both salinity and dietary lipid/EFA had a significant effect on survival and was in the range of 31.66–91.66%. The correlation coefficient for WG among the salinities tested in our study showed almost similar values, whereas wide variations were found in growth between the experimental diets containing different

Table 8
Effect of dietary lipid level on carcass macro-mineral composition of *Litopenaeus vannamei* reared at different saline water (g/kg wet basis).

Treatments	Carcass macro-minerals				
	Calcium	Magnesium	Phosphorus	Potassium	Sodium
Salinity					
2‰	6.88^{b}	0.26^{c}	2.26^{c}	2.06^{b}	1.68^{c}
25‰	7.51^{a}	0.60^{b}	2.44^{a}	2.74^{a}	2.58^{b}
50‰	5.24^{c}	0.65^{a}	2.36^{b}	1.77^{c}	8.66^{a}
Lipid/EFA level					
30/6	6.28^{c}	0.52^{a}	2.31^{b}	2.08^{c}	4.24^{c}
60/12	6.58^{b}	0.51^{b}	2.33^{b}	2.21^{b}	4.40^{a}
90/24	6.78^{a}	0.48^{c}	2.43^{a}	2.28^{a}	4.29^{b}
Interactions					
2 × 30/6	5.61^{e}	0.23^{g}	1.99^{d}	1.65^{f}	1.21^{g}
2 × 60/12	6.97^{c}	0.25^{f}	2.22^{c}	2.20^{c}	1.90^{f}
2 × 90/24	8.05^{a}	0.30^{e}	2.58^{a}	2.33^{b}	1.94^{f}
25 × 30/6	8.03^{a}	0.65^{c}	2.56^{a}	2.74^{a}	2.75^{c}
25 × 60/12	6.97^{c}	0.57^{d}	2.38^{b}	2.74^{a}	2.53^{d}
25 × 90/24	7.53^{b}	0.57^{d}	2.37^{b}	2.73^{a}	2.47^{e}
50 × 30/6	5.19^{f}	0.68^{b}	2.38^{b}	1.86^{d}	8.75^{a}
50 × 60/12	5.80^{d}	0.70^{a}	2.37^{b}	1.68^{f}	8.78^{a}
50 × 90/24	4.75^{g}	0.56^{d}	2.33^{bc}	1.77^{e}	8.47^{b}
P-value					
Salinity (A)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lipid/EFA level (B)	< 0.001	< 0.001	0.004	< 0.001	< 0.001
A x B	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SEM (±)	0.006	0.001	0.003	0.001	0.001

Mean bearing same superscript in a column within main effects and interactions between the categories do not differ significant ($P > 0.05$).

lipid/EFA level. Survival across salinities showed a significant variation and was relatively high in shrimp at 25‰ (84.44%) compared to 2‰ (46.66%) and 50‰ (40.55%). This clearly indicates that L. *vannamei* has indeed, a limited capability to tolerate the extreme hypo and hyper saline conditions.

The level of hemolymph triglycerides, cholesterol and total protein significantly increased with an increase in dietary lipid/EFA levels, whereas the increase of salinity significantly decreased triglycerides and cholesterol, but did not affect total protein. Decreased body moisture content with increased salinity is consistent with our earlier findings (Jannathulla et al., 2017), where L. *vannamei* was exposed to 3–60‰. Sang and Fotedar (2004) documented that shrimp catabolise energy sources from their body to fulfil the energy demand during stress conditions. This would be a reason for obtaining higher crude protein content at 25‰ (161.58 g/kg) compared to 2 and 50‰ (128.34 and 152.38 g/kg, respectively). Contrary to the above hypothesis, shrimp reared at 25‰ had a lower level of ether extract (5.66 g/kg) than other treatments (6.08–6.43 g/kg). A similar higher deposition of lipid has been reported earlier in euryhaline crab, *Chasmagnathus granulata* (Luvizotto-Santos et al., 2003) due to salinity stress. The difference in protein and lipid was not so pronounced among the treatments, but the evident difference was noticed in the total ash content at hyper osmotic condition (50‰) regardless of dietary lipid/EFA levels. Its level significantly increased from 25.74 to 41.89 g/kg with increasing salinity.

The fatty acids like C16:0, C18:0, C18:1, C18:2, C18:3, C20:5 and C22:6 were the major ones in experimental diets (Table 2) and were well reflected in the shrimp body. This result is in agreement with earlier findings in Indian white shrimp, *Fenneropenaeus indicus* (Colvin, 1976) and *P. monodon* (Deering et al., 1997). Romano et al. (2014) reported an increasing trend of n-3 LC-PUFA in *Scylla serrata* with a decrease in salinity. However, in our study, the shrimp reared at 25‰ have shown a higher deposition of fatty acids regardless of dietary lipid/EFA levels compared to 2 and 50‰. The results of the present investigation appear to support for supplementing n-3 LC-PUFA to enhance the osmoregulatory abilities of the aquatic species, as already described in *P. monodon* (Rees et al., 1994) and L. *vannamei* (Hurtado et al., 2006). The fatty acid content in the body significantly increased with increasing dietary lipid/EFA levels in all three salinities (2, 25 and 50‰). However, the utilization of fatty acids differed in shrimp reared at both the stress conditions (2 and 50‰). The body fatty acid profiles being lower in shrimp at 2‰ than in those reared at 50‰, indicating that fatty acids could be effectively utilized by shrimp reared at hypo-osmotic stress than hyper-osmotic stress. This could probably be a reason for better growth and survival at 2‰ compared to 50‰ (Table 4). The changes in fatty acid content of aquatic species can alter the osmoregulation process by modulating permeability of water and ions and the activity of an enzyme, Na^+/K^+ ATPase (Turner et al., 2003). This is in agreement with the findings of Morris et al. (1982), who reported the effect of permeability of water in an amphipod exposed to diluted sea water by using the changes in the fatty acid composition of gills. Palacios et al. (2004) has observed the fatty acid change in L. *vannamei* reared at 10‰ compared to 30‰ with PUFA-enriched diet, but not on Na^+/K^+ ATPase activity. In our study, n-3 PUFAs, in particular C20:5 and C22:6 were positively correlated with dietary lipid/EFA level while, the deposition of fatty acids varied according to the rearing salinity. This result suggests that though dietary lipid/EFA influences the body fatty acid composition, preferential utilization differs according to the salinity.

It was observed that both salinity and dietary lipid/EFA levels significantly affected the minerals in shrimp. The level of body calcium increased from 6.88 to 7.51 g/kg (wet basis) with the increase of salinity from 2 to 25‰ regardless of dietary lipid/EFA level and was significantly reduced to 5.24 g/kg at hyper saline condition (50‰). Stevenson (1985) reported that crustaceans store calcium in the body as well in hemolymph when cultured in freshwater for cuticular calcification, whereas marine penaeids may not need to store calcium, as it is readily available in the environment. This could be a possible reason for obtaining lower deposition of calcium in the shrimp body at 50‰ in our study. The levels of magnesium, potassium and phosphorus increased significantly with an increase in salinity and decreased with the increase in dietary lipid/EFA level. The increase of sodium was more pronounced with increasing water salinity compared to other elements in experimental water (Table 3) and the same was reflected in the shrimp body (Table 8). It is reported in earlier studies (Jannathulla et al., 2017) that L. *vannamei* had a better homeostasis process and tissue mineralization for sodium at low salinity compared to high saline conditions, which could be a possible reason for the deposition of higher sodium content in shrimp at 50‰.

5. Conclusion

It could be concluded that a combination of 25‰ salinity and 60/12 g/kg of dietary lipid/EFA would be ideal for obtaining a higher growth performance in L. *vannamei*. The salinity stress could be ameliorated to a certain extent by increasing the level of lipid/EFA in the diet, however, a better tolerance was observed in shrimp reared at 2‰ (hypo) compared to 50‰ (hyper) salinity.

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References

Akiyama, D.M., Dominy, W.G., Lawrence, A.L., 1991. Penaeid shrimp nutrition for the commercial feed industry. In: Fast, A.L., Lester, L.J. (Eds.), Marine Shrimp Culture: Principles and Practices. Elsevier Science Publishers BV, Amsterdam, The Netherlands, pp. 535–568.

AOAC, 1997. Official Methods of Analysis of Association of Analytical Chemists, 18th edn. Benjamin Franklin Station, Washington DC Association of Official Analytical Chemists.

APHA, 2012. Standard Methods for the Examination of Water and Waste Water, 22nd edn. American Public Health Association, New York.

Aziz, N.A., Azlan, A., Ismail, A., Mohd Alinafiah, S., Razman, M.R., 2012. Quantitative determination of fatty acids in marine fish and shellfish from warm water of Straits of Malacca for nutraceutical purposes. Biomed. Res. Int. 1–12. https://doi.org/10.1155/2013/284329.

Castille, F.L., Lawrence, A.L., 1981. The effect of salinity on the osmotic, sodium, and chloride concentrations in the hemolymph of the freshwater shrimps, *Macrobrachium ohione* Smith and *Macrobrachium rosenbergii* de Man. Comp. Biochem. Physiol. Part-A. 70, 47–52. https://doi.org/10.1016/0300-9629(81)90320-0.

Chen, K., Li, E., Gan, L., Wang, X., Xu, C., Lin, H., Qin, J.G., Chen, L., 2014. Growth and lipid metabolism of the pacific white shrimp *Litopenaeus vannamei* at different salinities. J. Shellfish Res. 33, 825–833. https://doi.org/10.2983/035.033.0317.

Chim, L., Lemaire, P., Delaporte, M., Le Moullac, G., Galois, R., Martin, J.L., 2001. Could a diet enriched with n-3 highly unsaturated fatty acids be considered a promising way to enhance the immune defences and the resistance of Penaeid prawns to environmental stress? Aquac. Res. 32, 91–94. https://doi.org/10.1046/j.1365-2109.2001.00534.x.

Colvin, P.M., 1976. Nutritional studies on penaeid prawns: protein requirements in compounded diets for juvenile *Penaeus indicus* (Milne Edwards). Aquaculture 7, 315–326. https://doi.org/10.1016/0044-8486(76)90128-9.

Cruz-Suarez, L.E., Ricque-Marie, D., Pinal-Mansilla, J.D., Wesche-Ebelling, P., 1994. Effect of different carbohydrate sources on the growth of *P. vannamei*: economic impact. Aquaculture 123, 349–360. https://doi.org/10.1016/0044-8486(94)90070-1.

Dayal, J.S., Rajaram, V., Ambasankar, K., Ahamad Ali, S., 2011. Sunflower oil cake as a replacement for fishmeal in feeds of Tiger shrimp, *Penaeus monodon* reared in tanks and in net cages. Indian. J. Mar. Sci. 40, 460–470.

Deering, M.J., Fielder, D.R., Hewitt, D.R., 1997. Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. Aquaculture 151, 131–141. https://doi.org/10.1016/S0044-8486(96)01480-9.

Diaz, F., Farfan, C., Sierra, E., Re, A.D., 2001. Effects of temperature and salinity

fluctuation on the ammonium excretion and osmoregulation of juveniles of *Penaeus vannamei*, Boone. Mar. Freshwater Behav. Physiol. 34, 93–104. https://doi.org/10.1080/10236240109379062.
Finlayson, A.J., 1964. Amino acid recovering in the analysis of some feed samples. Can. J. Plant Sci. 45, 184–188.
Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Garling, D.L., Wilson, R.P., 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. J. Nutr. 106, 1368–1375. https://doi.org/10.1093/jn/106.9.1368.
Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. Optimising the essential fatty acids in the diet for weight gain of the prawn, *Penaeus monodon*. Aquaculture 204, 85–99. https://doi.org/10.1016/S0044-8486(01)00644-5.
Hidalgo, M.C., Sanz, A., Garcia Gallego, M., Saurez, M.D., de la Higuera, M., 1993. Feeding of the European eel *Anguilla anguilla*. I. Influence of dietary carbohydrate level. Comp. Biochem. Physiol. 105A, 165–169. https://doi.org/10.1016/0300-9629(93)90192-7.
Huang, K., Wang, W., Lu, J., 2003. Protein requirements in compounded diets for *Litopenaeus vannamei* juveniles. J. Fish. Sci. China 10, 308–321.
Hurtado, M.A., Racotta, I.S., Arjona, O., Hernández-Rodríguez, M., Goytortúa, E., Civera, R., Palacios, E., 2006. Effect of hypo and hyper saline conditions on osmolarity and fatty acid composition of juvenile shrimp *Litopenaeus vannamei* (Boone, 1931) fed low and high HUFA diets. Aquac. Res. 37, 1316–1326. https://doi.org/10.1111/j.1365-2109.2006.01568.x.
Jajic, I., Krstovic, S., Glamocis, D., Jaksis, S., Abramovic, B., 2013. Validation of an HPLC method for the determination of amino acids in feed. J. Serb. Chem. Soc. 78, 839–850. https://doi.org/10.2298/JSC120712144J.
Jannathulla, R., Syama Dayal, J., Chitra, V., Ambasankar, K., Muralidhar, M., 2017. Growth and carcass mineralization of Pacific whiteleg shrimp *Penaeus vannamei* Boone 1931 in response to water salinity. Indian J. Fish. 64, 22–27.
Jannathulla, R., Dayal, J.S., Ambasankar, K., Eugine, A.C., Muralidhar, M., 2018. Fungus, *Aspergillus niger*, fermented groundnut oil cake as a fishmeal alternative in the diet of *Penaeus vannamei*. Aquac. Res. 49, 2891–2902. https://doi.org/10.1111/are.13756.
Li, E.C., Arena, L., Lizama, G., Gzxiola, G., Cuzon, G., Rosas, Carlos, Chen, L.Q., Van Wormhoudt, A., 2011. Glutamate dehydrogenase and Na+-K+ ATPase expression and growth response of *Litopenaesus vannamei* to different salinities and dietary protein levels. Chin. J. Oceanol. Limnol. 29, 343–349.
Li, E., Wang, X., Chen, K., Xu, C., Qin, J.G., Chen, L., 2015. Physiological change and nutritional requirement of Pacific white shrimp *Litopenaeus vannamei* at low salinity. Rev. Aquacult. 7, 1–19. https://doi.org/10.1111/raq.12104.
Luvizotto-Santos, R., Lee, J.T., Branco, Z.P., Bianchini, A., Nery, L.E., 2003. Lipids as energy source during salinity acclimation in the euryhaline crab *Chasmagnathus granulata* dana, 1851 (crustacea-grapsidae). J. Exp. Zool. Part-A 295, 200–205. https://doi.org/10.1002/jez.a.10219.
Macias-Sancho, J., Poersch, L.H., Bauer, W., Romano, L.A., Wasielesky, W., Tesser, M.B., 2014. Fishmeal substitution with Arthrospira (*Spirulina platensis*) in a practical diet for *Litopenaeus vannamei*: effects on growth and immunological parameters. Aquaculture 426, 120–125. https://doi.org/10.1016/j.aquaculture.2014.01.028.
Merican, Z.O., Shim, K.F., 1996. Qualitative requirements of essential fatty acids for juvenile *Penaeus monodon*. Aquaculture 147, 275–291. https://doi.org/10.1016/S0044-8486(96)01379-8.
Metcalfe, L.D., Schimitz, A., Pelka, J.R., 1966. Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. Annexe Chem. 38, 524–535. https://doi.org/10.1021/ac60235a044.
Morris, R.J., Lockwood, P.M., Dawson, M.E., 1982. An effect of acclimation salinityon the fattyacid composition of the gill phospholipids and water flux of the amphipod crustacean *Gamarus duebeni*. Comp. Biochem. Physiol. 497–503. https://doi.org/10.1016/0300-9629(82)90114-1. 72A.
MPEDA, 2017. India's Seafood Export at all-Time High in 2016–17. http://www.mpeda.org.in (accessed 18 July 2018).
Nordgarden, U., Hemrem, G.I., Hansen, T., 2002. Growth and body composition of Atlantic salmon (*Salmo salar* L.) parr and smolt fed diets varying in protein and lipid contents. Aquaculture 207, 65–78. https://doi.org/10.1016/S0044-8486(01)00750-5.
Palacios, E., Bonilla, A., Luna, D., Racotta, I.S., 2004. Survival: Na^+/K^+-ATPase and lipid responses to salinity challenge in fed and starved white Pacific shrimp (*Litopenaeus vannamei*) postlarvae. Aquaculture 234, 497–511. https://doi.org/10.1016/j.aquaculture.2003.12.001.
Pieper, A., Pfeffer, E., 1980. Studies on the effects of increasing proportion of sucrose or gelatinized maize starch in diet for rainbow trout (*Salmo gairdneri*, R) on the utilization of dietary energy and protein. Aquaculture 20, 333–342. https://doi.org/10.1016/0044-8486(80)90094-0.
Ponce-Palafox, J., Martinez-Palacios, C.A., Ross, L.G., 1997. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture 157, 107–115. https://doi.org/10.1016/S0044-8486(97)00148-8.
Ponce-Palafox, J.T., Pavia, Á.A., López, D.G., Arredondo-Figueroa, J.L., Lango-Reynoso, F., Del Refugio Castañeda-Chávez, M., Esparza-Leal, H., Ruiz-Luna, A., Páez-Ozuna, F., Castillo-Vargasmachuca, S.G., Peraza-Gómez, V., 2019. Response surface analysis of temperature-salinity interaction effects on water quality, growth and survival of shrimp *Penaeus vannamei* postlarvae raised in biofloc intensive nursery production. Aquaculture 503, 312–321. https://doi.org/10.1016/j.aquaculture.2019.01.020.
Rees, J.F., Curé, K., Piyatiratitivorakul, S., Sorgeloos, P., Menasveta, P., 1994. Highly unsaturated fatty acid requirements of *Penaeus monodon* postlarvae: an experimental approach based on Artemia enrichment. Aquaculture 122, 193–207. https://doi.org/10.1016/0044-8486(94)90510-X.
Romano, N., Zeng, C., Noordin, N.M., Ng, W.K., 2012. Improving the survival, growth and hemolymph ion maintenance of early juvenile blue swimmer crabs, *Portunus pelagicus*, at hypo-and hyper-osmotic conditions through dietary long chain PUFA supplementation. Aquaculture 15, 24–30. https://doi.org/10.1016/j.aquaculture.2012.02.013.
Romano, N., Wu, X., Zeng, C., Genodepa, J., Elliman, J., 2014. Growth, osmoregulatory responses and changes to the lipid and fatty acid composition of organs from the mud crab, *Scylla serrata*, over a broad salinity range. Mar. Biol. Res. 10, 460–471. https://doi.org/10.1080/17451000.2013.819981.
Sang, H.M., Fotedar, R., 2004. Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities. Aquaculture 234, 601–614. https://doi.org/10.1016/j.aquaculture.2004.01.008.
Sastry, C.S.P., Tammuru, M.K., 1985. Spectrophotometric determination of tryptophan in protein. J. Food Sci. Technol. 22, 146–147.
Söderhäll, K., Smith, V.J., 1983. Separation of the haemocyte populations of Carcinusmaenas and other marine decapods, and prophenoloxidase distribution. Dev. Comp. Immunol. 7, 229–239. https://doi.org/10.1016/0145-305X(83)90004-6.
Stevenson, J.R., 1985. Dynamics of the integument. In: Bliss, D.E., Mantel, L.H. (Eds.), Integument Pigments and Hormonal Processes. The Biology of Crustacean. Academic Press, New York, USA, pp. 2–42.
Sui, L., Ma, G., Deng, Y., 2015. Effect of dietary protein level and salinity on growth, survival, enzymatic activities and amino-acid composition of the white shrimp *Litopenaeus vannamei* (Boone, 1931) juveniles. Crustaceana 88, 82–95.
Tseng, Y.C., Hwang, P.P., 2008. Some insights into energy metabolism for osmoregulation in fish. Comp. Biochem. Physiol. Part C 148, 419–429. https://doi.org/10.1016/j.cbpc.2008.04.009.
Turner, N., Else, P.L., Hulbert, A.J., 2003. Docosahexaenoic acid (DHA) content of membranes determines molecular activity of the sodium pump: implications for disease states and metabolism. Naturwissenschaften 90, 521–523. https://doi.org/10.1007/s00114-003-0470-z.
Walker, S.J., Neill, W.H., Lawrence, A.L., Gatlin, D.M., 2009. Effect of salinity and body weight on ecophysiological performance of the Pacific white shrimp (*Litopenaeus vannamei*). J. Exp. Mar. Biol. Ecol. 380, 119–124. https://doi.org/10.1016/j.jembe.2009.08.014.
Wang, X.D., Li, E.C., Wang, S.F., Qin, J.G., Chen, X.F., Lai, Q.M., Chen, K., Xu, C., Gan, L., Yu, N., Du, Z.Y., 2015. Protein-sparing effect of carbohydrate in the diet of white shrimp *Litopenaeus vannamei* at low salinity. Aquac. Nutr. 21, 904–912. https://doi.org/10.1111/anu.12221.
Xu, C., Li, E., Liu, Y., Wang, S., Wang, X., Chen, K., Qin, J.G., Chen, L., 2018. Effect of dietary lipid level on growth, lipid metabolism and health status of the Pacific white shrimp *Litopenaeus vannamei* at two salinities. Aquac. Nutr. 24, 204–214. https://doi.org/10.1111/anu.12548.
Zhu, X.Z., Liu, Y.J., Tian, L.X., Mai, K.S., Zheng, S.X., Pan, Q.J., Cai, M.C., Zheng, C.Q., Zhang, Q.H., Hu, Y., 2010. Effects of dietary protein and lipid levels on growth and energy productive value of pacific white shrimp, *Litopenaeus vannamei*, at different salinities. Aquac. Nutr. 16, 392–399. https://doi.org/10.1111/j.1365-2095.2009.00677.x.