

Effect of different dietary lipid levels on growth, digestive enzyme activity and immune status of the Pacific white shrimp *Litopenaeus vannamei* under two salinity levels

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Abstract: The purpose of the current study was to examine the effect of five varying lipid levels (6%, 8%, 10%, 12%, and 14%) combined with two different salinity levels (15 and 30 ppt) on growth performance, survival, serum antioxidant response and digestive enzyme activity of white leg shrimp, *Litopenaeus vannamei*. The study was conducted in thirty rectangular Separate tanks (measuring 66 x 47 x 44 cm, holding 50 L) reinforced with water salinity levels of 15 and 30 ppt over a period of 90 days. There were 3 replicates for each treatment; each tank held 30 post-larvae with a range body weight of 0.02g. Shrimp were fed experimental diets contain almost 35% crude protein four times a day, equivalent to 14% of their initial body weight, which was slowly decreased to 5% by the end of the study. The best treatment, which contained 6% lipid and salinity of 30 ppt, showed the best result in growth performance and survival (%). The levels of trypsin activity in the intestines were notably higher in white shrimp in the group exposed to a salinity of 30 compared to those in the group exposed to a salinity of 15. The greatest amount of lipase activity was observed when shrimp were fed diets with 6, 8, and 10% lipid levels. It was determined that shrimp fed a diet containing 14% lipid at 15 and 30 ppt had the highest crude lipid content, while shrimp at 15 ppt exhibited the highest GSH-Px activity. These findings were observed under experimental circumstances.

Keywords: growth performance, survival (%), serum antioxidant, trypsin, lipase and white shrimp.

INTRODUCTION

Litopenaeus vannamei, commonly known as the Pacific white shrimp, is a highly significant species in global shrimp farming (Hu *et al.*, 2004). Currently, the cultivation of *L. vannamei* in inland salty water is growing as a new industry because of its ability to tolerate a broad range of salinity levels from 0.5 to 50‰ (Saoud *et al.*, 2003). Even though farming *L. vannamei* in low salinity can be profitable, there are challenges and risks in commercial production like slow growth, low survival rates, weak immune system, and low tolerance to water contaminants. Decreased salt levels in the body can limit salt movement from the blood to tissues or surroundings, causing cell swelling as water is taken in (Davis *et al.*, 2002). During periods of high salinity, water-dwelling creatures must adjust to the changing water conditions through osmoregulation, achieved by altering different enzymes and transporters, which is known to require a significant amount of energy (Tseng and Hwang, 2008). The favourable characteristics of this species make it the top option for shrimp farming, with one being that shrimp can regulate their internal osmotic concentration when faced with varying water salinity levels, aiding in their adaptation to their environment (Jannathulla *et al.*, 2020). In order to sustain shrimp populations and promote their development in the wild, the ability to adapt physiologically to changes in salinity is crucial for their survival and food intake in different growth conditions (Jaffer *et al.*, 2020). Research has assessed the ideal dietary lipid levels for *L. vannamei* at various salinity levels, indicating that the optimum lipid level in the diet varies depending on the salinity (Zhu *et al.*, 2010). Past research has indicated that increased dietary lipids can enhance the growth and immunity of *L. vannamei* in low

salinity conditions (Zhu *et al.*, 2010 and Zhang *et al.*, 2013). However, an overabundance of dietary fat can hinder shrimp growth and lead to higher mortality rates because of nutrient imbalance and lipid toxicity from lipid oxidation (Akiyama *et al.*, 1992). The intermittent studies show clear but inconsistent results on how dietary lipid levels and salinities affect physiological impact. In addition, the health condition and lipid breakdown are two important factors used to evaluate how organisms react to changes in dietary lipids when faced with low salinity stress. TGL and CPT-1, which are important enzymes for breaking down triglycerides and oxidizing long-chain fatty acids in mitochondria, have been studied in various reports on crustaceans (Chen *et al.*, 2015). TNF- α has the ability to control several cellular functions such as immune response, inflammation, cell death, cell specialization, growth, and immune system components activation (Goetz *et al.*, 2004 and Sun *et al.*, 2013). Yet, there is limited study on the variation in lipid breakdown and immune or inflammatory components in *L. vannamei* when given various lipid levels in low salinity environments.

This research examined five varied lipid diets (6, 8, 10, 12, and 14%) affected *L. vannamei* post-larvae under two salinity levels (15 and 30 ppt), focusing on growth performance, survival, muscle composition, antioxidant response in serum, and enzyme activity.

MATERIALS AND METHODS

Experimental diets preparation and feeding regime

Five diets containing the same amount of nitrogen (35% crude protein) were formulated, by adding each different levels of crude lipid (6, 8, 10, 12, and 14%). The diets were formulated by weighing each component separately and carefully blending the minerals, vitamins, and

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additives with corn. This blend was mixed with the ingredients along with the oil. Water was added until the mixture reached the right consistency for forming granules. The damp mixture was fed through a CBM granule machine that had a diameter of 2 mm. The pellets that were made were air-dried and frozen before the experiment began. A portion of the manufactured feed was ground to a diameter of 600 to 900 and 1200 microns to be suitable for feeding shrimp, post-larvae, at this stage. The experimental diets' ingredient and proximate composition are shown in Table (1). Analysis of diets was performed three times to determine their proximate composition based on the (AOAC,2019) method. Shrimp were given experimental diets at a rate of 14% of their initial body weight four times a day, with the amount gradually reduced to 5% by the end of the study. The daily percentage of food given for each treatment was determined and modified by using the average biomass measured every 15 days.

Experimental Design:-

The post-larvae of *L. vannamei* white leg shrimp were acquired from a shrimp hatchery in Berket Ghalioun, Kafr Al-Sheikh, Egypt. Shrimps were carried in polythene bags with two layers and oxygen. Upon arrival at the laboratory, the shrimp were transferred to acclimation tanks containing seawater filtered through a plankton net (50µm) to exclude unwanted substances

and suspended particles. Fresh water was added to adjust the salinity to (15 and 30 ppt). Before beginning the experiment, the shrimps were adjusted to the laboratory environment for a period of two weeks and fed on a commercial diet (38% crude protein) twice a day. Samples were initially collected right after post-larvae were obtained from the hatchery, and a final sample was obtained from each tank at the end of the experiment to determine chemical body composition.

Following a two-week acclimatization period, shrimp post-larvae were distributed to all tanks in three replicates. Prior to putting the shrimp in tanks, we measured the shrimp's weight, starting with their body weight (in grams). During the experiment, the following parameters were observed to keep the water quality optimal for shrimp culture.

Daily parameters: salinity and pH were measured daily at 10:00h, water temperature (°C) was measured daily at 13:00h using multi parameter analyser.

Biweekly parameters: water sample (100ml) were collected from each tank and filtered by filter papers to analyse total ammonium nitrogen (TAN), nitrite-N (NO₂-N), nitrate-N (NO₃-N) using spectrophotometer model (JENWAY 6100) (APHA, 1998).

Table (1): Formulation and proximate composition of the experimental diets (Dry matter base)

| Ingredients | Treatments | | | | |
|--|------------|---------|----------|----------|----------|
| | T1 (6%) | T2 (8%) | T3 (10%) | T4 (12%) | T5 (14%) |
| fish meal (70%) protein | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Soybean meal (44%) protein | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Yellow corn | 32.50 | 30.50 | 28.50 | 26.50 | 24.50 |
| Sun flour oil | 3.00 | 5.00 | 7.00 | 9.00 | 11.00 |
| Cholesterol | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin mixture ¹ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Mineral mixture ² | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Molasses | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Proximate analysis | | | | | |
| Moisture | 9.61 | 8.96 | 8.51 | 9.54 | 8.84 |
| Protein | 35.71 | 35.54 | 35.37 | 35.19 | 35.02 |
| Lipids | 6.67 | 8.60 | 10.53 | 12.46 | 14.39 |
| Ash | 5.23 | 5.21 | 5.18 | 5.16 | 5.13 |
| Fiber | 3.46 | 3.42 | 3.37 | 3.33 | 3.28 |
| NFE ³ | 48.93 | 47.23 | 45.55 | 43.86 | 42.18 |
| Gross energy (Kcal /100g) ⁴ | 465.89 | 476.19 | 486.56 | 496.84 | 507.21 |

¹ Each Kg vitamin contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg.

² Each Kg mineral mixture premix contained Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

³ Nitrogen Free Extract = 100 - (%Protein + %Fat + %Fiber + %Ash).

⁴ Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to (NRC, 2011)

Body Composition: Following the experiment, *L. vannamei* were deprived of feed for 24 hours and subsequently both counted and weighed. Three random pooled sample of shrimps were sedated in a cold bath from every container, and then haemolymph was drawn with a heparin-free

tuberculin syringe. Following an overnight clotting period at 4 °C and centrifugation at 7000 rpm at 4 °C for 15 minutes, haemolymph samples were preserved at -80 °C in a refrigerator for assessing antioxidant and immune activities. The *L. vannamei* were then cut open on an ice tray right after collecting their

haemolymph. The muscle, hepatopancreas and intestinal tissues were collected and stored at $-80\text{ }^{\circ}\text{C}$ in the refrigerator. These tissues were analysed for muscle composition (AOAC, 2019).

Growth performance parameters : Shrimp post-larvae weight (g) was measured at the beginning of the experiment and biweekly by taken randomize number of shrimps from each tank and weighted in an analytical digital balance and then returned to their tanks while the experiment. Shrimp weight gain (WG), specific growth rate (SGR) and survival % (S) were calculated based on the following formulas:

- Weight gain (WG) = Final body weight (g) - Initial body weight (g).
- Specific growth rate (SGR, %/days) = $[(\ln \text{FBW} - \ln \text{IBW}) / \text{day of experiment}] \times 100$
- Survival (%) = (Final number of shrimp / Initial number of shrimp) $\times 100$.

Digestive Enzyme Activity: The REMI Equipment mechanical homogenizer with Teflon coating was utilized to create a 5% tissue homogenate using a chilled 0.25 M sucrose solution in ice-cold conditions to preserve enzyme activity. After the tissue homogenates were ready, they were centrifuged at 2800 g for 10 minutes at 4°C using a Heraeus Megafuge 8R Centrifuge, a refrigerated centrifuge machine from Thermo Fisher Scientific. The collected supernatant was transferred into 2 ml Eppendorf tubes and kept in a deep freezer at -20°C until enzyme assays were conducted. If necessary, appropriate dilution of the samples was performed while conducting assays for various enzymes (Reitman and Frankel, 1957).

Antioxidant and Immune Status of Shrimp: Protein levels, MDA, SOD, GSH-Px were assessed using assay kits from Nanjing Jiancheng Bioengineering Institute in Nanjing, China. Serum was thinned with normal saline based on earlier experiments prior to the formal testing. Following various procedures as instructed by the kits, the levels of TG, total protein, and MDA in serum were assessed by measuring the absorbance of the supernatant at 500, 562, and 532 nm, respectively, using a 96-well plate. The methods of Nebot *et al.* (1993), Reiners *et al.* (1991), and Reitman and Frankel (1957) were used to measure the levels of SOD, GSH-Px, AST, and ALT in serum.

Statistical analysis: The data were statistically analyzed by a completely randomized design with SAS (2004) through the following model: $Y_{ij} = \mu + T_i + E_{ij}$, where μ is the overall mean; T_i is the fixed effect of i^{th} treatments, and E_{ij} is the random error. Difference between treatments were tested at the 5% probability level using (Duncan, 1955).

RESULTS

Water quality: The daily and biweekly water quality parameters monitored during the experimental period are shown in Table (2). No significant differences were observed among experimental groups for all water quality parameters.

Growth performance: In the present study, the dietary lipid levels significantly affected the growth of *L. vannamei*, as shown in Table (3). The group of the white shrimp fed a diet containing 6% lipid had a significantly ($P < 0.05$) higher FBW, WG and SGR compared to other experimental groups. Additionally, the group that was fed a diet containing 14% lipid had the lowest values of FBW, WG and SGR. Moreover, the survival rates were influenced by the lipid and salinity levels. Survival rates were at their highest for shrimp at a salinity of 30ppt with diets containing 6% and 8% lipid. In contrast, the lowest survival rate was observed for shrimp fed a diet containing 14% lipid at a 15ppt salinity. In the present study, *L. vannamei* at 30ppt salinity had a better growth performance than at the 15ppt salinity (Zidan *et al.*, 2024).

Muscle Composition: No variations were observed in muscle moisture and body crude protein content across all treatments listed in Table (4). There was a significant increase in muscle crude lipid with the rise in dietary lipid content at 30 ppt ($p < 0.05$). Salinity did not impact the lipid composition in muscle. The shrimp fed a diet containing 14% lipid at 30 ppt had the highest crude lipid content. A notable variation ($p > 0.05$) was observed in muscle ash levels across five diets, with shrimp having higher ash content at 14% lipid and 30 ppt compared to shrimp at 10% lipid and 15 ppt. Such as raw fat, serum has the highest level of its content.

Digestive Enzyme Activity: Table (5) demonstrates the levels of amylase, lipase, and trypsin in shrimp intestines. The trypsin activity in the intestines of white shrimp was notably higher ($p < 0.05$) when exposed to a salinity of 30 ppt compared to 15 ppt. Amylase activity was found to be highest at a salinity of 15 ppt compared to a salinity of 30 ppt. Moreover, the intestinal lipase function of *L. vannamei* showed a significant difference among groups that consumed 6, 8, 10, 12, and 14% lipid levels ($p > 0.05$). No variations were detected in the serum levels of SOD across all treatments. The level of GSH-Px in the shrimp's serum was higher at 15 ppt compared to 30 ppt ($p < 0.05$), with the lowest GSH-Px content in shrimp fed 10% lipid at both salinity levels.

Antioxidant and Immune Status of Shrimp: The levels of serum MDA in shrimp fed diets containing 8, 10, 12, and 14 % lipid were significantly higher compared to those fed 6% lipid, regardless of salinity ($p < 0.05$) Table (6).

Table (2): Effect of different levels of lipid and salinity on tanks water quality of *Litopenaeus vannamei* fed experimental diets

| Items | | Water quality parameters | | | | |
|-----------|----------------|--------------------------|----------|------------|------------------------|------------------------|
| lipid (%) | Salinity (ppt) | Temperature °C | pH | TAN (mg/l) | NO ₂ (mg/l) | NO ₃ (mg/l) |
| 6 | 30 | 27.8±1.53 | 7.8±0.20 | 0.03±0.01 | 0.02±0.000 | 0.03±0.001 |
| 8 | 30 | 28.5±1.53 | 7.8±0.16 | 0.03±0.01 | 0.02±0.001 | 0.03±0.001 |
| 10 | 30 | 28.1±1.13 | 7.8±0.32 | 0.03±0.01 | 0.03±0.030 | 0.02±0.001 |
| 12 | 30 | 28.5±1.03 | 7.9±0.30 | 0.03±0.01 | 0.02±0.001 | 0.03±0.001 |
| 14 | 30 | 27.4±0.53 | 7.8±0.30 | 0.03±0.01 | 0.03±0.001 | 0.03±0.002 |
| 6 | 15 | 28.0±1.48 | 7.9±0.48 | 0.02±0.01 | 0.02±0.000 | 0.02±0.008 |
| 8 | 15 | 28.1±0.47 | 7.7±0.10 | 0.02±0.01 | 0.03±0.018 | 0.02±0.005 |
| 10 | 15 | 28.2±1.19 | 7.8±0.19 | 0.02±0.01 | 0.02±0.001 | 0.02±0.008 |
| 12 | 15 | 28.7±2.45 | 7.9±0.33 | 0.02±0.01 | 0.03±0.001 | 0.02±0.001 |
| 14 | 15 | 28.6±2.30 | 7.8±0.33 | 0.02±0.01 | 0.02±0.001 | 0.02±0.008 |

Data are presented as means ±SD.

Table (3): The growth performance and survival of *Litopenaeus vannamei* fed diets with different levels of lipid and salinity

| Items | | Growth parameters | | | | |
|-----------|----------------|-------------------|------------------------|------------------------|------------------------|-------------------------|
| lipid (%) | Salinity (ppt) | IBW (g) | FBW (g) | WG (g) | SGR (%/day) | SR (%) |
| 6 | 30 | 0.02 | 8.63±0.91 ^a | 8.61±0.91 ^a | 6.74±0.67 ^a | 94.66±6.41 ^a |
| 8 | 30 | 0.02 | 8.52±0.07 ^a | 8.50±0.06 ^a | 6.73±0.69 ^a | 94.66±4.41 ^a |
| 10 | 30 | 0.02 | 8.30±1.40 ^a | 8.28±1.3 ^a | 6.70±0.67 ^a | 92.68±3.55 ^a |
| 12 | 30 | 0.02 | 7.84±0.69 ^b | 7.82±0.69 ^b | 6.63±0.41 ^b | 92.01±3.48 ^a |
| 14 | 30 | 0.02 | 7.29±1.55 ^c | 7.27±1.54 ^c | 6.55±0.65 ^c | 91.67±3.60 ^b |
| 6 | 15 | 0.02 | 7.52±0.70 ^b | 7.50±0.70 ^b | 6.59±0.52 ^b | 91.11±5.35 ^b |
| 8 | 15 | 0.02 | 7.11±1.56 ^c | 7.09±1.55 ^c | 6.53±0.64 ^c | 90.66±5.00 ^b |
| 10 | 15 | 0.02 | 6.64±0.82 ^c | 6.62±0.83 ^c | 6.45±0.61 ^c | 87.19±1.95 ^c |
| 12 | 15 | 0.02 | 6.32±0.06 ^d | 6.30±0.06 ^d | 6.40±0.59 ^d | 80.20±3.42 ^c |
| 14 | 15 | 0.02 | 6.14±1.50 ^d | 6.12±1.3 ^d | 6.36±0.42 ^d | 75.11±7.03 ^d |

Data are presented as means ±SD. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

Table (4): Effect of different levels of lipid and salinity on muscle composition of *Litopenaeus vannamei* fed experimental diets

| Items | | Moisture (%) | Crude protein (%) | Crude lipid (%) | Ash (%) |
|-----------|----------------|--------------|-------------------|-----------------|------------------------|
| Lipid (%) | Salinity (ppt) | | | | |
| 6 | 30 | 75.20 ± 9.9 | 11.48 ± 1.8 | 4.4 ± 1.6 | 4.1 ± 4.4 ^d |
| 8 | 30 | 74.20 ± 3.9 | 11.34 ± 2.4 | 4.6 ± 4.5 | 4.6 ± 1.4 ^d |
| 10 | 30 | 74.75 ± 1.7 | 10.09 ± 1.8 | 4.7 ± 3.2 | 5.0 ± 1.8 ^c |
| 12 | 30 | 75.12 ± 3.7 | 11.18 ± 4.8 | 4.9 ± 2.7 | 5.4 ± 0.5 ^b |
| 14 | 30 | 74.17 ± 5.6 | 10.34 ± 5.0 | 5.4 ± 1.5 | 6.1 ± 0.3 ^a |
| 6 | 15 | 75.10 ± 1.9 | 11.49 ± 1.5 | 4.4 ± 2.6 | 5.8 ± 1.4 ^b |
| 8 | 15 | 76.20 ± 2.5 | 10.44 ± 1.9 | 4.5 ± 4.7 | 5.0 ± 1.2 ^c |
| 10 | 15 | 74.20 ± 3.8 | 11.32 ± 3.6 | 4.2 ± 3.3 | 5.2 ± 0.6 ^c |
| 12 | 15 | 75.10 ± 6.9 | 10.07 ± 1.4 | 4.6 ± 1.8 | 5.6 ± 0.6 ^b |
| 14 | 15 | 74.20 ± 8.7 | 11.17 ± 1.6 | 5.0 ± 3.8 | 4.9 ± 0.3 ^d |

Data are presented as means ±SD. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

Table (5): Digestive, metabolic and oxidative stress enzyme activities of *Litopenaeus vannamei* diets containing different lipid levels at two salinities

| Items | | Trypsin (millimole of/mg protein) | Amylase (min/mg protein)) | Lipase (unit/h/mg protein) |
|-----------|-------------------|--------------------------------------|------------------------------|-------------------------------|
| lipid (%) | Salinity (ppt) | | | |
| 6 | 30 | 0.38 ± 0.091 ^a | 8.15 ± 0.01 ^b | 0.35 ± 0.001 ^f |
| 8 | 30 | 0.42 ± 0.00 ^a | 9.13 ± 1.5 ^b | 0.37 ± 0.00 ^e |
| 10 | 30 | 0.47 ± 0.003 ^a | 8.78 ± 0.45 ^b | 0.40 ± 0.01 ^d |
| 12 | 30 | 0.50 ± 0.00 ^a | 8.20 ± 0.67 ^b | 0.44 ± 0.003 ^c |
| 14 | 30 | 0.46 ± 0.053 ^a | 7.50 ± 0.11 ^c | 0.40 ± 0.002 ^d |
| 6 | 15 | 0.24 ± 0.01 ^c | 9.99 ± 0.21 ^a | 0.30 ± 0.004 ^j |
| 8 | 15 | 0.30 ± 0.00 ^b | 10.66 ± 0.32 ^a | 0.48 ± 00.01 ^b |
| 10 | 15 | 0.31 ± 0.01 ^b | 10.27 ± 0.25 ^a | 0.54 ± 0.003 ^a |
| 12 | 15 | 0.26 ± 0.01 ^c | 10.20 ± 0.51 ^a | 0.47 ± 0.002 ^b |
| 14 | 15 | 0.26 ± 0.02 ^c | 9.05 ± 0.04 ^a | 0.44 ± 0.093 ^c |

Data are presented as means ±SD. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

Table (6): Serum contents of MDA and activities of SOD and GSH- Px of *Litopenaeus vannamei* fed diets containing different lipid levels at two salinities

| Items | | MDA (nmol/ml) | SOD (U/ml) | GSH-Px (U) |
|-----------|---------------|---------------------------|-------------|--------------------------------|
| lipid (%) | Salinity(ppt) | | | |
| 6 | 30 | 9.46 ± 3.24 ^c | 5.94 ± 0.10 | 1,706.15 ± 49.90 ^c |
| 8 | 30 | 14.05 ± 1.93 ^a | 5.60 ± 0.14 | 1,696.37 ± 44.36 ^c |
| 10 | 30 | 14.20 ± 1.30 ^a | 5.71 ± 0.02 | 1,685.30 ± 41.30 ^d |
| 12 | 30 | 14.35 ± 1.70 ^a | 5.89 ± 0.25 | 1,715.96 ± 206.81 ^c |
| 14 | 30 | 14.60 ± 1.23 ^a | 5.96 ± 0.27 | 1,776.72 ± 106.71 ^c |
| 6 | 15 | 9.94 ± 1.25 ^c | 5.93 ± 0.09 | 1,913.58 ± 41.09 ^a |
| 8 | 15 | 11.26 ± 0.22 ^b | 5.60 ± 0.14 | 1,811.84 ± 42.46 ^a |
| 10 | 15 | 12.26 ± 0.31 ^b | 5.75 ± 0.14 | 1,800.14 ± 12.46 ^b |
| 12 | 15 | 15.66 ± 2.02 ^a | 5.99 ± 0.28 | 1,964.45 ± 89.86 ^a |
| 14 | 15 | 16.62 ± 1.04 ^a | 5.54 ± 0.20 | 1,985.34 ± 49.12 ^a |

MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase.

Data are presented as means ±SD. Values in the same Column with different superscript letters are significantly different ($P < 0.05$).

DISCUSSION

The purpose of the study was to determine the ideal lipid levels in the diet of *L. vannamei* juveniles at salinities of 15 and 30 ppt. Inland saline water provides a different aquaculture option for rearing euryhaline species such as *L. vannamei*. This research assessed shrimp performance, including growth, nutrient utilization, and physiological-metabolic responses, was influenced by varying levels of dietary lipid under rearing conditions with salinities of 15 and 30 ppt. The quality of water is crucial because it directly or indirectly determines the survival of organisms in the aquatic ecosystem. The current research found that the water temperature, pH, and dissolved oxygen in the

culture system were all at levels conducive to the cultivation of *L. vannamei* as noted by Bett and Vinatea (2009). *L. vannamei* is able to withstand a broad range of salinities from 0.5 to 40 ppt due to its ability to regulate osmosis effectively (Saoud *et al.*, 2003). In contrast, the isosmotic point for *L. vannamei* falls between 21.1 and 26.1 ppt according to Jaffer *et al.* (2020). The study kept the water salinity between 15 and 30 ppt, and the levels of total ammonium-nitrogen, nitrite-nitrogen, and nitrate-nitrogen were within the recommended range according to (Talukdar *et al.* 2020 and Zidan *et al.*, 2024).

In the present study, dietary lipid levels significantly affected the growth of *L. vannamei*, as

shown in Table (3). The group of the white shrimp fed a diet containing 6% lipid had significantly ($P < 0.05$) recorded a higher FBW, WG and SGR compared to the other experimental groups, while the group that was fed a diet containing 14% lipid had lower values of FBW, WG and SGR. The highest WG and SGR of the post larval *L. vannamei* were observed at 6% lipid, consistent with findings reported by (González *et al.* 2002 and Zidan *et al.*, 2024), where a dietary lipid level of 60g kg⁻¹ at 25ppt salinity was regarded as optimal for *L. vannamei*. However, at 3 ppt, *L. vannamei* fed 90g kg⁻¹ lipid had the best growth performance (Xu *et al.*, 2018). There is a wide variety for the dietary lipid requirement of *L. vannamei*. In some research, *L. vannamei* samples were fed diets containing 81.0– 112.0g/kg of lipids for 8 weeks (Wouters *et al.*, 2001), or diets containing 30.0– 90.0g/kg lipids for 6 weeks (González *et al.*, 2002). There is a wide variety for the dietary lipid requirement of *L. vannamei* in the study research. Another interesting study indicated that shrimp fed a diet of 120.0g/kg lipid level for 30 days and 100.0g/kg lipid level for 60 days were optimal for the growth of *L. vannamei* (Zhang *et al.*, 2013). The dietary lipid requirement may be complex and related to many factors, such as diet composition, temperature, and salinity (Xie *et al.*, 2019). Moreover, The WG and SGR of shrimp at 30ppt recorded a higher value than those fed with the same diet at 15ppt ($P < 0.05$). Salinity had a significant influence on the survival of the test shrimps ($P < 0.05$), as reported by (Walker *et al.*, 2009 and Zidan *et al.*, 2024), *L. vannamei* had a higher growth rate at 28 ppt than shrimps at 2 ppt. The distinctive results may be related to the initial shrimp size, moult frequency, or the different dietary nutrient compositions (Ding *et al.*, 2008), but more work should be further conducted to demonstrate the difference in these reports. Table (4) demonstrates that shrimp displayed the highest body crude lipid content when fed a diet containing 14% lipid under 15 and 30 ppt conditions. Other research indicates that shrimp and certain other crustaceans have a constrained ability to process high levels of dietary fat (Glencross *et al.*, 2001), which can hinder shrimp growth due to lipid peroxidation and nutritional imbalance (Nogueira *et al.*, 2003). Endogenous antioxidant defenses are essential in protecting cell membranes from oxidative damage caused by peroxidation of unsaturated fatty acids. The primary defence against ROS is antioxidant enzymes, with SOD transforming superoxide radical into peroxide and GSH-Px neutralizing peroxide and organic hydroperoxide (Halliwell, 1994 and Di *et al.*, 1995). There were no variations observed in serum SOD activity across all treatments, while GSH-Px activity in shrimp was higher at 15 ppt compared to 30 ppt. The reason the SOD enzyme is not working to remove superoxide anion is that the superoxide anion in the hemolymph remains stable with all treatments following prolonged exposure to low salinity. The increased GSH-Px activity in shrimp at 15 ppt could result from higher levels of peroxide and organic hydroperoxide in shrimp exposed to low salinity.

Endogenous antioxidants are vital for safeguarding cell membranes against oxidative harm from peroxidation of unsaturated fatty acids. Antioxidant enzymes are the main protection against ROS, with SOD converting superoxide radical into peroxide and GSH-Px deactivating peroxide and organic hydroperoxide (Halliwell, 1994 and Di *et al.*, 1995). No changes were seen in serum SOD levels with different treatments, but GSH-Px levels were higher in shrimp at 15 ppt than at 30 ppt. The SOD enzyme is ineffective in eliminating the superoxide anion in the hemolymph due to the fact that the superoxide anion remains steady despite various treatments after being exposed to low salinity for an extended period. The higher GSH-Px activity in shrimp at 15 ppt may be due to elevated peroxide and organic hydroperoxide levels in shrimp exposed to low salinity. Reduced lipase activity in groups fed lower dietary lipids may be because there is a limited substrate available in the gastrointestinal tract, and it is possible that higher dietary carbohydrates could inhibit the protease activity in these groups. Excessive dietary lipid may reduce the activity of proteolytic and lipolytic enzymes, as evidenced by decreased protease and lipase activity in groups fed very high levels of lipid. Alternatively, the higher and lower levels of amylase activity observed in the groups fed with higher and lower lipid diets in this study could be a result of differences in digestible carbohydrate intake (Deng *et al.*, 2021).

CONCLUSION

It could be concluded that the treatment containing 6% lipid at salinity 30ppt was the best in growth performance and the shrimp that was fed on diet containing 14% lipid had the highest crude lipid levels at levels of salinity 15 and 30 ppt. Additionally, the shrimp showed the highest GSH-Px activity at level of salinity 15 ppt in the experiment.

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تأثير مستويات الدهون الغذائية المختلفة على النمو، نشاط الإنزيمات الهاضمة والحالة المناعية للجمبري الفانمي تحت مستويين من الملوحة

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المستخلص: أجريت هذه التجربة لدراسة تأثير خمس مستويات مختلفة من الدهون (6%، 8%، 10%، 12% و 14%) تحت مستويين مختلفين من الملوحة (15 و 30 جزء في الألف) على أداء النمو، معدلات الاعاشة، استجابة مضادات الأكسدة و النشاط الانزيمي للجمبري الفانمي. نفذت التجربة في 30 تانك مستطيل (66 × 47 × 44 سم، 50 لتر لكل منها) ومملوء بماء ملوحتة 15 و 30 جزء في الألف لمدة 90 يوم. كان لكل معاملة 3 مكررات؛ يحتوي كل تانك على 30 يرقة بمتوسط وزن 0.02 جم. تم تغذية الجمبري على العلائق التجريبية التي تحتوي على حوالي 35% بروتين أربع مرات يوميًا بمعدل تغذية 14% من وزن الجسم الابتدائي وتم إعادة ضبطها تدريجيًا إلى 5% في نهاية التجربة. أظهرت أفضل معاملة والتي احتوت على 6% دهون وملوحة 30 جزء في الألف أفضل نتيجة في أداء النمو والبقاء على قيد الحياة (%). كانت مستويات نشاط التربسين في الأمعاء أعلى بشكل ملحوظ في الجمبري في المجموعة المعرضة لملوحة 30 مقارنة بالمجموعة المعرضة لملوحة 15. وقد وجد أن أعلى نشاط لانزيم الليباز في الجمبري الذي تم تغذيته على علائق تحتوي على مستويات دهون 6 و 8 و 10%. تم تحديد أن الجمبري الذي تم تغذيته على نظام غذائي يحتوي على 14% دهون عند 15 و 30 جزء في الألف كان لديه أعلى محتوى من الدهون الخام، في حين أظهر الجمبري عند 15 جزء في الألف أعلى نشاط GSH-Px. وقد لوحظت هذه النتائج في ظل الظروف التجريبية.

الكلمات الداله:- معدلات النمو، معدلات الاعاشة، مضادات الاكسدة، التربسين، الليباز و الجمبري الفانمي