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The Selenium Supplementation Dietary Effect on the Nutritional Value and Immune Response of the Pacific White Shrimp *Litopenaeus vannamei* (Boone, 1931)

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ABSTRACT

While Selenium (Se) deficiency results in the corruption of antioxidants' efficacy, any increase in its level causes toxicity and subsequently weakens growth rate and immune responses. Thus, it is important to assess the appropriate levels of Se supplementation in the diet, either inorganic or organic form, to precisely comprehend its impact on growth and health of experimental samples. Samples of *Litopenaeus vannamei* were fed dietry supplemented with different levels of organic and inorganic selenium (seleno-L methionine and sodium selenate). For 90 days, shrimps were fed on a diet formulated as pellets mixed with 0.3, 0.4 and 0.5 mg of organic Se and represented mainly by selenomethionine, and the same concentrations were considered for the inorganic selenium (sodium selenite). The effect of the formulated diet on the proximate composition of basic biochemical constituents of the cultured *Litopenaeus vannamei* was evaluated. After the feeding trial, shrimps were used for protein and fat composition, amino acids and fatty acids analysis. Detection of vibrio count in hepatopancreas and haemolymph count were also measured. The results revealed that the Se type and concentrations affecting amino acid ratio indicate the priority of inorganic Se over organic Se, and 0.3mg/ kg concentration over the higher ones (0.4 and 0.5mg/ kg). The highest recorded fatty acids % were associated with organic Se dietary (oleinic acid and elaidic acid) (24.1% and 14.9, respectively), which significantly increased at 0.5mg/ kg organic Se (31.2% and 17.9% respectively). Generally, the organic Se scored higher fatty acid ratios than basic and inorganic Se diets, especially at 0.4mg/ kg Se. *L. vannamei* fed Se in its diets recorded lower bacteraemia than control ones; this was mainly observed in those fed 0.4 and 0.5mg/ kg organic Se ($P< 0.05$). Furthermore, the incorporation of organic selenium (OS) in the diet stimulated the proliferation rate of total haemocytes cells (THCs) in the studied species.

INTRODUCTION

Prawns, crabs, crayfish, and lobster are examples of edible crustaceans that are among the main sources of nutrient-rich food for people. Numerous research supports the intake of crustaceans **(Marques *et al.*, 2008, Wardiatno and Mashar, 2010, Wardiatno *et al.*,**

2012). A crustacean's nutritional value is determined by its biochemical makeup, which includes its protein, amino acids, lipids, fatty acids, carbohydrates, vitamins and minerals. Shrimps make up around 20% of the total volume of the global seafood market **(Bhavan *et al.*, 2010).** *L. vannamei* is rapidly replacing other shrimp species in Egypt; the main explanation for this shift is that *L. vannamei* has a faster growth rate, higher inventory rate and yield, wide tolerance to disease and good survival **(Cuzon *et al.*, 2004; Jaspe *et al.*, 2011)**.

The rising demand for shrimp products and the associated increase in production has increased the demand for appropriate nutrient supply **(Qiuran, *et al.*, 2021)** that meet the demands of different breeding environments such as variable temperatures, pH, high ammonia, water pollution, etc. Even though the wonderful growth and resistance that distinguish *vannamei* from the other shrimps, poor and stunted growth has been recorded in some shrimp farming sectors **(Balasubramanian *et al.*, 2018)**

While most of the research to date has focussed on the use of dietary trace elements to improve the productivity of crustaceans, limited information is available on the use of an organic and inorganic form of selenium. Se is a component of selenoproteins, which are important for Se-dependent enzymes with Se-cysteine at their active sites **(Stadtman, 1996)**. Se-dependent enzymes have a critical enzyme catalytic effect on organisms. For instance, the selenoenzyme glutathione peroxidase (GPx), an imperative antioxidative enzyme in animals, can protect cell membranes against oxidative destruction **(Dröge, 2002; Lin & Shiau, 2005)**.

Although Se deficiency leads to distraction and corruption of the ability of the antioxidants, equally, its increase leads to toxicity and reduces growth rates as well as weakens immune responses and lowers the diet conversion efficiency of animals **(Maier & Knight, 1994; Wang *et al.*, 2012; Yu *et al.*, 2021).** Thus, it is important to establish the appropriate levels of Se supplementation in the diet, either inorganic or organic form **(Maier & Knight, 1994; Wang *et al.*, 2012)**. Before this study was initiated, our survey on local shrimp feed producers revealed that no Se supplement was added to the premix used to produce shrimp feed. They considered that the Se present in natural feed ingredients (fish meal, soybean meal, etc.) and seawater (0.0009 ppm in 3.5% salinity) was sufficient to satisfy the shrimp's nutritional requirement **(Turekian, 1968)**. However, due to the beneficial effects of Se supplementation described above for other animal feeds including crustaceans feed, the current study was accomplished to precisely comprehend the growth and health implications of selenium (Se) supplementation in the diets of *L. vannamei* by evaluating the effect of varying levels of organic and inorganic selenium (seleno-L methionine and sodium selenate) in the formulated diet on the proximate composition of basic biochemical constituents of the cultured *Litopenaeus vannamei.*

MATERIALS AND METHODS

Experimental design

The experiment was performed in Delta production sector (Berket Ghalioun), Kafr El-Shekh, in a fish and shrimp hatchery; it lasted 90 days from 13 December 2019 to 11 March 2020.

Shrimps used in this work were at the juvenile stage, their weights (5 ± 0.76 g) and lengths &8.4± 0.55 mm) were measured. Shrimp specimens were reared in cylindrical fiberglass tanks (250L capacity). Each tank was supplied by a double air-stone to maintain the dissolved oxygen at 6- 8mg/ L. The temperature was kept at 28- 32°C using individual automatic heaters in each tank. The pH was kept at 8- 8.2, and salinity was at 25g/L. NO_2-N, NO_3-N and NH_3/NH^{+4} were monitored every 2 days using chemical test kits (Aquarium Pharmaceuticals™, Inc., McLean, Virginia, USA). Specimens were acclimated for 1 week, then randomly distributed into triplicate groups (n = 30). During the culture period, water was changed at a rate of 10 % per day to evaluate its quality.

Diets formulation

The basal diet was formulated as shown in Table (1); basal diet pellets of 0.5mm diameter and 1mm to 3 mm length were prepared by mixing all ingredients of basal diet at the selected levels for the specific trial. One kg of basal diet was approximately mixed with 0.3, 0.4 and 0.5 mg of organic Se and represented mainly by selenomethionine **(Burdock & Cousins, 2010),** and the same concentrations were considered for the inorganic selenium (sodium selenite). All diets were pelletized using a mincer and then dried under direct sunlight. Dried pellets were then allowed to cool at room temperature, packed and stored in a dark room before being used. The *L. vannamei* samples in each tank were fed either the test or basal diet at a proportion of its average body weight every day (ranging from 5% at weight 5 g and up to 1.8 % at weight 25g). The source of organic and inorganic selenium was from LANXESS AG Headquarters: Kennedyplatz1, 50569 Cologne Germany, which was denoted by Canada **(Revanasiddappa & Dayananda, 2006)**. Shrimps were fed four times per day (at 08:00, 11:00, 16:00 and 23:00), and uneaten food and faeces were siphoned out before the next feeding. Sufficient freshwater was added to maintain the constant volume of water tanks. At the end of the experiment, the nutritional value of enriched *Litopenaeus vannamei* was measured.

Chemical analysis

At the end of the experiment (90 days), the body crude protein, lipid, moisture and ash contents were determined according to standard methods **(AOAC, 2012)**. Samples were dried to a constant weight at 105°C. Crude protein levels were determined using the Kjeldahl method (Elementar, Germany). Crude lipids were measured after diethyl ether extraction using the Soxhlet method (Buchi 36,680, Switzerland). Ash content was determined after combustion in a muffle furnace at 550°C for 10h.

Amino acid composition

After samples (faecal and dietary) were hydrolyzed in 6 N HCl for 24 hours at 110°C, amino acids were identified according to **Antoine *et al.* (1999)**. Materials were then derivatized using o-phthaldialdehyde (OPA). HPLC (Knauer, Germany) was used to measure the total amino acids using a C18 column at a flow rate of 1ml min^{1} and a fluorescence detector (RF-530, Knauer, Germany).

Table 1. Ingredients (g/kg) and proximate composition of the basal diet of *L. vannamei*

Ingredient	Content (g/kg)
Wheat bran	**300**
Fish meal	**123**
Chicken meal	**111**
Soybean	**220**
Rice bran	**60**
DDGS	**150**
Shrimp vitamins	**1.5**
Shrimp salts	**1.5**
Soybean oil	**12**
Mono calcium phosphate	**7**
Phospho lipids	**5**
Legno bond DD	**1**
Proximate composition	**%**
Crude proteins	**34%**
Crude fat	**6%**
Fibers	**3%**
Ash	**12%**

Fatty acids composition

A Philips PU 4400 gas chromatograph (GC) (Phillips Scientific, Cambridge, United Kingdom), outfitted with a fused silica capillary column BPX-70 (length 25m, film thickness 0.2 to 0.5 µm) and FID detector was used to examine samples of fatty acid methyl esters (FAME). Helium served as the carrier gas. The temperature programme contained a gradient with an increase rate of 1.5°C min^{-1} from 160 to 230°C. By using established purified standards, FAMEs were found. The average of the three replicates injections served as the final values.

Detection of vibrio count in hepatopancreas

After 90 days of feeding regimes, 0.1g from hepatopancreas tissues (each sample consisted of HP from five specimens) was taken and put in a homogenizer with 900µl

sterilized seawater. Serial dilutions for samples up to a concentration of 0.1 were prepared. 15ml of TCBS media was poured into the plates, then 100µl was taken by electronic pipette and stretched on the Petri dishes and incubated in the biological incubator at 30°C for 10 – 16h.

Detection of haemolymph count

100µl of haemolymph was drawn from the shrimp samples and mixed with 100µl of the anticoagulant. 20µl of rose Bengal stain was added to the samples. 10 to 20µl was taken from the mix and put on the haemocytometer and examined under the microscope.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of different groups. If there is a significant difference between means, Tukey's method and Fisher LSD method for multiple comparisons were used to detect all pairwise differences between groups means to determine specifically which means are different. For all statistical tests, *P*-values < 0.05 was statistically significant. Data and statistical analysis were performed using Excel 365 (Microsoft Corporation, USA), and Minitab version 19. In all Tables and Figs., means that do not share a letter are significantly different ($P< 0.05$).

RESULTS

1. Moisture and ash

Moisture of *L. vannamei* was ~74.3%±0.6 with shrimps fed basic dietary, and no effect of both types or concentrations of Se was detected on the moisture ratio ($P>0.001$). The ash content was calculated as 1.17%±0.21 of *vannamei* fed the basic diet and was 1.14%± 0.27; 1.21± 0.07 with *vannamei* fed organic and inorganic Se, respectively.

2. Crude protein and fat analysis

As shown in Fig. (1), the shrimp fed the diet supplemented with both types of selenium showed higher body protein content %, compared to the animals fed the control diet. The shrimp fed organic Se recorded significant crude protein than inorganic ones at 0.5mg/ kg Se (21.83±0.24 and 20.49±0.14, respectively). While, the crude fat content in the shrimp showed the opposite trend, where inorganic Se scored significant higher ratio of crude fat, compared with organic Se ($P<0.05$) at 0.5mg/ kg Se (Fig. 2). Treatments and concentrations grouping information using Fisher LSD method and 95% confidence emphasize the above analysis.

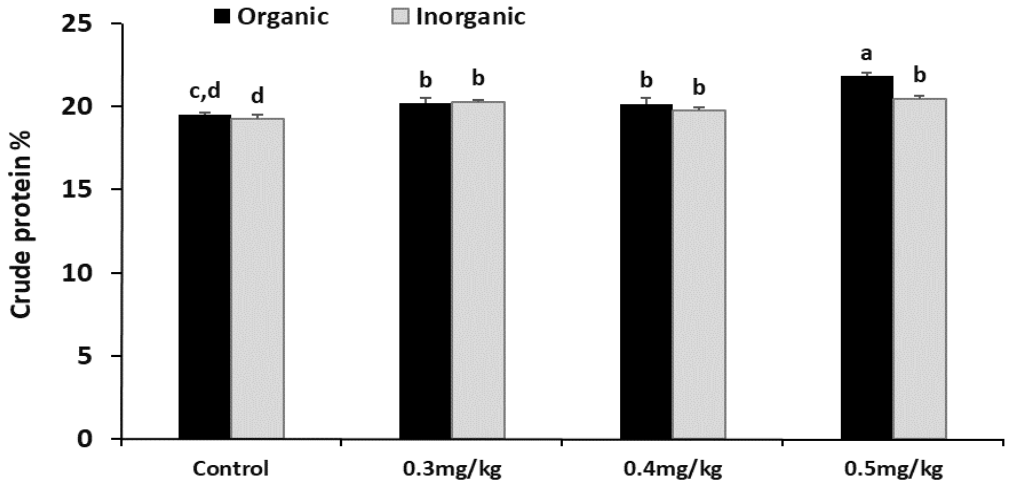


Fig. 1. The effect of dietary organic and inorganic selenium levels on the percentage of crude protein of *L. vannamei* after 90 days. Error bars represent the means ± SE (n = 30)

As shown in Fig. (2), no correlation was recorded in the ratio of crude fat % and the type of Se or its concentration. At 0.5mg/ kg inorganic Se, the crude fat ratio was significantly higher than shrimps manipulated with the rest of other Se types (P<0.001). On the other side, inorganic Se of 0.3 and 0.4 and 0.3mg/ kg organic Se concentrations recorded the lowest fat %.

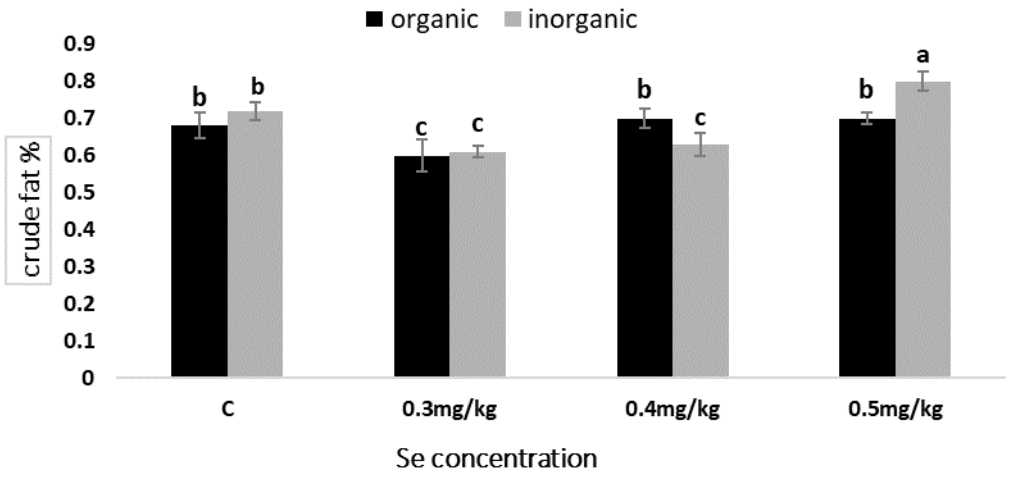


Fig. 2: The effect of dietary organic and inorganic selenium levels on the percentage of crude fat of *L. vannamei* after 90 days. Error bars represent the means ± SE (n = 30)

3. Amino acids analysis

Fifteen amino acids were recorded in the tested specimens fed with organic and inorganic Se dietary for 90 days (Tables 2, 3). The results indicate the presence of 9 essential amino

acids (arginine, histidine, lysine, threonine, methionine, leucine, isoleucine, valine and phenylalanine) and accumulated 6 non-essential amino acids (Proline, tyrosine, alanine, serine, glutamic acid and aspartic acid), the Glycine was undetected (Table 2).

The highest amino acid ratio was Glutamine (7. 5%). Organic Se diet had no effect on glutamine % while inorganic Se scored significant accumulation (P<0.1) than the other diets; at the same time, no effect of inorganic Se concentrations was recorded on the Glutamine ratio. The second and third amino acids were asparagine (5.6 and 5.4% for organic and inorganic Se, respectively) and arginine (5.4 and 5.26% for organic and inorganic Se, respectively).

Arginine % accumulation recorded significant differences only at 0.3 and 0.4mg/ kg inorganic Se. The effect of organic Se slightly appeared only with asparagine, glutamine, methionine, isoleucine, leucine and lysine. The effect was detected mainly at 0.4mg/ kg, followed by 0.5mg/ kg.

Table 2. Amino Acids % of *L. vannamei* fed with organic Se for 90 days

Amino Acid %	**Control**	**0.3**	**0.4**	**0.5**
Asparagine	5.6±0.26	5.46±0.25	5.03±0.25*	5.36±0.12
Threonine	2.13±0.15	2.23±0.15	1.9±0.1	1.96±0.15
Serine	2.13±0.25	2.16±0.12	1.93±0.15	2.1±0.17
Glutamine	7.53±0.1	7.5±0.2	6.83±0.21*	7.35±0.33
Alanine	3.36±0.15	3.16±0.1	3.03±0.25	3.26±0.25
Valine	2.43±0.12	2.36±0.21	2.13±0.15	2.33±0.15
Methionine	1.36±0.15	1.56±0.12	1.93±0.06*	2.13±0.1*
Isoleucine	2±0.1	2.33±0.15	2.8±0.26*	3.13±0.15*
Leucine	3.33±0.25	4.06±0.16*	3.76±0.25	4.23±0.251*
Tyrosine	2.16±0.1	2.13±0.1	1.96±0.21	2.13±0.15
Phenylalanine	2.5±0.2	2.43±0.15	2.2±0.2	2.36±0.15
Histidine	1.3±0.2	1.33±0.1	1.23±0.15	1.3±0.2
Lysine	4.76±0.25	4.56±0.2	4.2±0.17*	4.43±0.25
Arginine	5.4±0.3	5.53±0.25	5.16±0.21	5.03±0.21
Proline	4.56±0.15	4.46±0.15	4.13±0.25*	5.66±0.12*

*Values are expressed as means ± SE (n=3). Data in the same row assigned with (*) are significantly different (P<0.05)

The Se type and concentrations affecting the amino acid ratio are illustrated in Table (4), which indicates the priority of inorganic Se over organic Se and 0.3 mg/kg concentration over the higher ones (0.4 and 0.5 mg/kg inorganic Se).

Table 3. Amino Acids % of *L. vannamei* fed with inorganic Se for 90 days

Amino Acid	Control	0.3	0.4	0.5
Asparagine	5.4±0.1	6.56±0.21*	6.33±0.15	5.53±0.35
Threonine	2.06±0.08	2.56±0.12*	2.53±0.15	2.06±0.06
Serine	2.16±0.15	2.53±0.06	2.7±0.2*	2.26±0.15
Glutamine	7.36±0.20	8.13±0.15	8.16±0.21*	8.06±0.21
Alanine	3.3±0.2	3.93±0.06*	3.76±0.25	3.73±0.15
Valine	2.4±0.1	2.86±0.05	2.73±0.25*	2.56±0.15
Methionine	1.53±0.15	1.83±0.06	1.83±0.15	2.2±0.44*
Isoleucine	2.33±0.21	2.63±0.057	2.66±0.15	2.76±0.15*
Leucine	4.1±0.14	3.83±0.06	4.76±0.05	4.93±0.25*
Tyrosine	2.1±0.17	2.63±0.15*	2.35±0.07	2.23±0.25
Phenylalanine	2.26±0.06	2.63±0.21	2.73±0.06	2.66±0.15
Histidine	1.46±0.15	1.66±0.12	1.6±0.17	1.36±0.15
Lysine	4.6±0.26	5.3±0.26*	5.06±0.25	4.76±0.12
Arginine	5.26±0.21	6.5±0.17*	6.36±0.15*	4.96±0.15
Proline	4.3±0.1	4.25±0.07	3.8±0.2	4.36±0.12

*Values are expressed as means ± SE (n=3). Data in the same row assigned with (*) are significantly different (P<0.05)

Table 4. Se concentrations affecting the amino acids ratio in *L. vannamei* fed with organic and inorganic Se dietary for 90 days

Amino acid	Se type	Concentration mg/kg	With Se %	Control %
Glutamine	**inorganic**	0.3	8.16	7.63
Asparagine	**inorganic**	0.3	6.56	5.4
Arginine	**inorganic**	0.3	6.5	5.26
Lysine	**inorganic**	0.3	5.3	4.6
Proline	**Organic**	0.5	5.66	4.5
Leucine	**inorganic**	0.5	4.93	4.1
Alanine	**inorganic**	0.3	3.93	3.3
Threonine	**inorganic**	0.3	2.56	2.06
Tyrosine	**inorganic**	0.3	2.63	2.1
Phenylalanine	**inorganic**	0.3	2.63	2.26
Serine	**inorganic**	0.4	2.7	2.16
Valine	**inorganic**	0.4	2.73	2.4
Isoleucine	**organic**	0.5	3.13	2
Methionine	**inorganic**	0.5	2.2	1.35
Histidine	**inorganic**	0.3	1.66	1.33

4. Fatty acids analysis

Approximately, 37 fatty acids % in *L. vannamei* fed with organic and inorganic Se dietary for 90 days are represented in Tables (5, 6). Only 22 fatty acids were detected, while the other 17 were not spotted. The highest recorded fatty acid % with organic Se dietary was oleinic acid (24.1%), which significantly increased at 0.5mg/ kg (31.2%). Oleinic was followed by palmitic acid (19.36%), which significantly decreased at 0.5mg/ kg. The third fatty acid was elaidic acid (14.9), which significantly increased at 0.5mg/ kg organic Se. Stearic acid scored 8.4% and increased at all concentrations of organic Se. The lowest fatty acid was decanoic acid (0.18%), which significantly increased at 0.3 and 0.4mg/ kg, and then decreased to 0.15% at 0.5mg/ kg organic Se. Decanoic was followed by arachnidic acid (0.23%) that gradually increased at 0.3, 0.4 and 0.5mg/ kg (0.39, 0.41 and 0.79 respectively). In some cases, there was no effect of Se addition on fatty acid concentration; for example, Se had no effect on linolic and cis-11-eicosenoic. On the other side organic Se had negative effect on the fatty acid ratio of eicosatrienoic acid and behenic acid ratio.

In the case of inorganic Se dietary, the oleinic acid (25.97%) also recorded the highest conc. as organic Se dietary but significantly decreased with Se concentrations to reach 18.44% at 0.5mg/ kg. The second rank was that of the palmitic acid (20.4%), which didn't significantly differ with the gradient concentrations of Se. The third acid conc. was elaidic acid (15%), which increased significantly with inorganic Se to reach 23.3% at 0.5mg/ kg. The 4th was stearic acid (9.67%), which slightly increased at 0.5mg/ kg (11.95%). For the organic Se dietary, the lowest fatty acid was decanoic acid (0.27%) at control diet, which completely disappeared at 0.4 and 0.5mg/ kg inorganic Se. The highest conc. of some fatty acids with Se are given in Table (7).

Table 5. Fatty acids % of *L. vannamei* fed with organic Se dietary for 90 days

Fatty Acid %	Control	0.3	0.4	0.5
(C4:0) Butyric Acid	3.47±0.26	2.32±0.14*	6.06±0.19*	0.91±0.06*
(C6:0) Hexanoic Acid	0.37±0.04	0.57±0.04*	0.58±0.03*	0.27±0.03*
(C8:0) Octanoic Acid	0.45±0.03	0.89±0.02*	1.35±0.1*	0.38±0.03
(C10:0) Decanoic Acid	0.18±0.01	0.34±0.02*	0.63±0.04*	0.15±0.01
(C12:0) Dodecanoic Acid	0.69±0.02	1.35±0.11*	2.71±0.06*	0.11±0.02*
(C14:0) Myristic Acid	0.4±0.02	0.49±0.02*	0.56±0.05*	0.32±0.02*
(C15:1)Cis-10-Pentadecanoic Acid	0.47±0.04	1.03±0.07	2±0.13*	0.15±0.19
(C16:0) Palmitic Acid	19.36±0.4	20.76±0.88	19.68±0.55	16.21±0.7*
(C16:1) Palmitoleic Acid	0.3±0.04	0.34±0.03	0.51±0.03*	0.32±0.02
(C17:0) Heptadecanoic Acid	0.64±0.04	0.72±0.03*	0.618±0.03	0.686±0.03
(C18:0) Stearic Acid	8.08±0.56	13.49±0.914*	12.65±0.79*	10.26±0.78*
(C18:1n9t) Elaidic Acid	14.94±0.64	16.32±0.61	15.02±0.76	17.85±0.9*

(C18:1n6c) Linolic Acid (OMEGA6)	2.07±0.22	2.28±0.12	2.010±0.1	2.35±0.12
(C18:2n6t) Linolelaidic Acid	1.17±0.10	0.3±0.028	0.39±0.036	0.14±0.030
(C18:2n9c) Oleinic Acid	24.1±0.62	20.79±0.9	18.93±0.69	31.23±0.88*
(C18:3n3) α-Linolenic Acid (OMEGA3)	0.95±0.07	0.79±0.06*	0.76±0.03*	0.84±0.11
(C20:0) Arachidic Acid	0.23±0.02	0.39±0.03*	0.41±0.03*	0.79±0.07*
(C20:1) cis-11- Eicosenoic Acid	0.77±0.04	0.83±0.04	0.63±0.03	0.89±0.09
(C20:2) cis-11,14 Eicosadienoic Acid	2.06±0.11	2.04±0.14	1.89±0.13	1.97±0.13
(C20:3n6)cis-8,11,11,14-Eicosatrienoic Acid	3.16±0.13	2.9±0.15	2.37±0.09	1.99±0.11
(C22:0) Behenic Acid	7.57±0.34	6.08±0.16	5.51±0.1	4.87±0.11
(C24:1) cis-15-Tetracosenoic Acid	5.53±0.24	4.35±0.1	4.17±0.11	3.02±0.1

*Values are expressed as means ± SE (n=3). Data in the same row assigned with (*) are significantly different (P<0.05).

Table 6. Fatty acids % of *L. vannamei* fed inorganic Se dietary for 90 days

Fatty Acid	**Control**	**0.3**	**0.4**	**0.5**
(C4:0) Butyric Acid %	2.12±0.228	2.69±0.237	4.63±0.390*	3.97±0.241*
(C6:0) Hexanoic Acid %	0.49±0.008	0.49±0.02	0.47±0.088	0.56±0.041*
(C8:0) Octanoic Acid %	0.61±0.010	0.71±0.031	0.59±0.110	0.76±0.044
(C10:0) Decanoic acid%	0.27±0.001	0.35±0.03	0	0
(C12:0) Dodecanoic Acid %	1.02±0.045	1.41±0.067	0.99±0.107	0.93±0.090
(C14:0) Myristic Acid %	0.44±0.008	0.64±0.050*	0.47±0.067	1.02±0.078*
(C15:1) Cis-10-pentadecanoic Acid	0.73±0.003	1.26±0.217*	0.77±0.085	0.91±0.108
(C16:0) Palmitic Acid %	20.38±1.15	20.178±0.83	18.785±0.823	19.17±0.837
(C16;1) palmitoleic Acid %	0.42±0.003	0.37±0.038	0.96±0.206*	1.347±0.069*
(C17:0) Heptadecanoic Acid %	0.4±0.016	0.77±0.032*	0.74±0.050*	0.56±0.042
(C18:0) Stearic Acid %	9.67±0.288	11.75±0.44*	11.95±0.39*	10.29±0.75
(C18:1n9t) Elaidic Acid %	15.04±0.3	16.9±0.77	19.14±0.68*	23.27±0.663*
(C18:1n6c) Linolic Acid % (OMEGA6)	1.81±0.184	2.08±0.159	2.11±0.301*	2.33±0.050*
(C18:2n6t) Linolelaidic Acid %	0.78±0.111	5.19±0.49*	0.82±0.038	0.86±0.044
(C18:2n9c) Oleinic Acid %	25.96±0.54	20.44±0.65	21.04±0.66	18.46±0.56*
(C18:3n3) α-Linolenic Acid %(OMEGA3)	1.76±0.15	1.16±0.169	0.9±0.04*	1.02±0.09
(C20:0) Arachidic Acid %	0.34±0.04	0.36±0.04	0.4±0.019	0.45±0.05
(C20:1) cis-11- Eicosenoic Acid %	0.39±0.04	0.654±0.057	0.814±0.13*	0.87±0.035*

(C20:2) cis-11,14-Eicosadienoic Acid %	1.52±0.082	1.77±0.35	1.876±0.051	1.64±0.09
(C20:3n6) cis-8,11,11,14-Eicosatrienoic acid%	1.83±0.199	1.97±0.26	2.37±0.05*	2.93±0.169*
(C22:0) Behenic Acid %	5.37±0.25	5.16±0.334	6.08±0.35*	5.18±0.209
(C24:1) cis-15-Tetracosenoic Acid %	4.3±0.15	4.24±0.35	4.1±0.34	3.85±0.155

*Values are expressed as means ± SE (n=3). Data in the same row assigned with (*) are significantly different ($p<0.05$).

Table 7. Se concentrations affecting ratios of fatty acids in *L. vannamei* fed organic and inorganic Se dietary for 90 days

Fatty acid	Se type	Control %	With Se %	Se Conc. mg/kg
Butyric acid	Organic	3.5	6	0.4
Hexanoic acid	Organic	0.37	0.58	0.3
Octanoic acid	Organic	0.45	1.35	0.4
Decanoic	Organic	0.18	0.63	0.4
Dodecanoic acid	Organic	0.69	2.71	0.4
Myristic acid	Inorganic	0.44	1.02	0.5
Cis-10-pentadecanoic acid	Organic	0.47	1.99	0.4
Palmitoleic acid	Organic	0.42	1.35	0.5
Stearic acid	Organic	8	13.4	0.3
Elaidic acid	Inorganic	15	23.3	0.5
Linolelaidic acid	Inorganic	0.77	5.19	0.3
Oleinic acid	Organic	24.1	31.23	0.5
Arachidic acid	Organic	0.23	0.79	0.5
cis-8,11,11,14-Eicosatrienoic acid	Inorganic	1.8	2.93	0.5

From the above Tables (5-7), 10 fatty acids scored significant ratio with organic Se, while the rest (4 fatty acids) recorded high ratio with inorganic Se. In the case of amino acids (Table 4), inorganic Se dominated the high ratio (13 amino acids), 9 amino acids at 0.3, 2 at 0.4 and 2 at 0.5gm/ kg dietary, while only two amino acids scored significant increases at 0.5mg/ kg.

5. Vibrio count in *L.* vannamei fed with organic and inorganic Se diet

After 90 days, *L. vannamei* fed basal diet had higher bacteraemia than those fed Se as shown in Fig. (3). Among *L. vannamei* fed Se in the diets, the lowest bacteraemia was observed in those fed 0.4 and 0.5mg/ kg organic Se, which was significantly lower ($P<$ 0.05) than the control. Moreover, there was a significant difference between organic and

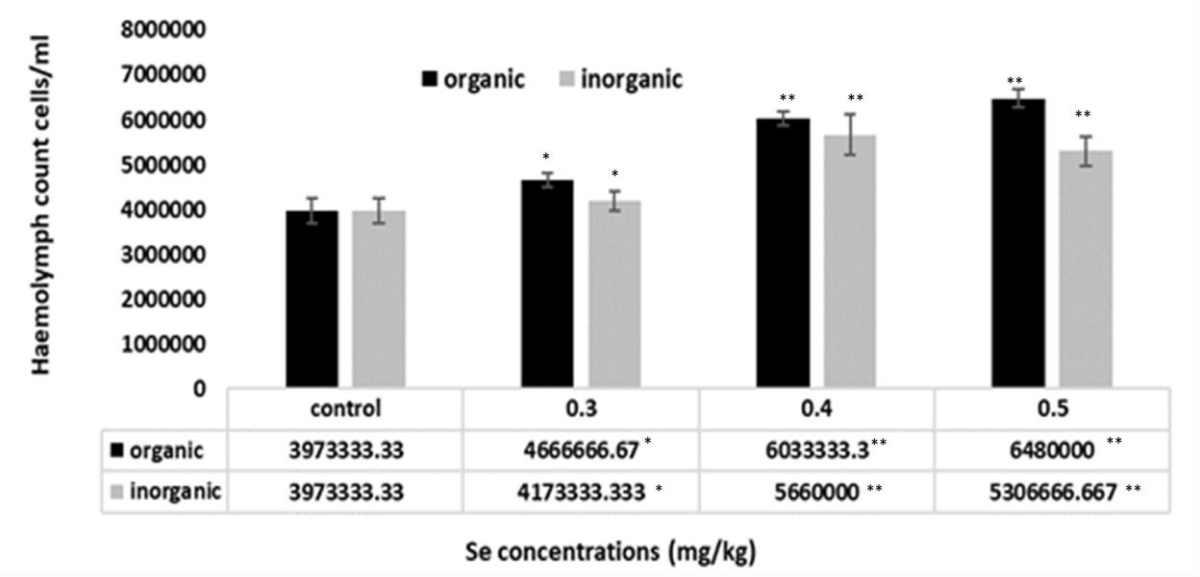
90,000

inorganic Se in each concentration, with inorganic Se bacterial number duplicated, compared to organic Se.

Fig. 3. Vibrio count in the hepatopancreas of *L. vannamei* fed Se dietary for 90 days means ± SE (n= 5 specimens).

6.Haemolymph total count in *L. vannamei* fed with Se dietary for 90 days

The incorporation of OS in the diet stimulated and increased the proliferation rate of haemocytes and the subsequent higher THCs (total haemolymph count) in the studied species. As shown in Fig. (4), the haemolymph count cells/ml increased significantly with Se addition, and there were significant differences between organic and inorganic Se (P<0.05) dietary.



	control	0.3	0.4	0.5
organic	3973333.33	4666666.67 *	6033333.3**	6480000 **
inorganic	3973333.33	4173333.333 *	5660000 **	5306666.667 **

Fig. 4. The haemolymph count cells/ml of *litopenaeus vannamei* fed organic and inorganic Se dietary for 90 days

DISCUSSION

The nutritional composition of feeds influences the body composition of aquatic species **(Jannathulla *et al.*, 2018)**, particularly Se which can promote the nutrition regulation and antioxidant activity in shrimp **(Jiang, 2020)**. In the present study, Se concentrations were chosen between 0.2- 0.5mg/ kg **(Davis, 1990)** and the levels determined in the study of **Chen *et al.* (2013)** who found that the optimum selenium content range for aquatic animals is 0.2– 1.8mg Se/ kg diet.

Moisture of *L. vannamei* was ~74.3%±0.6, and no effect of Se additions was detected on the moisture ratio. Moisture value is generally reported as 75 to 80% **(Sambhu & Jayaprakash, 1994; Yanar & Celik, 2006)**. **Gunalan *et al.* (2013)** recorded slightly higher moisture percentage (76.2%) with *L. vannamei*. Ash content of shrimp is generally

1 to 1.5%, the ash content of the present species was 1.17%±0.21. It is corroborated with the findings of **Yanar and Celik (2006)** and **Gokoglu *et al.* (2008)**; they found that ash in black tiger and white shrimps were 0.95 and 1.47%, respectively. These values are very close to the findings of the present study.

According to the study of **Sriraman (1978)**, the protein content of crustaceans was around 20%. In the present investigation, the protein content of *L. vannamei* showed 19.24% with control diet. In **Gunalan *et al.* (2013)** investigation, the protein content of *L. vannamei* showed 35.69%. Similar findings were recorded in the studies of **Sriraman (1978)**, **Nair and Prabhu (1990)**, **Reddy and Shanbhogue (1994)**, **Ravichandran (2000)** and **Silva and Chamul (2000). Olsen *et al*. (2014)** correlated the yield differences to the biological characteristics of the shrimps and dites. In general, lipid act as major food reserves along with protein and subjected to periodic fluctuations influenced by environmental variables like temperature **(Johnstene, 1917; Pillay & Nair, 1973)**.
The shrimps fed the diet supplemented with both types of selenium showed improvement in the body protein content %, compared to the animals fed the control diet. Organic Se dietary attained a significant increase over inorganic Se, especially at 0.5mg/ kg (21.83±0.24) as a high-range protein containing nutrient like fish.

While, the crude fat content in the shrimp under investigation showed the opposite shift. **Wang *et al.* (2013)** found that high Se levels promote the deposition of protein synthesis increasing the crude protein levels and decreasing the crude lipid levels in *L. vannamei* **(Li *et al.*, 2016)**. **Davis (1990)** found that, selenium incorporated into proteins to make selenoproteins such as glutathione peroxidase (GPx), an important antioxidant enzyme. On the opposite, higher dietary levels of Se can inhibit cell protein synthesis and induce cell damage **(Hamilton, 2004; Wang *et al.*, 2006)**. Fat values in the present shrimp (0.7%) were lower compared to the findings of **Dinçer and Aydin (2014)** who recorded fat values ranging from 1.07-1.3% in *jinga* shrimp. *P. monodon* has been found to contain protein and fat more than the current species of *L. vannamei* (24.58% and 8.32%, respectively) **(Jeyasanta & Patterson, 2017)**. While, lower values of crude fat contents were recorded in the pond cultured shrimp *Penaeus monodon* (6.3% DM) and *Penaeus vannamei* (5.7% DM), as reported by **Sriket et al. (2007)** than the current species.
Using Se, the fat content was slightly affected by Se addition at 0.3mg/kg (0.6mg/kg) and increased only at 0.5mg/ kg inorganic Se to reach 0.8mg/ kg. This outcome coincides with that of **Yu *et al.* (2022)** who recorded no significant differences in *Penaeus vannamei* fat after 8 weeks of different dietary selenium sources. Herein species is an extremely very low in fat and calories, making it a healthy food choice for consumers **(Binsan *et al.*, 2008)**. The compositions of *L. vannamei* as other types of shrimp may change depending on the diet, location and condition (maturity stages of the female); the sex of the animal may also affect its fatty acid content, and furthermore, the proximate composition **(Karakoltsidis *et al.*, 1995)**. In general, lipid and proteins are influenced by

environmental variables like temperature **(Pillay & Nair, 1973**). In the studied shrimp, the proportion of protein was greater, followed by lipid and carbohydrate in the muscle of *L. vannamei* with or without Se addition.

Amino acids are the building blocks of proteins and serve as a source of energy **(Bhavan *et al.*, 2010)**. Presently, a new concept has been arrived with functional amino acids (FAA), they mainly helped to regulate the key metabolic pathways to improve the health, growth and reproduction in addition to preventing various diseases and disorders **(Wu, 2013)**. All FAA amino acids, except tryptophan and cystine, were in considerable quantity in *L. vannamei* reared in the present study, especially with Se addition.

The analysis induced the existence of 9 essential amino acids (threonine, valine, arginine, methionine, isoleucine, leucine, lysine, phenylalanine, and histidine) and 6 non-essential amino acids (Proline, tyrosine, alanine, serine, glutamic acid and aspartic acid). In aquaculture prawn *Macrobrachium rosenbergii*, nineteen amino acids were detected, among these eleven are essential, and eight are non-essential amino acids **(Bhavan *et al.* 2010)**, while nine essential amino acids and nine non-essential were detected by **Hamdi (2011)** in edible muscles of *Procambarus clarkii* and *Erugosquilla massavensis*. In this respect, according to **Sikorski (1999)**, glycine, alanine, serine, and threonine give tasty sweetness, while arginine, leucine, valine, methionine, phenylalanine, and histidine give a bitter taste. In the present study, the levels of arginine, glutamic acid and aspartic acid were high in respect to the other amino acids in the muscle tissues, especially with Se additives. Lysine % was significantly high in all trials of inorganic Se dietary than the others fed organic Se, which influenced the improving of immune functions, antioxidant defense systems and energy metabolism **(Safari *et al.*, 2015)**. The same was concluded in the same species (*Litopenaeus vannamei*) according to **Zhou *et al.* (2017)**. Proline amino acid also recorded its highest ratio at 0.5mg/ kg organic Se dietary, which has a good influence on growth, antioxidant system, and stress resistance as mentioned by **Xie *et al.* (2015)** in their study on the same species (*Litopenaeus vannamei*).

In the present study, twenty-two saturated and non-saturated fatty acids were detected. Organic selenium dietary was the most effective diet on the concentrations of the fatty acids. The highest recorded fatty acid % with organic Se dietary was oleinic acid (31.2%), Palmitic acid (19.36%), elaidic acid (14.9 %) and stearic acid scored 8.4%. The use of organic Se in dietary supplements improving fatty acids ratio has been reported in aquatic animals **(Lin & Shiau, 2005**) and in the crucian carp *Carassius auratus* **(Han *et al.*, 2011)**. This result meets the fact stated in the study of **Caravaggi *et al.* (1970)** who postulated that, inorganic selenium has the disadvantage of a low-absorption rate, environmental pollution and high toxicity.

Palmitic acid (19.36%) significantly decreased at 0.5mg/ kg of organic selenium diet; this may be due to the high concentration of selenium which may cause toxicity since Se associates with sulfur-containing amino acids cysteine (Cys) and methionine (Met),

where it is translationally incorporated as selenocysteine (SeCys) and selenomethionine (SeMet) in the protein **(Plateau *et al.*, 2015).**

Generally, previous studies revealed the importance of dietary Se in maintaining an effective immune system **(Kong *et al.*, 2017)**. **Le and Fotedar, (2014a, b)** have studied the role of Se dietary in increasing growth and resistance to *Vibrio anguillarum* in yellowtail kingfish. In crustaceans, the immune system mainly depends on non-specific immune processes for pathogen resistance, such as the prophenoloxidase-activating (Propo) system **(Iwanaga & Lee, 2005).**

Among *L. vannamei* fed Se in the diets, the lowest bacteraemia was observed in those fed 0.4 and 0.5 g/kg organic Se which was significantly lower ($P< 0.05$) than the control. Additionally, there was a significant difference between organic and inorganic Se, at each concentration, with inorganic Se bacterial number duplicated when compared with organic Se. This may be because the increase of the prophenoloxidase-activating (proPO) system is critical to antibacterial immune defence and is a sensitive indicator that reflects the immune status of crustacean species according to **Perazzolo and Barracco (1997)** and **Qiao *et al.* (2011)**. **Kong *et al.* (2017)** revealed according to his study of the selenium dietary effect on *Macrobrachium nipponense* that, the lowest PO activity was found in prawn-fed Se-deficient or excessive diets, reflecting that dietary Se deficiency or excess could decrease prawn innate immune ability.

The incorporation of organic selenium (OS) in the diet stimulated and increased the total haemocyte counts in the studied species, which indicated the positive effect of increasing the concentration of OS on the immunological response of *L. vannami*. However, our results disagree with those of **Kim *et al.* (2006)** and **Qiao *et al.* (2017)** who showed that high concentrations of selenium induce apoptosis. In addition, **Coskun *et al.* (2020)** also revealed a decrease in the THC of larvae of *Galleria mellonella* fed a selenium diet which can be prevented by adding vitamin E to the diet. **Watanabe *et al.* (1989)**, **Bell and Sargent (2003)** and **Osborn and Akoh (2002)** mentioned in their review article that n-9 fatty acids found as oleinic acids (C18:1 n-9) play a moderate role in the body and increase the stress tolerance. In the present study, the considerable ratio of n-3 PUFA, particularly, linoleic, EPA and DHA with organic Se indicated better action, tolerance and survival of *L. vannamei* in the culture.

CONCLUSION

The addition of organic selenium to *L. vannamei* diet increased total protein body composition and fatty acid ratios of samples under study, compared to basic and inorganic Se diets, especially at 0.4mg/ kg Se. In addition, Se supplementation enhanced the immune response of *L. vannamei* fed organic Se in its diets since the the samples under study showed lower bacteraemia than the control ones; This was mainly observed

in those fed 0.4 and 0.5mg/ kg organic Se ($P < 0.05$). Furthermore, it increased the proliferation rate of total haemocytes cells (THCs) in the studied species.

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