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Reproductive performance and vitellogenin mRNA transcript abundance in the hepatopancreas of female *Litopenaeus vannamei* fed diets with different soy lecithin content

Khaleg Maneii[a], Amin Oujifard[a,b,**], Ahmad Ghasemi[c], Mansour Torfi Mozanzadeh[d,*]

[a] *Department of Fisheries, Faculty of Agriculture and Natural Resources, Persian Gulf University, Bushehr, 7516913817, Iran*
[b] *Department of Fisheries, Faculty of Marine Science and Technology, Persian Gulf University, Bushehr, 7516913817, Iran*
[c] *Department of Fisheries, Persian Gulf Research Institute, Persian Gulf University, Bushehr, Iran*
[d] *Agriculture Research, Education and Extension, South Iran Aquaculture Research Center, Iran Fisheries Science Research Institution (IFSRI), Ahwaz, Iran*

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ABSTRACT

A 30-day nutritional study was conducted for determining the effects of supplementing soy lecithin (SL) in the diet of female *Litopenaeus vannamei*. Four isonitrogenous and isoenergetic diets were supplemented with graded amounts of SL including 0 (control), 20, 40 and 60 g Kg^{-1}. The brooding specimens fed the 40 g SL/kg diet had the greatest gonadosomatic index (3.4 ± 0.2 %) and the shortest latency period from eyestalk ablation to the first spawning (5 days). In addition, there was a larger content of long chain polyunsaturated fatty acids (LC-PUFA) in the hepatopancreas of brooding specimens fed with the 20 and 40 SL/kg diets compared to the other treatments. Furthermore, brooding specimens fed with the 40 g SL/kg diet had the greatest hemolymph cholesterol and high density lipoprotein concentrations. The abundance of vitellogenin mRNA transcript was greater in the hepatopancreas of brooding specimens fed with the 40 and 60 g SL/kg diets compared with the other groups. Results indicate that supplementing diets with 40 g SL/kg can improve growth and reproductive performance in *L. vannamei* female brooding specimens probably by enhancing LC-PUFA deposition and enhancing vitellogenin gene expression, as indicated by a greater abundance of mRNA transcript for vitellogenin, in the hepatopancreas.

1. Introduction

The western whiteleg shrimp (*Litopenaeus vannamei,* Boone 1931) with 4.2 million tons produced accounted for 53% of the world's total crustacean production in the aquaculture sector in 2016 (FAO, 2018). During recent years, the propagation of this species has been based on domesticated brooding specimens produced in biosecure settings utilizing selective breeding programs (Ceballos-Vázquez et al., 2010). The reproductive performance of domesticated brooding specimens are commonly less than that of specimens obtained from their natural habitat wild-caught ones mainly due to the apparent nutritional deficiencies of the specimens produced using aquaculture practices (Palacios et al., 2000).

* Corresponding author.
** Corresponding author at: Department of Fisheries, Faculty of Agriculture and Natural Resources, Persian Gulf University, Bushehr, 7516913817, Iran.
E-mail addresses: oujifard@pgu.ac.ir (A. Oujifard), m.torfi@areeo.ac.ir (M.T. Mozanzadeh).

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Generally, the feeding regimen for shrimp brooding specimens in the hatcheries still depends on various fresh or frozen food such as squid, bivalves, marine polychaetes and crustaceans as well as beef or pork liver (Hoa et al., 2009). The commercial use natural fresh food (*e.g.* squid, polychaete, crab, artemia biomass, mussels, clams and oysters) has been considered unfavorable for shrimp propagation in biosecure settings due to the risk of disease transmission, laborious application of such a feeding regimen and the inconsistent nutritional quality of feed sources (Wouters et al., 2001a, b; Vijayan et al., 2005). Replacement of fresh food with formulated feeds, therefore, is pivotal for safe and economical propagation of domesticated shrimp broodstocks. Results from previous research indicated the effect of formulated diets on successful spawning of shrimp brooding specimens relies on species and dietary ingredients (Wouters et al., 2002; Goodall et al., 2016). In addition, dietary requirements of shrimp brooders increase during sexual maturation and essential nutrients should be supplied in additional amounts during this developmental period (Arshadi et al., 2018).

Results of previous research indicate the lipid fraction of natural fresh foods contain functional components such as phospholipids (PL), which induce the secondary vitellogenesis and stimulate ovarian maturation in female shrimp brooding specimens (Mendoza et al., 1997). Furthermore, the PL especially phosphatidylcholine and phosphatidylethanolamine are the primary PL components in shrimp ovaries (Wouters et al., 2001b). The biosynthesis of PL through a *de novo* pathway has been reported to occur in most crustaceans; however, the synthesis rate is less than that needed to meet the nutritional requirements of shrimp brooding specimens especially during the period of gonad maturation (Kanazawa et al., 1985). Phospholipids are structural components of cell membranes and important precursors of eicosanoids, which are important regulatory factors of many cell functions. Furthermore, as an emulsifier and components of lipoproteins, PL have important functions in lipid absorption and transport from the hepatopancreas (HP) to the other organs (Coutteau et al., 1997; Tocher et al., 2008; Sui et al., 2009).

Soy lecithin (SL) is the most accessible and commercial source of PL in aquafeeds (Tocher et al., 2008). It has been confirmed that the incorporation of SL in diets of shrimp can lead to sexual maturation and promote reproductive performance by enhancing lipid mobilization from the HP to the ovaries (Cahu et al., 1994). It, therefore, it has been suggested that dietary PL can enhance long chain polyunsaturated fatty acid (LC-PUFA) and cholesterol retention in crustacean tissues (Teshima et al., 1986; Kontara et al., 1997; González-Félix and Perez-Velazquez, 2002; González-Félix et al., 2002; Li et al., 2016). Furthermore, Wang et al. (2013) reported that sufficient amount of SL in diets could augment the vitellogenin (*vtg*) gene expression and induce development of ovaries in *Cherax quadricarinatus* indicating that there is a positive effect of dietary SL on vitellogenesis during gonadal development of crustaceans. In the present study, the aim was to determine the effect of a graded content of dietary SL on reproduction, *vtg* gene expression, as determined by abundance *vtg* mRNA transcript in the HP and hemolymph metabolites and electrolytes of female *L. vannamei.*

2. Materials and methods

2.1. Diets

Four isonitrogenous (*ca.* 500 g/kg protein) and isolipidic (*ca.* 120 g/kg lipids) diets were supplemented with a graded content of

Table 1
Ingredients and composition of experimental diets.

Ingredients (g/kg)[a]	SL (g/kg)			
	0	20	40	60
Fish meal[b]	500	500	500	500
Shrimp meal[b]	120	120	120	120
Wheat gluten meal[b]	120	120	120	120
Corn gluten meal[b]	120	120	120	120
Beef gelatin	25	25	25	25
Yeast	10	10	10	10
Fish oil[b]	62	42	22	2
Soy lecithin[c]	0	20	40	60
Cholesterol[d]	5	5	5	5
Vitamin and mineral premix[e]	20	20	20	20
Vitamin C[f]	3	3	3	3
Di-calcium phosphate[b]	15	15	15	15

[a] Composition of ingredients [fish meal (520 g/kg crude protein, 180 g/kg crude lipid), shrimp meal (380 g kg^{-1} crude protein, 80 g kg^{-1} crude lipid), wheat gluten meal (500 g/kg crude protein, 30 g/kg crude lipid), corn gluten meal (520 g/kg crude protein, 30 g/kg crude lipid), beef gelatin (850 g/kg crude protein, 42 g/kg crude lipid).

[b] Havorash (Bushehr, Iran).

[c] Behpak Industrial Company, Behshahr, Mazandaran, Iran.

[d] Merck, Germany.

[e] Sumchun pure chemical, South Korea.

[f] Vitamin A, 5,000,000 IU; vitamin D3, 500,000 IU; vitamin E, 3000 mg; vitamin K3, 1500 mg; vitamin B1, 6000 mg; vitamin B2, 24,000 mg; vitamin B5, 52,000 mg; vitamin B6, 18,000 mg; vitamin B12, 60,000 mg; Folic acid, 3000 mg; nicotinamide 180,000 mg; antioxidant, 500 mg, copper, 3000 mg; zinc, 15,000 mg; manganese, 20,000 mg; Iron, 10,000 mg; potassiumiodate, 300 mg, career up to1 kg, Damloran pharmaceutical company, Broujerd, Iran.

SL including 0 (control), 20, 40 and 60 g/kg (Table 1). Diets contained approximately 80 g/kg moisture, 100 g/kg ash and 18.5 MJ/kg energy to meet all nutritional requirements of *L. vannamei* brooding specimens based on results from previous studies (Goimier et al., 2006; Arshadi et al., 2018). Fish, shrimp, wheat and corn gluten meal were the main protein sources, whereas FO and SL served as the main lipid ingredients in the experimental diets. After blending the dry ingredients (30 min), enough distilled water was poured on the feed to form a dough, which subsequently was extruded with a meat grinder to produce 3 mm pellets. The pellets were subsequently dried (50 °C for 24 h) and kept in a freezer (−20 °C) until the beginning of the feeding experiment.

2.2. Husbandry settings

Hawaiian domesticated *L. vannamei,* with a six generation (SPF6) linage were transported from a private greenhouse pond (surface: 1000 m^2, Delvar, Bushehr, Iran; 28°76́' N, 51°07′ E) to the Aquatic Research Laboratory of Persian Gulf University (Bushehr, Iran; 28°96′ N, 50°83′ E). Shrimp brooding specimens were disinfected with formalin (100 ppm, 30 s) as recommended by Alday-Sanz (2010). The shrimp population was stocked in two 4000 L circular fiberglass tanks (30 shrimp/m^3), and acclimated to the husbandry system for a week. Healthy shrimp brooding specimens (n = 120; 28.6 ± 0.2 g, mean ± SEM) including males and females (1:1 ratio) were stocked into 12 black circular polyethylene tanks with a volume of 250 L at a density of 10 shrimp per tank (five females and five males in each tank). Tanks were supplied with 200 L of seawater that was filtered with sand and activated carbon filters and disinfected using a UV system. For increasing and stabilizing water temperature, each tank was equipped with a 300-watt aquarium heater. The physicochemical parameters of water including temperature (28.9 ± 1.4 °C), salinity (35.2 ± 3.1‰), dissolved oxygen (5.5 ± 0.2 mg/L) and pH (8.2 ± 0.2) were determined two times a day (0800 h and 1700 h). The experimental photoperiodic pattern was 12L:12D (light: darkness) during the experiment. In this study, fresh food were not used for feeding of brooders due to the high levels of PL in these foodstuff (*e.g.* squid and blood worm). Triplicate groups of shrimp were fed one of the experimental diets two times daily (1200 h and 2300 h) at 5% of their biomass during 30 days of husbandry trial according to Arshadi et al. (2018).

2.3. Sampling

At the end of the experimental period, brooding specimens were not supplied feed for a day and before sampling the shrimp were placed in chilled water (4 °C, 10 min) for reducing manipulation stress, then their body weight (BW_f) and length were determined. After 30 days of feeding, the left eyestalk of females brooding specimens (n = 5 shrimp/tank) were ablated by cutting the base of the eye peduncle, then the wound was burned. For evaluating latency period after eyestalk ablation (ESA), development of ovaries was examined daily by shining the beam from a flashlight into the dorsal surface of exoskeleton to monitor the size and color of the gonads (Alday-Sanz, 2010). The visual maturation stage of female brooding specimens was characterized as being in three stages: 1) mature, having bright orange gonads with lobules occupying most of the cephalothorax and extending in a thick line along the abdominal region; 2) maturing, having yellowish opaque gonads without lobules and 3) immature, with transparent or white gonads indistinguishable from the intestine through the exoskeleton. When the eyestalk-ablated female brooding specimens developed to their final maturation stage (IV), their hemolymph was collected (three samples per tank) as described by Arshadi et al. (2018), then the shrimp were placed in chilled water and the hepatopancreas (HP) was dissected and weighed (three samples per tank) and stored at −80 °C to determine the abundance of *vtg* mRNA transcript and fatty acids profile in the HP. For examining absolute fecundity, about 0.1 g of tissue from the cranial, mid and caudal sections of the ovaries was collected from the other female shrimp in each tank (two samples per tank) and stored in Gilson's fluid for 2 months (Simpson, 1951). A light microscope equipped with a micrometer was used for measuring egg diameter (μm) with the applied magnification of X40.

2.4. Fatty acid (FA) profile

Total lipids were extracted from the ingredients of the experimental diets (n = 1, Table 2) and HP (nine samples per treatment) using chloroform/methanol (2:1, v/v) solution utilizing the procedure previously published by Folch et al. (1957). Acidic methanolysis (sulphuric acid in methanol) of lipid extracts was conducted to prepare FA methyl esters using previously published procedures (Christie, 1993). The FA profile of samples was evaluated using a gas chromatography (GC, Agilent technologies 7890 N, USA), equipped with aflame ionization detector (FID) and a cyanopropyl–phenyl capillary column (DB-225MS, 30 m × 0.250 mm ID × 0.25 μm Film thickness, USA). The identification of FA was accomplished by using an external commercial standard mixture (GLC-68d, NuChek Prep., MN, USA) as described by Agh et al. (2014).

2.5. Biochemical analyses

Values for hemolymph biochemical variables were determined using an autoanalyser (Technicon RA-1000, Technicon Instruments, NewYork, NY, USA) utilizing clinical diagnostic kits (Pars Azmoon Kit, Tehran, Iran) for quantification of glucose (Glu), total protein (TP), calcium (Ca^{2+}), total cholesterol (Chol), high-density lipoprotein (HDL) and triglyceride (Tg). Plasma sodium, chloride (mmol/L) and total osmolality (mOsmol/kg) were determined using an electrolyte analyzer (Caretium, XI-921, China) and a freezing point osmometer (Knauer, K-7400, Germany), respectively.

Table 2
Fatty acid composition of experimental diets (% total fatty acids).

	SL (g/kg)			
Fatty acids	0	20	40	60
14:0	2.5	1.8	2.5	1.1
16:0	23.3	22.9	28.0	23.9
18:0	6.7	7.1	5.6	6.9
20:0	0.4	0.5	0.6	0.8
22:0	0.6	0.4	0.4	0.6
24:0	0.7	1.0	1.1	0.8
SFA[a]	34.2	33.7	38.2	34.1
14:1n-5	0.4	0.3	0.4	0.4
16:1n-7	5.1	4.2	4.8	3.4
18:1n-7	2.8	2.5	3.1	2.1
18:1n-9	27.4	26.3	20.1	23.6
20:1n-9	1.6	1.3	0.7	0.4
22:1n-9	1.1	0.4	1.3	0.2
MUFA[b]	38.4	35.0	30.4	30.1
18:2n-6, LNA[c]	12.5	16.4	21.7	25.8
20:2n-6	0.4	0.1	0.4	0.2
20:4n-6, ARA[d]	1.0	0.9	0.4	0.6
n-6 PUFA[e]	13.9	17.4	22.5	26.6
18:3n-3, ALA[f]	1.1	1.7	2.5	2.9
20:5n-3, EPA[g]	3.4	2.4	1.2	0.8
22:6n-3, DHA[h]	7.1	6.8	4.9	5.1
n-3 PUFA[i]	11.6	10.9	8.6	8.8
LC-PUFA[j]	11.9	10.2	6.9	6.7
n-3/n-6	0.9	0.6	0.4	0.3
ARA/EPA	0.3	0.4	0.3	0.7
DHA/EPA	2.1	2.9	4.2	6.0

[a] SFA: saturated fatty acids.
[b] MUFA: monounsaturated fatty acids.
[c] LNA; linoleic acid.
[d] ARA; arachidonic acid.
[e] n-6 PUFA: n-6 polyunsaturated fatty acids.
[f] ALA; α-linoleic acid.
[g] EPA; eicosapentaenoic acid.
[h] DHA; docosahexaenoic acid.
[i] n-3 PUFA: n-3 polyunsaturated fatty acids.
[j] LC-PUFA; long chain polyunsaturated fatty acids.

2.6. Evaluation of relative hepatopancreatic vtg mRNA transcript abundance

Total RNA in hepatopancreas was isolated using a RNXTM kit (Cinnagen, Tehran, Iran) according to instructions described by the manufacturer. The concentration of RNA was evaluated spectrophotometrically (Biotech Photometer, WPA) at 260, and samples with the RNA ratios (A260:A280) of greater than 1.8 were selected for further analyses. To verify RNA integrity, RNA was evaluated using a 1% agarose. Reverse transcription (RT) was conducted using 1 μg of total RNA with the random hexamers and M-MuLV reverse transcriptase enzyme kit (Vivantis) following the protocol provided by the manufacturer. Beta-actin was used as the internal housekeeping gene. The Primer sequencing and GenBank accession numbers for beta-actin and vitelogenin genes included in the assay are provided in Table 3. Real-time quantitative RT-PCR was performed using a real-time PCR machine (Rotor Gene-3000, Sydney, Australia) in a total volume of 12.5 μl containing 6.25 μl of SYBR Green qPCR Master Mix (2×) (Cinnagen, Iran), 0.5 μl of cDNA, 0.5 μl of each primer(12.5 mM), and 4.75 μl of DNase free water. The conducting of the real-time quantitative RT-PCR procedure included a denaturation step at 94 °C for 2 min, followed by 40 amplification cycles of 15 s denaturation at 94 °C, 30 s annealing at 60 °C, 30 s extension at 72 °C and final extension at 72 °C for 5 min followed by conducting the melting curve procedure. After PCR amplification, melt-curve analysis was conducted to confirm that there was only one amplified product. The data for

Table 3
Primers used in the real-time quantitative RTPCR analyses.

Reference	Amplicon size	Gen Bank Number	Sequence (5′- 3′)	Primer name
Chen et al. (2014)	218	AY321153	Forward: GGTGTTG CTGTTGCTGCTGTGAA	VTG-F
			Reverse: TTGACTAACTGAGATGAAG AGAAC	VTG-R
Wang et al. (2017)	140	AF300705	Forward: CGCGACCTCACAGACTACCT	β-actin RT-F
			Reverse: GTGGTCATCTCCTGCTCGAA	β-actin RT-R

Table 4
Morphometric and reproductive variables of *L. vannamei* females fed diets supplemented with a soy lecithin (mean ± SEM, n = 3).

	SL (g/kg)			
	0	20	40	60
Morphometric variables				
BW_i(g)[a]	28.7 ± 0.0	28.8 ± 0.2	28.5 ± 0.4	28.4 ± 0.1
BW_f(g)[b]	31.2 ± 0.1^{b}	31.5 ± 0.1^{b}	33.0 ± 1.1^{a}	31.8 ± 0.3^{b}
WG (%)[c]	8.8 ± 0.2^{b}	9.4 ± 0.3^{b}	15.8 ± 2.3^{a}	12.0 ± 1.8^{ab}
SGR (% BW/day)[d]	0.28 ± 0.0^{b}	0.32 ± 0.0^{b}	0.5 ± 0.1^{a}	0.38 ± 0.0^{ab}
K (%)[e]	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
Survival (%)[f]	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Reproductive variables				
HPI (%)[g]	3.3 ± 0.3	3.6 ± 0.2	3.5 ± 0.1	3.5 ± 0.1
GSI (%)[h]	2.8 ± 0.1^{b}	2.9 ± 0.3^{b}	3.4 ± 0.2^{a}	2.7 ± 0.3^{b}
Absolute fecundity ($\times 10^3$)	159.5 ± 5.8	175.5 ± 12.4	180.7 ± 14.5	187.1 ± 10.0
Relative fecundity ($\times 10^3$)[i]	5.1 ± 0.2	5.6 ± 0.3	5.5 ± 0.3	5.6 ± 0.2
Eggs diameter (μm)	96.6 ± 5.6^{b}	93.3 ± 9.5^{b}	103.3 ± 8.0^{a}	106.7 ± 9.9^{a}
Latency period (days after eye stalk ablation to spawning)	9.0 ± 0.0^{c}	6.0 ± 0.6^{b}	5.0 ± 0.2^{a}	6.0 ± 0.6^{b}

Note: A different superscript in the same row denotes differences ($P < 0.05$).
[a] BW_i: initial body weight.
[b] BW_f: final body weight.
[c] WG: weight gain = $(BW_f - BW_i)/BW_i) \times 100$.
[d] SGR: specific growth rate = $[(\ln BW_f - \ln BW_i)/t] \times 100$, where t is experimental period = 30 days.
[e] K: Fulton's condition factor = $(BW_f\ (g)/TL_f\ (cm^3) \times 100$.
[f] Survival = (number of specimens in each group remaining on day 30/initial number of specimens) × 100.
[g] HPI: hepatopancreatic index = (hepatopancreas weight (g)/BW_f (g)) × 100.
[h] GSI: gonadosomatic index = (gonad weight (g)/BW_f (g)) × 100.
[i] Relative fecundity = ((number of eggs ($\times 10^3$)/BW_f (g)).

relative abundance of mRNA transcript were obtained by conducting three independent biological replicates, which were performed in duplicate to calculate the threshold cycle (Ct) value. Data analysis of the real-time PCR values was performed in triplicate using the Rotor-Gene, RG-3000 (Australia) software. The comparative CT ($2^{-\Delta\Delta CT}$) method was used (Livak and Schmittgen, 2001).

2.7. Statistics

There was analysis of data using SPSS (Ver. 16.0, Chicago, IL, USA). After the testing for normality of the data distribution (Shapiro–Wilk's test) and homogeneity of variance (Levene's test), a one-way analysis of variance was conducted. A Duncan's *posthoc* test was used for multiple comparisons. The significance level was considered to be $P \leq 0.05$.

3. Results

There was not any mortality during the feeding trial (Table 4). Shrimp brooding specimens fed with the 40 g SL/kg diet had more weight gain (15.8 ± 2.3%) and specific growth rate (0.5 ± 0.1%) than those fed with the control and 20 g SL/kg diets, whereas brooding specimens fed with the 60 g SL/kg diet had intermediate values for these variables. Shrimp brooding specimens fed the 40 g SL/kg diet had the greatest gonadosomatic index ($P < 0.05$). There were no differences in hepatopancreatic index as well as absolute and relative fecundities among groups ($P > 0.05$). The spawning specimens fed 40 or 60 g SL/kg diets had a greater egg diameter than the specimens of the other groups ($P < 0.05$). The shortest and the longest latency periods between ESA to final maturation were observed in spawning specimens fed the 40 g SL/Kg and the control diet, respectively ($P < 0.05$).

For the FA profile of experimental diets, the concentrations of linoleic (LNA) and α-linolenic (ALA) acids increased as the dietary content of SL increased, whereas the monounsaturated fatty acids (MUFA), arachidonic acid (ARA), n-3 PUFA (mainly eicosapentaenoic (EPA) and docosahexaenoic acids (DHA)), LC-PUFA concentrations and n-3/n-6 ratio progressively decreased (Table 2). The FA composition of the HP was markedly altered as a result of the different diets (Tables 5). The concentrations of saturated fatty acids (SFA) was greatest in brooding specimens fed with the 60 g SL/kg diet ($P < 0.05$). The amount of MUFA gradually increased in the HP of brooding specimens with a greater dietary SL content and brooding specimens fed with the 60 g SL/kg diet had the greatest MUFA concentrations ($P < 0.05$). Brooding specimens fed with the 60 g SL/kg diet had the greatest concentrations of n-6 PUFA especially the LNA and ARA acids. There were the greatest and least contents of LC-PUFA, especially DHA were found in the HP of brooding specimens fed 20 and 60 g SL/kg diets, respectively ($P < 0.05$). The n-3 to n-6 PUFA ratio was markedly less in the HP of brooding specimens fed with the 60 g SL/kg diet.

In the present study, there was the greater hemolymph GLU in brooding specimens fed with SL supplemented diets compared to the control ($P < 0.05$, Table 6). Furthermore, female brooding specimens fed with the 40 and 60 g SL/kg diets had the greatest and least hemolymph cholesterol and HDL, respectively ($P < 0.05$). The content of triglycerides in spawning specimens fed with the 20 and 40 g SL/kg diets was higher than other treatments. The concentrations of TP and electrolytes including calcium, phosphorus,

Table 5
Fatty acid composition of the hepatopancreas of *L. vannamei* fed different experimental diets (% total fatty acids; mean ± SEM, n = 3).

	SL (g/kg)			
Fatty acids	0	20	40	60
14:0	0.8 ± 0.0[b]	1.0 ± 0.0[a]	0.8 ± 0.0[b]	0.7 ± 0.0[b]
16:0	19.3 ± 0.4	20.4 ± 1.1	19.7 ± 1.0	20.5 ± 9.5
18:0	12.9 ± 0.6[ab]	9.6 ± 0.6[b]	10.6 ± 0.3[ab]	13.5 ± 1.7[a]
20:0	0.7 ± 0.1[b]	1.1 ± 0.1[a]	0.7 ± 0.1[b]	0.9 ± 0.1[ab]
22:0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
SFA[a]	34.0 ± 1.3[ab]	32.4 ± 0.8[b]	32.0 ± 2.7[b]	35.9 ± 2.3[a]
14:1n-5	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
16:1n-7	3.3 ± 0.2	2.9 ± 0.5	3.4 ± 0.2	2.9 ± 0.5
18:1n-7	4.6 ± 0.4[a]	3.1 ± 0.4[b]	4.2 ± 0.2[ab]	4.6 ± 0.3[a]
18:1n-9	20.8 ± 1.2[b]	20.6 ± 0.3[b]	22.9 ± 1.7[ab]	24.9 ± 1.1[a]
20:1n-9	0.3 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.1[b]	0.8 ± 0.3[a]
22:1n-9	0.1 ± 0.0[b]	0.4 ± 0.1[a]	0.1 ± 0.0[b]	0.3 ± 0.0[ab]
MUFA[b]	29.4 ± 0.9[ab]	27.7 ± 1.3[b]	31.2 ± 1.6[ab]	33.7 ± 2.2[a]
18:2n-6, LA[c]	6.4 ± 0.5[b]	7.3 ± 0.3[ab]	6.0 ± 0.1[b]	8.1 ± 0.3[a]
20:2n-6	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	1.1 ± 0.1
20:3n-6	0.7 ± 0.1[a]	0.4 ± 0.0[b]	0.5 ± 0.0[b]	0.5 ± 0.1[b]
20:4n-6, ARA[d]	7.7 ± 0.1[b]	7.3 ± 0.3[b]	7.8 ± 0.5[b]	10.1 ± 0.1[a]
n-6 PUFA[e]	15.8 ± 0.5[b]	15.9 ± 0.8[b]	15.2 ± 1.0[b]	19.8 ± 1.8[a]
18:3n-3, LNA[f]	0.5 ± 0.0[a]	0.6 ± 0.1[a]	0.3 ± 0.0[b]	0.6 ± 0.1[a]
20:3n-3	0.2 ± 0.0[b]	0.1 ± 0.0[b]	0.2 ± 0.0[b]	0.5 ± 0.1[a]
20:5n-3, EPA[g]	6.3 ± 0.6[a]	7.2 ± 0.2[a]	7.5 ± 0.3[a]	3.7 ± 0.4[b]
22:6n-3, DHA[h]	8.5 ± 0.6[b]	11.5 ± 1.6[a]	9.2 ± 0.7[b]	5.4 ± 0.2[c]
n-3 PUFA[i]	15.5 ± 0.3[b]	19.4 ± 1.1[a]	17.2 ± 3.2[ab]	10.2 ± 0.9[c]
LC-PUFA[j]	24.0 ± 0.9[b]	27.3 ± 0.6[a]	26.0 ± 0.1[a]	21.3 ± 1.1[c]
n-3/n-6	1.0 ± 0.1[a]	1.2 ± 0.0[a]	1.1 ± 0.1[a]	0.5 ± 0.0[b]
ARA/EPA	1.3 ± 0.2[b]	1.0 ± 0.1[b]	1.0 ± 0.0[b]	2.8 ± 0.1[a]
DHA/EPA	1.4 ± 0.0[ab]	1.6 ± 0.1[a]	1.2 ± 0.1[b]	1.5 ± 0.1[ab]

Note: A different superscript in the same row denotes differences (P < 0.05).
[a] SFA: saturated fatty acids.
[b] MUFA: monounsaturated fatty acids.
[c] LNA; linoleic acid.
[d] ARA; arachidonic acid.
[e] n-6 PUFA: n-6 polyunsaturated fatty acids.
[f] ALA; α-linoleic acid.
[g] EPA; eicosapentaenoic acid.
[h] DHA; docosahexaenoic acid.
[i] n-3 PUFA: n-3 polyunsaturated fatty acids.
[j] LC-PUFA; long chain polyunsaturated fatty acids.

Table 6
Plasma biochemical variables of *L. vannamei* females fed experimental diets (mean ± SEM, n = 3).

	SL (g/kg)			
Biochemical variables	0	20	40	60
Total protein (g/dL)	10.1 ± 0.8	9.9 ± 0.2	9.5 ± 0.8	7.8 ± 1.4
Glucose (mg/dL)	35.8 ± 1.5[b]	40 ± 0.6[a]	42.7 ± 0.8	41.4 ± 0.8[a]
Lactate (mg/dL)	50.3 ± 0.7	47.0 ± 0.8	46.2 ± 1.7	49.2 ± 0.7
Cholesterol (mg/dL)	35.3 ± 1.6[ab]	37.5 ± 2.2[ab]	44.7 ± 2.0[a]	21.0 ± 0.2[b]
Triglyceride (mg/dL)	55.6 ± 7.9[b]	108.3 ± 11.4[a]	92.5 ± 2.3[a]	43.5 ± 1.2[b]
HDL (mg/dL)	10.0 ± 0.3[ab]	11.7 ± 1.1[ab]	12.9 ± 0.9[a]	6.7 ± 0.1[b]
Calcium (mg/dL)	13.2 ± 0.5	12.8 ± 0.3	12.9 ± 0.4	12.6 ± 0.1
Inorganic phosphorus (mg/dL)	2.4 ± 0.0	1.7 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Sodium (mmol/L)	310 ± 2.3	296.5 ± 12.4	314.5 ± 13	309.5 ± 14
Chlorine (mmol/L)	164.5 ± 3.8	162 ± 1.7	180.5 ± 8.9	174 ± 1.2
Total osmolality (mOsm/kg)	453 ± 3.4	445.5 ± 18.8	460 ± 6.9	456.3 ± 6.9

Note: A different superscript in the same row denotes differences (P < 0.05).

sodium, chloride and total osmolality in hemolymph; however, were not affected by dietary SL in female brooding specimens ($P > 0.05$).

The relative abundance of *vtg* mRNA transcript was greater in the HP of female shrimp fed with the 40 and 60 g SL/kg diets compared to those fed with the control and 20 g SL Kg^{-1} diets, whereas there was the greatest relative abundance of *vtg* mRNA transcript in shrimp fed with the 60 g SL/kg diet ($P < 0.05$, Fig. 1).

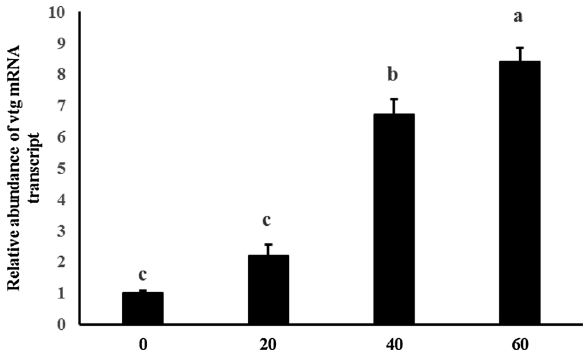

Fig. 1. Relative abundance of vitellogenin mRNA transcript in *L. vannamei* examined using real-time quantitative RT-qPCR; B-actin gene was used as an internal control to calibrate the cDNA template for all the samples (mean ± SEM, n = 3); Different superscripts on the bars denotes differences ($P < .05$).

4. Discussion

Soybean lecithin provides phosphatidylcholine, which is the main substrate for the formation of lipoproteins and has growth-promoting effects in crustacean species. In addition, it can positively induce lipid mobilization in the body that may enhance energy availability for growth (Coutteau et al., 1997). The findings from conducting the present study indicate dietary SL at 40 g/kg improved growth performance in *L. vannamei* brooding specimens. Results from previous studies in *L. vannamei* at the post-larval (15 g SL/kg diet, Coutteau et al., 1996) and juvenile (30 − 50 g SL/kg diet, Gong et al., 2001; González-Félix and Perez-Velazquez, 2002; González-Félix et al., 2002; Sanchez et al., 2014) stages also indicate there was a positive effect of dietary SL on growth of this species.

The *L. vannamei* spawning specimens have a restricted capacity for PL biosynthesis (Cahu et al., 1994). Cahu et al. (1994) reported dietary PL and LC-PUFA deficiencies lead to a decreased frequency of spawning, number of spawning specimens and egg count in *L. vannamei* brooding specimens. In the present study, there was the greatest GSI and lesser latency period in brooding specimens fed with the 40 g SL/kg diet, which coincided with greatest hemolymph cholesterol and HDL concentrations, indicating there was an increased mobilization of lipids from the HP to ovaries in this group as previously reported in *Marsupenaeus japonicas* (Alava et al., 1993) and *E. sinensis* (Sui et al., 2009) female brooding specimens.

A plethora of studies in crustaceans have indicated the FA profile of the HP and ovaries generally is consistent with the FA composition of the diets (Cahu et al., 1994; Sui et al., 2009; Wang et al., 2013; Li et al., 2016). In the present study, the concentration of n-6 PUFA, especially LNA and ARA, increased in the HP with an increased dietary SL content indicating that there is an induction of LNA to ARA bioconversion, especially in the 60 g SL/kg group. In addition, the retention of LC-PUFA, especially DHA, in the HP of the brooding specimens fed with the 20 and 40 g SL/kg diets was greater indicating the positive effects of dietary SL in selective deposition of these FA as previously reported in other crustacean species (Wu et al., 2007; Wang et al., 2013). These results indicate there is an improvement of the reproductive performance in brooding specimens fed the 40 g SL/kg diet that is partially related to the retention of LC-PUFA which is consistent with the enhancement of HDL concentrations in the hemolymph as well as enhanced hepatopancreatic *vtg* gene expression as indicated by *vtg* mRNA transcript abundance.

Results of the present study indicate the TP in hemolymph was not affected by dietary SL inclusion, indicating the greater content of the SL did not have health promoting effects in brooding specimens. The concentration of GLU was greater in the brooding specimens fed SL-supplemented diets indicating that there was an enhanced nutritional status of these specimens as compared with those fed the control diet. Results of the present study indicate the shrimp brooding specimens fed with the 40 g SL/kg diet had the greatest cholesterol and HDL content in the hemolymph indicating an augmentation in mobilization of FA and cholesterol from the HP to the developing ovaries. It, therefore, has been confirmed that the PL can enhance the emulsification of dietary lipid and facilitate the transportation of assimilated lipids into hemolymph (Coutteau et al., 1997). Similar to results from the present study, it has been reported that supplementing the diet with phospholipids increased cholesterol content in the hemolymph of *Hornurns amencunus* (Baum et al., 1990) and *P. trituberculatus* (Han et al., 2018). The significant reduction in lipid concentrations in hemolymph of the brooding speciments fed with the 60 g SL/kg diet; however, may be related to marked reduction in dietary n-3 LC-PUFA. The n − 3 LC-PUFA can induce lipoprotein synthesis by decreasing the concentration of enzymes associated with cholesterol synthesis (Abbey et al., 1990).

Phospholipids have an important function in cell membrane structure and can modulate ion permeability and the function of certain membrane-related enzymes involved in osmoregulation processes (i.e., Na^+/K^+ ATPase, Gong et al., 2004). Osmotic pressure and electrolyte concentrations in hemolymph modulate the capacity of osmoregulation in crustacean species. Results of the current study indicate the dietary SL did not change osmoregulation capacity in *L. vannamei* brooding specimens, indicating PL might be enough in all experimental diets to support osmoregulatory functions. Gong et al. (2004) noted that the extra inclusion of lecithin along with cholesterol, K^+, Mg^{2+} and NaCl in feed leads to an improved osmoregulatory capacity in *L. vannamei*.

There is very little information about nutritional regulation of PL on vitellogenesis at the molecular level. In the present study, there was a greater hepatopancreatic *vtg* gene expression, as indicated by the increased abundance of the vtg mRNA transcript, in female brooding specimens fed with the 40 or 60 g SL/kg diet indicating secondary oocyte development and subsequent ovarian maturation was induced by inclusion of these amounts in diets. The greater GSI value in brooding specimens fed with 40 g SL/kg diet might have resulted from a greater hepatopancreatic *vtg* gene expression as well as optimal concentrations of LC-PUFA in the diet. Results from previous research also indicate there are beneficial effects of the PL in enhancing HP *vtg* gene expression in *C. quadricarinatus* (Wang et al., 2013) and *P. trituberculatus* female brooding specimens (Ding et al., 2017).

5. Conclusion

In summary, results from the present study, when there was inclusion of 40 g SL/kg in a diet, indicate there was improvement in growth and reproductive performance in *L. vannamei* female brooding specimens as a result of enhanced LC-PUFA deposition and *vtg* gene expression in the HP as well as induced lipid mobilization (*e.g.,* cholesterol, HDL and triglycerides) through hemolymph transport from the HP to the ovaries. In addition, inclusion of 60 g SL/kg of diet markedly reduced the LC-PUFA content in the HP which may lead to a reduction of lipoprotein synthesis and consequently decreased reproductive performance in terms of the GSI and latency period. Further research is required to evaluate the proteomic mode of action and pathways in which dietary phospholipids can modulate reproductive performance in *L. vannamei*.

Data availability statement

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

Declaration of Competing Interest

I hereby confirm that all authors have approved the final version the manuscript and agreed with its submission to Animal Reproduction Science. There is no conflict of interest among authors in submission of the manuscript.

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References

Abbey, M., Clifton, P., Kestin, M., Belling, B., Nestel, P., 1990. Effects of fish-oil on lipoproteins, lecithin: cholesterol acyltransferase and lipid transfer proteins activity in humans. Arteriosclerosis 85, 85–94.

Agh, N., Jasour, M.S., Noori, F., 2014. Potential development of value-added fishery products in underutilized and commercial fish species: comparative study of lipid quality indicators. J. Am. Oil Chem. Soc. 91, 1171–1177.

Alava, V.R., Kanazawa, A., Teshima, S., Sakamoto, S., 1993. Effect of dietary phospholipids and n-3 highly unsaturated fatty acids on ovarian development of kuruma prawn. Nip. Sui. Gak. 59, 345–351.

Alday-Sanz, V., 2010. The Shrimp Book (p. 899). Nottingham University Press, U.K.

Arshadi, A., Yavari, V., Oujifard, A., Mousavi, S.M., Gisbert, E., Mozanzadeh, M.T., 2018. Dietary nucleotide mixture effects on reproductive and performance, ovary fatty acid profile and biochemical parameters of female Pacific shrimp *Litopenaeus vannamei*. Aqua. Nutr. 24, 515–523.

Baum, N.A., Conklin, D.E., Chang, E.S., 1990. Effect of dietary lecithin in combination with casein or crab protein on cholesterol uptake and transport in the lobster *Homarus americanus*. J. World. Aqua. Soc. 21, 277–287.

Cahu, C., Guillaume, J.C., Stephan, G., Chim, L., 1994. Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg and tissue composition in *Penaeus vannamei* fed semi-purified diets. Aquaculture 126, 159–170.

Ceballos-Vázquez, B.P., Palacios, E., Aguilar-Villavicencio, J., Racotta, I.S., 2010. Gonadal development in male and female domesticated whiteleg shrimp, *Litopenaeus vannamei*, in relation to age and weight. Aquaculture 308, 116–123.

Chen, T., Zhang, L.P., Wong, N.-K., Zhong, M., Ren, C.-H., Hu, C.-H., 2014. Pacific white shrimp (*Litopenaeus vannamei*) vitellogenesis-inhibiting hormone (VIH) is predominantly expressed in the brain and negatively regulates hepatopancreatic vitellogenin (VTG) gene expression. Biol. Reprod. 47, 1–10.

Christie, W.W., 1993. Preparation of ester derivatives of fatty acids for chromatographic analysis. In: Christie, W.W. (Ed.), Advances in Lipid Methodology. Oily Press, Dundee, Scotland, pp. 69–111.

Coutteau, P., Camara, M.R., Sorgeloos, P., 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval *Penaeus vannamei*. Aquaculture 147, 261–273.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture 155, 149–164.

Ding, L.-Y., Jin, M., Sun, P., Lu, Y., Ma, H.-N., Yuan, Y., Fu, H.-Y., Zhou, Q.-C., 2017. Cloning, tissue expression of the fatty acid-binding protein (Pt-FABP1) gene, and effects of dietary phospholipid levels on fabp and vitellogenin gene expression in the female swimming crab *Portunus trituberculatus*. Aquaculture 474, 57–65.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. FAO, Rome.

Folch, j., Less, M., Stainley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226, 497–509.

Goimier, Y., Pascual, C., Sanchez, A., Gaxiola, G., Sanchez, A., Rosas, C., 2006. Relation between reproductive, physiological, and immunological condition of *Litopenaeus setiferus* pre-adult males fed different dietary protein levels (Crustacea; Penaeidae). Anim. Reprod. Sci. 92, 193–208.

Gong, H., Lawrence, A.L., Gatlin, D.M.I.I.I., Jiang, D.H., Zhang, F., 2001. Comparison of different types and levels of commercial soybean lecithin supplemented in semipurified diets for juvenile *Litopenaeus vannamei* Boone. Aqua. Nutr. 7, 11–17.

Gong, H., Jiang, D.H., Lightner, D.V., Collins, C., Brock, D., 2004. A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. Aqua. Nutr. 10, 227–236.

González-Félix, M.L., Perez-Velazquez, M., 2002. Current status of lipid nutrition of pacific white shrimp *Litopenaeus vannamei*. In: Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxola-Cortez, M.G., Simoez, N. (Eds.), Advances En Nutricion Acuicola. Three and Six of the September, pp. 35–45 Cancun, Mexico.

González-Félix, M.L., Gatlin, D.M.I.I.I., Lawrence, A.L., Perez-Velazquez, M., 2002. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture 207, 151–167.

Goodall, J.D., Wade, N.M., Merritt, D.J., Sellars, M.J., Salee, K., Coman, G.J., 2016. The effects of adding microbial biomass to grow-out and maturation feeds on the reproductive performance of black tiger shrimp, *Penaeus monodon*. Aquaculture 450, 206–212.

Han, T., Wang, J.T., Li, X.Y., Yang, Y.X., Yang, M., Tian, H.L., Wang, C.L., 2018. Effects of dietary phospholipid and cholesterol levels on growth and fatty acid composition of juvenile swimming crab, *Portunus trituberculatus*. Aqua. Nutr. 24, 164–172.
Hoa, N.D., Wouters, R., Wille, M., Thanh, V., Dong, T.K., Hao, N.V., Sorgeloos, P., 2009. A fresh-food maturation diet with an adequate HUFA composition for broodstock nutrition studies in black tiger shrimp *Penaeus monodon* (Fabricius, 1798). Aquaculture 297, 116–121.
Kanazawa, A., Teshima, S., Sakamoto, M., 1985. Effects of dietary lipids, fatty acids and phospholipids on the growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture 50, 39–49.
Kontara, E.K.M., Coutteau, P., Sorgeloos, P., 1997. Effect of dietary pohospholipid on requirements for and incorporation of n-3 highly unsaturated fatty acids in postlarval *Penaeus japonicus* Bate. Aquaculture 158, 305–320.
Li, X.Y., Wang, J.T., Han, T., Hu, S.X., Jiang, Y.D., Wang, C.L., 2016. Effects of phospholipid addition to diets with different inclusion levels of fish oil on growth and fatty acid body composition of juvenile swimming crab *Portunus trituberculatus*. Aqua. Res. 47, 1112–1124.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔC (T) Method. Methods 25, 402–408.
Mendoza, R., Revol, A., Fauvel, C., Patrois, J., Guillaume, J.C., 1997. Influence of squid extracts on the triggering of secondary vitellogenesis in *Penaeus vannamei*. Aqua. Nutr. 3, 55–63.
Palacios, E., Ibarra, A.M., Racotta, I.S., 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. Aquaculture 185, 353–371.
Sanchez, D.R., Fox, J.M., Gatlin, D., Lawrence, A.L., 2014. Dietary effect of fish oil and soybean lecithin on growth and survival of juvenile *Litopenaeus vannamei* in the presence or absence of phytoplankton in an indoor system. Aqua. Res. 45, 1367–1379.
Simpson, A.C., 1951. The fecundity of the plaice. Fisheries Investig. London 17, 1–27.
Sui, L.Y., Wu, X.G., Wille, M., Cheng, Y.X., Sorgeloos, P., 2009. Effect of dietary soybean lecithin on reproductive performance of Chinese mitten crab *Eriocheir sinensis* (H. Milne-Edwards) broodstock. Aqua. Inter. 17, 45–56.
Teshima, S., Kanazawa, A., Kakuta, Y., 1986. Effects of dietary phospholipids on growth and body composition of the juvenile prawn. Bull. Jpn. Sot. Sci. Fish. 52, 155–158.
Tocher, D.R., Bendiksen, E.Å., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280, 21–34.
Vijayan, K.K., Raj, V.S., Balasubramanian, C.P., Alavandi, S.V., Sekhar, V.T., Santiago, T.C., 2005. Polychaete worms – a vector for white spot syndrome virus (WSSV). Dis. Aqua. Organ. 63, 107–111.
Wang, L., Zuo, D., Lv, W., Li, J., Wang, Q., Zhao, Y., 2013. Effects of dietary soybean lecithin on gonadal development and vitellogenin mRNA expression in the female redclaw crayfish *Cherax quadricarinatus* (von Martens) at first maturation. Aqua. Res. 44, 1167–1176.
Wang, X., Li, E., Xu, Z., Li, T., Xu, C., Chen, L., 2017. Molecular response of carbohydrate metabolism to dietary carbohydrate and acute low salinity stress in Pacific white shrimp *Litopenaeus vannamei*. Turk. J. Fish. Aqua. Sci. 17, 153–169.
Wouters, R., Molina, C., Lavens, P., Calderon, J., 2001a. Lipid composition and vitamin content of wild female *Litopenaeus vannamei* in different stages of sexual maturation. Aquaculture 198, 307–323.
Wouters, R., Lavens, P., Nieto, J., Sorgeloos, P., 2001b. Penaeid shrimp broodstock nutrition: an updated review on research and development. Aquaculture 202, 1–21.
Wouters, R., Zambrano, B., Espin, M., Calderon, J., Lavens, P., Sorgeloos, P., 2002. Experimental broodstock diets as partial fresh food substitutes in white shrimp *Litopenaeus vannamei*. Aqua. Nutr. 8, 249–256.
Wu, X., Cheng, Y., Sui, L., Zeng, C., Southgate, P.C., Yang, X., 2007. Effect of dietary supplementation of phospholipids and highly unsaturated fatty acids on reproductive performance and offspring quality of Chinese mitten crab, *Eriocheir sinensis* (H. Milne-Edwards), female broodstock. Aquaculture 273, 602–613.