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Effects of dietary black cumin seed meal on growth performance, blood biochemistry and fatty acid composition of Pacific white shrimp *Litopenaeus vannamei*

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Abstract

This study examined the effects of black cumin seed (BCS) on growth performance, whole-body proximate and fatty acid composition, and blood biochemistry of Pacific white shrimp *Litopenaeus vannamei*. Six hundred shrimp (7.5 ± 0.2 g) were assigned into five treatments in three replicates. Five diets containing BCS at 0, 5, 15, 30 and 50 g/kg were prepared and named BCS0, BCS5, BCS15, BCS30 and BCS50, respectively. Shrimp were fed at a rate of 5% body weight three times daily for 90 days. Shrimp fed BCS5, BCS15 and BCS30 showed no adverse effects on growth, whereas shrimp fed BCS50 showed retarded growth parameters. Consistently, cortisol was significantly increased in BCS50 group. Moreover, cholesterol and triglyceride were significantly lower in shrimp that fed diets containing BCS compared to the control ($p < .05$). The highly unsaturated fatty acid (HUFA) was significantly higher in the control diet compared to other diets, but it was significantly higher in shrimp that fed BCS30 and BCS50 than in control shrimp with no significant increase in Δ6 fatty acyl desaturase transcript. Considering the growth parameters, the highest body HUFA contents, and the lowest haemolymph cholesterol and triglyceride levels, the supplementation of BCS in shrimp diet is suggested at 30 g/kg.

KEYWORDS

Δ6 fatty acyl desaturase, black cumin, fatty acid, growth, highly unsaturated fatty acid

1 | INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei*, is known as the main farmed shrimp species worldwide (Lin et al., 2012). From 80 million tons of total aquaculture in 2016, 4.1 million tons (53% of total crustaceans) belonged to *L. vannamei* (FAO, 2018). Resistance against wide ranges of salinities and white spot disease together with the development of new intensive aquaculture techniques are known as the most important reasons for the growth in *L. vannamei* production (FAO, 2006; Roy, Davis, Saoud, & Henry, 2007).

Developing cost-effective healthy feed has become an interesting topic for aquaculture. Many plant-derived compounds are known to have various positive effects such as antistress, growth-promoting, appetizer, immune stimulant and antimicrobial properties. Furthermore, many important drugs have been derived directly or indirectly from plants or from molecules of plant origin (Gurib-Fakim, 2006).

Black cumin seed (BCS) of *Nigella sativa* L. (Ranunculaceae) is an annual herbaceous plant (Atta, 2003) which has been successfully used in animals' feed and human's food. BCS has traditionally been used in medicine for various diseases owing to its rich and

diverse chemical composition including amino acids, proteins, carbohydrates, crude fibre, oils, minerals, alkaloids, saponin and others (Khoddami, Ghazali, Yassoralipour, Ramakrishnan, & Ganjloo, 2011). It also contains many bioactive constituents, such as antioxidant compounds (mainly represented by thymoquinone and dithymoquinone), flavonoids, sterols and polyunsaturated fatty acids (PUFAs) (Asgary, Sahebkar, & Goli-Malekabadi, 2015). The lowering effect of BCS on plasma cholesterol and triglyceride levels in animals is also well known (e.g. Al-Beitawi, El-Ghousein, & Nofal, 2009; Ghasemi, Kasani, & Taherpour, 2014; Yalçın, Yalçın, Uzunoglu, Duyum, & Elta, 2012). Moreover, dietary BCS can alter the blood parameters including proteins and cortisol in animals (Toghyani, Toghyani, Gheisari, Ghalamkari, & Mohammadrezaei, 2010; Tousson, El-Moghazy, & El-Atrsh, 2011). These parameters are considered as biomarkers for growth and performance of animals.

Black cumin seed is also believed to improve the fatty acid (FA) composition of animals by increasing the highly unsaturated fatty acid (HUFA) contents of animals utilizing BCS in the diet (Öz, Dikel, & Durmus, 2018; Yalçın, Erol, Buğdaycı, Özsoy, & Çakır, 2009). Oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) are known as two major FAs in BCS (Kaskoos, 2011). These C18 FAs are the substrate for the *Δ6* fatty acyl desaturases (*Δ6 FAD*), a key enzyme involved in converting C18 to HUFA (Lin, Hao, Zhu, Li, & Wen, 2017; Wu et al., 2018). Therefore, dietary BCS might increase the expression of *Δ6 FAD* and consequently lead to more HUFA biosynthesis in animals.

Black cumin seed has been widely used as a natural feed additive to improve growth performance, efficiency of feed utilization, FA composition and immune response of livestock animals and poultry. BCS has been also incorporated to the diet of some farmed fish species, for example rainbow trout *Oncorhynchus mykiss* (Altunoglu, Bilen, Ulu, & Biswas, 2017; Dorucu, Colak, Ispir, Altinterim, & Celayir, 2009; Öz, 2018) and Asian sea bass *Lates calcarifer* (Abdelwahab & El Bahr, 2012). However, these studies in aquaculture are mostly limited to evaluating the immune response. Despite the documented positive effects of BCS on human and many other animals, particularly on FA composition, to our knowledge, there is no previous report on the effect of BCS on shrimp; hence, this study is the first to investigate the effects of BCS powder on growth parameters; body composition; FA profile; haemolymph biochemical parameters including cholesterol, triglyceride, total protein and cortisol; and the mRNA expression of *Δ6 FAD* in Pacific white shrimp juvenile.

2 | MATERIALS AND METHODS

2.1 | Experimental diet preparation

Black cumin seed was obtained from a domestic farm in Isfahan, Iran. Its proximate composition was analysed in Isfahan University of Technology, Isfahan, Iran. It contained 223.1 g/kg protein, 382.7 g/kg lipid, 245.5 g/kg carbohydrate, 62.0g/kg moisture, 86.7 g/kg ash and 56.8 g/kg fibre (Table 1). The FA profile of BCS (Table 1) was analysed at the University of Tehran, Karaj, Iran.

TABLE 1 Proximate composition and fatty acid contents of black cumin (% total fatty acid)

Item	Value
Proximate composition (dry basis; g/kg)	
Protein	223.1
Lipid	382.7
Carbohydrate	245.5
Moisture	62.0
Ash	86.7
Fibre	56.8
Fatty acid profile (%)	
Lauric acid (C12:0)	0.00
Myristic acid (C14:0)	0.14
Pentadecanoic acid (C15:0)	0.00
Palmitic acid (C16:0)	12.34
Palmitoleic acid (C16:1)	0.15
Heptadecanoic acid (C17:0)	0.00
Stearic acid (C18:0)	3.12
Oleic acid (n-9) (C18:1)	24.31
Linoleic acid (C18:2) (n-6)	56.87
Linolenic acid (C18:3) (n-3)	0.09
Eicosatrienoic acid (C20:3) (n-3)	3.00
Arachidonic acid (C20:4) ARA (n-6)	0.00
Docosahexaenoic acid (C22:6) DHA (n-3)	0.00
Eicosapentaenoic acid (C20:5) EPA (n-3)	0.00

Five experimental diets containing 0 (control), 5 (BCS5), 15 (BCS15), 30 (BCS30) and 50 (BCS50) g/kg BCS were formulated using LINDO software 6.1 (USA) (Table 2). For preparation of the diets, the ingredients were mixed thoroughly for 20 min and distilled water was added to obtain soft dough that was pelleted by a meat grinder with diameter of 3 mm. The pellets were dried in front of a fan flaw for 48 hr and then stored in a freezer at −20°C until their use.

2.2 | Shrimp and experimental condition

Juveniles of *L. vannamei* were transferred from a private shrimp farm in Tiab, Iran, to Persian Gulf Biotechnology Park in Qeshm Island, Iran, and acclimated to the experimental condition. The experiment was conducted in accordance with the Iranian Society for the Prevention of Cruelty to Animals and the Canadian Council on Animal Care. The shrimp were fed with an ordinary commercial diet for about 3 weeks. A total of 600 healthy shrimp (7.5 ± 0.2 g) were distributed randomly into 15 circular polyethylene tanks (280 L) at a density of 40 shrimp in each tank.

There were five treatments including a control and four feeding groups, and each treatment consisted of three replicates (totally 120 shrimp for each treatment). The shrimps were fed at a rate of 4%

TABLE 2 The ingredients and chemical composition of the experimental diets

	Diets				
	Control	BCS5	BCS15	BCS30	BCS50
Ingredients (g/kg)					
Fish meal	300.0	300.0	300.0	300.0	300.0
Soybean meal	205.0	204.1	202.3	199.6	196.0
Wheat gluten meal	96.0	96.0	96.0	96.0	96.0
Shrimp meal	64.0	64.0	64.0	64.0	64.0
Wheat flour	209.0	206.9	202.7	196.4	188.0
Rice flour	26.0	26.0	26.0	26.0	26.0
Bentonite	10.0	10.0	10.0	10.0	10.0
Binder	10.0	10.0	10.0	10.0	10.0
Mineral mixture[a]	10.0	10.0	10.0	10.0	10.0
Vitamin mixture[b]	10.0	10.0	10.0	10.0	10.0
Fish oil[c]	40.0	38.7	36.0	32.0	26.7
Soybean oil	20.0	19.3	18.0	16.0	13.3
Black cumin powder	0.0	5.0	15.0	30.0	50.0
Chemical composition (g/kg)					
Crude protein[d]	395.8	383.8	392.6	384.3	385.8
Crude lipid	105.1	103.0	108.6	114.2	118.5
Carbohydrate	232.1	253.4	232	241	222.9
Energy (kJl/kg)[e]	17,072.8	17,149.4	17,149.4	17,372.0	17,256.1

[a]Five kg mineral mixture contains Fe, 4,000 mg; Zn, 12,000 mg; Se, 60 mg; Co, 2,000 mg; Cu, 51,000 mg; Mn, 4,000 mg; I, 80 mg; and choline chloride, 80,000 mg.

[b]Five kg vitamin mixture contains vitamin A, 8,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 150,000 mg; vitamin K3, 50,000 mg; vitamin B1, 50,000 mg; vitamin B2, 40,000 mg; vitamin B3, 150,000 mg; vitamin B5, 200,000 mg; vitamin B6, 80,000 mg; vitamin B9, 15,000 mg; vitamin B12, 50 mg; vitamin C, 500,000 mg; H2, 1,500 mg; inositol, 500,000 mg; and BHT, 1,000 mg.

[c]The proportion of fish oil to soybean oil was 2/1.

[d]In this mixture, the ratio of wheat to soybean was 70 to 30 and their protein percentage was equal with black cumin seed powder approximately (22.5%).

[e]Energy was calculated as 16.7, 16.7 and 37.7 kJ/kg of protein, carbohydrate and lipids, respectively (calculated from physiological fuel values).

body weight daily at four times (07:00, 11:00, 15:00 and 19:00) for a period of 12 weeks. About 90% of water exchange was implemented before first feeding every day. The amount of the diets was calculated every 2 weeks, according to the average weight of shrimp in each tank. Aeration was performed by two air stones in each tank. The average values for water dissolved oxygen, pH, salinity and temperature were 5.8 ± 0.4 mg/L, 8.1 ± 0.1, 36.5 ± 0.5‰ and 32 ± 2°C, respectively, and photoperiod was 14-hr light:10-hr darkness. At the beginning and at the end of experimental period, the mean weights of shrimps in each tank were measured. Moreover, total length, carapace length and carapace width of 10 shrimp in each tank were recorded. Then, the head and skin of six shrimp of each tank were dissected and the rest of the body was packed in two re-sealable plastic bags equally and stored in freezer at −20°C for assessment of proximate body composition and FA profile. To evaluate the concentration of cholesterol and triglyceride, haemolymph of three shrimps from each tank was taken from the base of the pleopods at the first abdominal segment near the genital pore.

2.3 | Growth parameters

Body weight was measured to the nearest 0.01 g. The carapace length and width were measured to the nearest 0.01 mm. Total length was assessed with 1 mm accuracy. Growth parameters and feed utilization were calculated according to the formulae given below:

$$\text{Weight gain(WG)} = \text{final weight} - \text{initial weight},$$

$$\text{Specific growth rate(SGR)} = 100 \times (\,(\text{Ln final weight} - \text{Ln initial weight})\,/\text{day}),$$

$$\text{Total length increase(TLI)} = \text{final length} - \text{initial length},$$

$$\text{Carapace length increase(CLI)} = \text{final carapace length} - \text{initial carapace length},$$

$$\text{Carapace width increase(CWI)} = \text{final carapace width} - \text{initial carapace width},$$

$$\text{Feed conversion ratio(FCR)} = \text{feed intake(g)}/\text{weight gain(g)},$$

Survival% =

100 × (initial shrimp number − dead shrimp number)/(initial shrimp number).

2.4 | Whole-body proximate composition

Proximate analysis of the diets was performed, and whole body of three shrimp from each tank was analysed using standard methods of the Association of Official Analytical Chemists (AOAC, 1995) at the laboratory of Isfahan University of Technology, Isfahan, Iran. Moisture was determined by drying in oven (Binder, USA) at 105°C for 24 hr to a constant weight. Crude protein was measured using a Kjeldahl system (Gerhardt, type VAP.40). Crude lipid was calculated with ether extraction in a Soxhlet extractor (Gerhardt, type SE-416), and crude ash was measured by incineration at 550°C in a muffle furnace for 8 hr. The carbohydrate (CHO) was calculated by the following formula (Merrill & Watt, 1973):

$$\text{CHO} = 100 - (\text{moisture} + \text{crude ash} + \text{crude lipid} + \text{crude protein}).$$

2.5 | Fatty acid composition

Fatty acid composition of the experimental diets and the three shrimp from each tank were analysed at the University of Tehran, Tehran, Iran. Total lipids were extracted using chloroform:methanol (2:1, v/v) according to AOAC (1995). FA methyl esters were prepared by acidic methanolysis of lipid extracts using BF3 in methanol. The FA methyl esters were recovered with *n*-hexane according to Metcalfe & Schmitz (1961) and analysed using a gas chromatograph (model: CP3800 Varian) with a flame ionization detector, equipped with a capillary column (BPX70 SGM; 60 m × 0.32 mm id., film thickness: 0.25 μm). Injector and detector temperatures were 210°C and 250°C, respectively. The column temperature was programmed from 160 to 180°C at a rate of 2°C/min, helium was used as the carrier gas, and the total run time was 85 min per sample. FA peaks were integrated using Varian Star Chromatography software (version 6.41), and identification was carried out with reference to known standards (Sigma-Aldrich).

2.6 | Blood biochemistry

Haemolymph was obtained by using 1-ml syringe along with 25-gauge needle, containing 0.3 ml of precooled anticoagulant (10 mM Tris-HCl, 250 mM sucrose, 100 mM sodium citrate, pH 7.6, 4°C) as previously described (Akbarzadeh, Pakravan, et al., 2019). The collected haemolymph was transferred to 1.5-ml vials and stored in freezer at −20°C. Then, haemolymph was thawed at room temperature and centrifuged (6000 *g*, 10 min, 4°C) until plasma was separated from haemocytes. Plasma was analysed by means of an autoanalyser (COBAS INTEGRA 400 PLUS) using clinical kits (Roche) to measure the amount of cholesterol, triglyceride, total protein and cortisol (Huang et al., 2015; Wang, Qu, Yan, et al., 2019; Wang, Qu, Zhuo, et al., 2019).

2.7 | Quantitative reverse transcription–PCR (qRT-PCR)

The mRNA expression of Δ6 FAD was quantified as previously described (Akbarzadeh, Pakravan, et al., 2019). Briefly, 50–100 mg of hepatopancreas tissue from five individuals of each feeding group was homogenized using 1.0 ml RNAx-Plus reagent (SinaClon BioScience), treated with DNase I (SinaClon BioScience), and one microgram of total RNA was used to synthesize first-strand cDNA using PrimeScript™ RT Reagent Kit (Takara) following the manufacturer's instructions. The transcripts of Δ6 FAD and the internal reference gene, beta-actin, were measured by quantitative real-time PCR on a QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems). The thermal cycling conditions and reaction components were set up as previously described (Akbarzadeh, Pakravan, et al., 2019). The specific qRT-PCR primers for Δ6 FAD gene were F-TACACCTTCCACGACGAT, R-ATCAGAATCATCCTCCAGTC (Chen et al., 2017), and the primers for beta-actin were F-CCACGAG ACCACCTACAAC, R-AGCGAGGGCAGTGATTTC (Wang, Wang, Kou, Lo, & Huang, 2007). qRT-PCR data were analysed, and amplification efficiencies were determined as previously described (Akbarzadeh, Pakravan, et al., 2019).

2.8 | Statistical analysis

Differences in values of growth parameters, body composition, FA contents, biochemical parameters of haemolymph and the mRNA expression of Δ6 FAD gene across all the treatments were analysed by a one-way analysis of variance (ANOVA) and Tukey post hoc test following the normality test of data (Kolmogorov–Smirnov) and heterogeneity for variance (Leven's test) of all the treatments. Differences were considered statistically significant at $p < .05$. All the statistical analyses were carried out using SPSS program version 20.0. Values were presented as mean ± standard error (*SE*), with the exception of cholesterol and triglyceride that were presented as mean ± standard deviation (*SD*).

3 | RESULTS

3.1 | Growth parameters

All the experimental diets were accepted by the shrimp. The effects of BCS on growth parameters of *L. vannamei* are presented in Table 3. There were no significant differences in carapace length and width gain, and survival between treatments ($p > .05$). However, final weight, weight gain, specific growth rate and feed conversion ratio of shrimp fed diet containing BCS50 were significantly lower than those of the control group ($p < .05$). Moreover, total length gain of shrimp fed diet with BCS50 was significantly lower than shrimp fed with BCS50 and control group ($p < .05$).

TABLE 3 Growth performance, feed utilization and survival rate of Pacific white shrimp *Litopenaeus vannamei* fed the experimental diets for 12 weeks

	Treatments				
Items	Control	BCS5	BCS15	BCS30	BCS50
IW (g)	7.54 ± 0.05	7.70 ± 0.30	7.45 ± 0.24	7.66 ± 0.27	7.50 ± 0.33
FW (g)	20.07 ± 0.91^{a}	18.96 ± 0.48^{ab}	18.9 ± 0.57^{ab}	18.62 ± 1.34^{ab}	17.58 ± 0.72^{b}
WG (g)	12.53 ± 1.17^{a}	11.26 ± 0.36^{ab}	11.45 ± 0.41^{ab}	10.96 ± 0.14^{ab}	10.08 ± 0.86^{b}
FI (g)	41.47 ± 0.57^{b}	40.54 ± 0.58^{b}	43.51 ± 0.56^{a}	41.98 ± 0.08^{ab}	42.44 ± 0.42^{ab}
SGR	1.135 ± 0.06^{a}	1.02 ± 0.02^{ab}	0.98 ± 0.01^{ab}	1.02 ± 0.04^{ab}	0.93 ± 0.06^{b}
TLI (cm)	4.05 ± 0.13^{a}	3.70 ± 0.14^{ab}	3.61 ± 0.15^{ab}	4.09 ± 0.12^{a}	3.25 ± 0.17^{b}
CLI (cm)	10.78 ± 0.56	9.46 ± 0.85	9.75 ± 0.93	11.52 ± 0.32	9.18 ± 0.63
CWI (cm)	4.38 ± 0.38	3.74 ± 0.18	3.70 ± 0.58	4.40 ± 0.57	3.28 ± 0.37
FCR	3.31 ± 0.49^{a}	3.60 ± 0.23^{a}	3.80 ± 0.15^{a}	3.83 ± 0.07^{a}	4.21 ± 0.20^{b}
PER	0.77 ± 0.1	0.71 ± 0.05	0.69 ± 0.03	0.68 ± 0.2	0.63 ± 0.05
Survival (%)	100	100	100	100	100

Note: Data (mean ± *SE*; *n* = 3) with different letters are significantly different among treatments according to ANOVA test ($p < .05$).
Abbreviations: CLI, carapace length increase; CWI, carapace width increase; FCR, feed conversion ratio; FI, feed intake; FW, final weight; IW, initial weight; PER, protein efficiency rate; SGR, specific growth ratio; TLI, total length increase; WG, weight gain.

TABLE 4 Proximate body composition (g/kg wet weight) of *Litopenaeus vannamei* fed the experimental diets for 12 weeks

	Treatments				
Items	Control	BCS5	BCS15	BCS30	BCS50
Protein	192.00 ± 0.70	191.10 ± 2.1	192.20 ± 1.70	186.70 ± 2.10	192.80 ± 0.60
Lipid	14.40 ± 0.90	13.10 ± 0.70	13.60 ± 0.40	14.80 ± 1.30	15.80 ± 0.70
Ash	17.50 ± 0.30	16.40 ± 0.70	17.70 ± 0.60	15.30 ± 0.60	16.50 ± 0.20
Carbohydrate[a]	46.50 ± 1.80	50.10 ± 1.90	46.80 ± 1.20	45.20 ± 3.70	42.00 ± 2.30
Moisture	729.60 ± 2.60	729.20 ± 0.90	729.70 ± 2.70	738.10 ± 1.60	731.90 ± 3.20

Note: Data (mean ± *SE*; *n* = 3) with different letters are significantly different among treatments according to ANOVA test ($p < .05$).
[a]Carbohydrate (CHO) was calculated by the formula: CHO = 100 − (moisture + crude ash + crude lipid + crude protein)

3.2 | Whole-body proximate composition

The whole-body proximate composition of *L. vannamei* fed diets containing different levels of BCS powder is presented in Table 4. There were no significant differences in whole-body protein, lipid, ash, carbohydrate and moisture among five experimental groups ($p > .05$). However, the amount of lipid contents showed an insignificant increase in shrimp fed diets containing BCS30 and BCS50 compared to control group.

3.3 | Fatty acid composition

Fatty acid composition for the experimental diets and whole body of *L. vannamei* fed diets containing different levels of BCS powder is shown in Tables 5 and 6. Saturated fatty acids (SFAs) were significantly lower in the diet supplemented with BCS30 compared to other experimental diets. The highest amount of SFAs was observed in diet supplemented with 5 g/kg BCS. On the other hand, monounsaturated fatty acids (MUFAs) including oleic acid, and PUFAs including linoleic acid, eicosadienoic acid (C20:2n-6) and eicosatrienoic acid (C20:3n-3) were significantly higher in diet containing BCS30 of BCS compared to other experimental diets. HUFAs including arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were significantly higher in control diet compared to diets containing BCS ($p < .05$). Similar to experimental diets, SFAs were significantly lower in the carcass of shrimp fed with the diet containing BCS30 compared to other groups. On the contrary, the contents of MUFAs including oleic acid; PUFAs including linoleic acid, linolenic acid (C18:3n-3) and eicosatrienoic acid; and HUFAs including ARA, EPA and DHA in the carcass of *L. vannamei* fed with BCS30 were significantly higher compared to the control group ($p < .05$).

3.4 | Haemolymph biochemical parameters

The results of haemolymph biochemical parameters of *L. vannamei* fed diets containing different levels of BCS are presented in Table 7.

TABLE 5 Fatty acid profile of experimental diets (% total fatty acid)

	Treatments				
Items	Control	BCS5	BCS15	BCS30	BCS50
C14:0	4.42 ± 0.01^{a}	4.32 ± 0.09^{a}	4.05 ± 0.05^{b}	2.41 ± 0.01^{d}	3.33 ± 0.04^{c}
C15:0	0.56 ± 0.01^{b}	0.64 ± 0.01^{a}	0.58 ± 0.00^{b}	0.37 ± 0.00^{d}	0.49 ± 0.01^{c}
C16:0	20.94 ± 0.07^{d}	23.86 ± 0.12^{a}	22.62 ± 0.11^{b}	11.20 ± 0.01^{e}	21.84 ± 0.10^{c}
C17:0	0.46 ± 0.04^{c}	0.89 ± 0.02^{a}	0.81 ± 0.00^{ab}	0.59 ± 0.06^{bc}	0.54 ± 0.18^{c}
C18:0	5.77 ± 0.04^{c}	6.49 ± 0.02^{a}	6.00 ± 0.01^{b}	3.22 ± 0.03^{d}	5.95 ± 0.01^{b}
C20:0	0.39 ± 0.02^{a}	0.45 ± 0.02^{a}	0.41 ± 0.03^{a}	0.22 ± 0.01^{b}	0.42 ± 0.01^{a}
C22:0	0.23 ± 0.01^{ab}	0.25 ± 0.03^{a}	0.23 ± 0.00^{ab}	0.18 ± 0.01^{b}	0.26 ± 0.01^{a}
C24:0	0.57 ± 0.01^{a}	0.42 ± 0.01^{b}	0.44 ± 0.04^{b}	0.40 ± 0.02^{b}	0.37 ± 0.01^{b}
SFA	33.32 ± 0.10^{c}	37.33 ± 0.13^{a}	35.14 ± 0.18^{b}	18.60 ± 0.02^{d}	33.21 ± 0.00^{c}
C16:1	4.85 ± 0.04^{a}	4.76 ± 0.02^{a}	4.40 ± 0.05^{b}	4.10 ± 0.06^{c}	3.54 ± 0.02^{d}
C17:1	0.74 ± 0.05^{a}	0.45 ± 0.03^{b}	0.39 ± 0.00^{b}	0.17 ± 0.01^{c}	0.23 ± 0.01^{c}
C18:1n-9	15.78 ± 0.00^{d}	16.94 ± 0.20^{c}	16.99 ± 0.12^{c}	24.66 ± 0.01^{a}	17.57 ± 0.02^{b}
C20:1	0.52 ± 0.01^{a}	0.42 ± 0.00^{b}	0.40 ± 0.02^{bc}	0.39 ± 0.01^{bc}	0.37 ± 0.00^{c}
C22:1	0.58 ± 0.04^{a}	0.55 ± 0.05^{a}	0.57 ± 0.02^{a}	0.56 ± 0.02^{a}	0.35 ± 0.00^{b}
MUFA	25.40 ± 0.142^{c}	26.08 ± 0.076^{b}	25.65 ± 0.069^{c}	33.10 ± 0.036^{a}	24.32 ± 0.03^{d}
C18:2n-6(LA)	23.67 ± 0.04^{d}	23.27 ± 0.02^{e}	25.91 ± 0.04^{c}	35.25 ± 0.13^{a}	30.22 ± 0.00^{b}
C18:3n-3(ALA)	3.24 ± 0.04^{a}	2.52 ± 0.00^{b}	2.58 ± 0.07^{b}	2.42 ± 0.04^{b}	2.05 ± 0.02^{c}
C20:2n-6	0.56 ± 0.01^{c}	0.65 ± 0.01^{b}	0.58 ± 0.03^{c}	0.87 ± 0.00^{a}	0.56 ± 0.01^{c}
C20:3n-3	0.11 ± 0.01^{c}	0.15 ± 0.01^{c}	0.26 ± 0.01^{b}	0.67 ± 0.02^{a}	0.72 ± 0.01^{a}
C20:3n-6	0.10 ± 0.01	0.10 ± 0.02	0.01 ± 0.00	0.01 ± 0.02	0.10 ± 0.01
C20:4n-6 (ARA)	1.26 ± 0.02^{a}	0.95 ± 0.01^{b}	0.97 ± 0.02^{b}	0.96 ± 0.00^{b}	0.94 ± 0.01^{b}
C20:5n-3 (EPA)	4.30 ± 0.01^{a}	3.07 ± 0.11^{b}	2.95 ± 0.10^{bc}	2.67 ± 0.10^{c}	2.62 ± 0.03^{c}
C22:4n-6 (DTA)	0.20 ± 0.04	0.214 ± 0.00	0.193 ± 0.03	0.21 ± 0.01	0.219 ± 0.01
C22:5n-3 (DPA)	1.03 ± 0.02^{a}	0.72 ± 0.03^{b}	0.69 ± 0.00^{bc}	0.68 ± 0.03^{bc}	0.60 ± 0.03^{c}
C22:6n-3 (DHA)	6.80 ± 0.05^{a}	4.96 ± 0.03^{b}	5.00 ± 0.05^{b}	4.49 ± 0.07^{c}	4.47 ± 0.00^{c}
PUFA	41.28 ± 0.04^{c}	36.59 ± 0.14^{e}	39.21 ± 0.11^{d}	48.31 ± 0.05^{a}	42.47 ± 0.03^{b}
HUFA	12.36 ± 0.02^{a}	8.98 ± 0.04^{b}	8.94 ± 0.01^{b}	8.13 ± 0.05^{c}	8.04 ± 0.02^{c}
n-3	15.50 ± 0.07^{a}	11.43 ± 0.10^{b}	11.49 ± 0.08^{b}	10.95 ± 0.17^{c}	10.47 ± 0.03^{d}
n-6	25.78 ± 0.04^{d}	25.16 ± 0.04^{e}	27.71 ± 0.02^{c}	37.36 ± 0.12^{a}	32.00 ± 0.01^{b}
n-3/n-6	0.60 ± 0.00^{a}	0.45 ± 0.00^{b}	0.41 ± 0.00^{c}	0.29 ± 0.01^{e}	0.33 ± 0.00^{d}
DHA/EPA	1.58 ± 0.01	1.62 ± 0.07	1.69 ± 0.04	1.69 ± 0.13	1.70 ± 0.02
ARA/EPA	0.29 ± 0.01^{b}	0.31 ± 0.02^{b}	0.329 ± 0.00^{ab}	0.36 ± 0.02^{a}	0.36 ± 0.00^{a}
n-3 LC-PUFA	11.09 ± 0.06^{a}	8.03 ± 0.08^{b}	7.96 ± 0.00^{b}	7.16 ± 0.09^{c}	7.09 ± 0.03^{c}
n-3 LC-PUFA/ARA	8.80 ± 0.21^{a}	8.50 ± 0.20^{ab}	8.18 ± 0.13^{b}	7.46 ± 0.07^{c}	7.51 ± 0.03^{c}

Note: Data (mean ± *SE*; *n* = 3) with different letters are significantly different among treatments according to ANOVA test ($p < .05$).

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HUFA, ARA + EPA+DHA; LA, linoleic acid; MUFA, monounsaturated fatty acid; n-3, LC-PUFA, EPA + DHA; ND, not detected; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

The contents of cholesterol and triglyceride in shrimp fed diets containing different levels of BCS were significantly lower than those of the control group ($p < .05$). There were no significant differences in total protein of shrimp fed diets containing different amounts of BCS and control group ($p > .05$). The concentration of cortisol was significantly higher in shrimp fed BCS50 compared to the control group ($p < .05$).

3.5 | Transcript levels of Δ6 FAD in L. vannamei

The mRNA expression of *Δ6 FAD* gene was detected in *L. vannamei* fed diets with different levels of BCS. The transcripts of *Δ6 FAD* were not statistically significant among experimental groups ($p > .05$). However, the expression of *Δ6 FAD* was higher in *L. vannamei* fed diets with BCS30 compared to control group (Figure 1).

TABLE 6 Fatty acid profile of whole body of *Litopenaeus vannamei* fed the experimental diets for 12 weeks (% total fatty acid)

	Treatments				
Items	**Control**	**BCS5**	**BCS15**	**BCS30**	**BCS50**
C12:0	4.47 ± 0.26^{b}	7.83 ± 0.32^{a}	7.27 ± 0.07^{a}	2.47 ± 0.01^{c}	4.90 ± 0.17^{b}
C14:0	6.90 ± 0.46^{b}	10.90 ± 0.92^{a}	10.29 ± 0.25^{a}	3.67 ± 0.06^{c}	6.89 ± 0.05^{b}
C15:0	3.97 ± 0.18^{ab}	5.12 ± 0.91^{a}	5.35 ± 0.38^{a}	1.51 ± 0.24^{b}	2.34 ± 0.30^{ab}
C16:0	23.07 ± 0.91^{bc}	26.26 ± 0.32^{a}	25.02 ± 0.28^{ab}	21.67 ± 0.05^{c}	24.03 ± 0.17^{abc}
C17:0	0.94 ± 0.02	1.45 ± 0.22	1.64 ± 0.11	1.39 ± 0.05	1.25 ± 0.10
C18:0	12.66 ± 0.41	16.83 ± 1.13	16.07 ± 0.74	13.81 ± 0.01	14.61 ± 0.24
SFA	52.02 ± 2.25^{bc}	68.38 ± 1.11^{a}	65.64 ± 1.06^{a}	44.51 ± 0.19^{c}	54.03 ± 0.43^{b}
C16:1	0.51 ± 0.13^{b}	ND	1.00 ± 0.07^{ab}	1.20 ± 0.11^{a}	1.26 ± 0.17^{a}
C18:1n-9	14.38 ± 0.72^{ab}	10.70 ± 0.28^{d}	11.02 ± 0.19^{cd}	16.41 ± 0.20^{a}	13.27 ± 0.27^{bc}
MUFA	14.90 ± 0.59^{b}	10.70 ± 0.28^{c}	12.02 ± 0.26^{c}	17.61 ± 0.10^{a}	14.53 ± 0.33^{b}
C18:2n-6 (LA)	20.81 ± 2.24^{a}	10.74 ± 0.39^{b}	10.39 ± 0.96^{b}	14.82 ± 0.31^{ab}	12.93 ± 0.42^{ab}
C18:3n-3 (ALA)	0.26 ± 0.01^{c}	ND	0.17 ± 0.01^{d}	0.47 ± 0.01^{a}	0.32 ± 0.01^{b}
C20:3n-3	1.33 ± 0.11^{bc}	0.36 ± 0.01^{d}	0.97 ± 0.13^{c}	1.82 ± 0.10^{ab}	1.96 ± 0.03^{a}
C20:4n-6 (ARA)	2.16 ± 0.08^{c}	2.38 ± 0.11^{c}	2.15 ± 0.12^{c}	4.50 ± 0.01^{a}	3.27 ± 0.14^{b}
C20:5n-3 (EPA)	4.75 ± 0.25^{c}	4.08 ± 0.36^{c}	4.66 ± 0.25^{c}	9.03 ± 0.11^{a}	7.08 ± 0.03^{b}
C22:6n-3 (DHA)	3.81 ± 0.35^{c}	3.36 ± 0.05^{c}	4.00 ± 0.17^{c}	7.24 ± 0.02^{a}	5.85 ± 0.17^{b}
PUFA	33.09 ± 1.66^{ab}	20.91 ± 0.83^{c}	22.34 ± 0.79^{c}	37.88 ± 0.10^{a}	31.44 ± 0.10^{b}
HUFA	10.71 ± 0.67^{c}	9.81 ± 0.42^{c}	10.82 ± 0.29^{c}	20.77 ± 0.09^{a}	16.20 ± 0.28^{b}
n-3	10.14 ± 0.50^{c}	7.79 ± 0.33^{d}	9.8 ± 0.29^{cd}	18.57 ± 0.21^{a}	15.24 ± 0.18^{b}
n-6	22.95 ± 2.16^{a}	13.12 ± 0.50^{b}	12.54 ± 1.09^{b}	19.31 ± 0.30^{ab}	16.20 ± 0.28^{ab}
n-3/n-6	0.46 ± 0.07^{b}	0.59 ± 0.01^{b}	0.81 ± 0.09^{ab}	0.96 ± 0.03^{a}	0.94 ± 0.03^{a}
DHA/EPA	0.80 ± 0.03	0.84 ± 0.09	0.86 ± 0.01	0.80 ± 0.01	0.83 ± 0.03
ARA/EPA	0.46 ± 0.01	0.59 ± 0.02	0.47 ± 0.05	0.50 ± 0.01	0.46 ± 0.02
n-3 LC-PUFA	8.55 ± 0.60^{c}	7.43 ± 0.31^{c}	8.66 ± 0.41^{c}	16.28 ± 0.02^{a}	12.93 ± 0.14^{b}
n-3 LC-PUFA/ARA	3.94 ± 0.14	3.12 ± 0.01	4.09 ± 0.43	3.62 ± 0.02	3.97 ± 0.13

Note: Data (mean ± *SE*; *n* = 3) with different letters are significantly different among treatments according to ANOVA test ($p < .05$).

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HUFA, ARA + EPA+DHA; LA, linoleic acid; MUFA, monounsaturated fatty acid; n-3, LC-PUFA, EPA + DHA; ND, not detected; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

TABLE 7 Plasma biochemical parameters (mg/dl) of *Litopenaeus vannamei* fed the experimental diets for 12 weeks

	Treatments				
Items	**Control**	**BCS5**	**BCS15**	**BCS30**	**BCS50**
Cholesterol	24.25 ± 8.66^{a}	14.17 ± 4.25^{b}	10.07 ± 4.31^{b}	8.81 ± 3.44^{b}	11.44 ± 3.84^{b}
Triglyceride	41.50 ± 10.46^{a}	27.17 ± 5.16^{b}	26.33 ± 14.33^{b}	18.56 ± 5.37^{b}	24.17 ± 10.13^{b}
Total protein	7.16 ± 1.20	6.33 ± 1.48	6.66 ± 1.71	5.60 ± 1.54	6.56 ± 1.05
Cortisol	0.16 ± 0.02^{b}	0.17 ± 0.02^{b}	0.17 ± 0.02^{b}	0.18 ± 0.02^{b}	0.28 ± 0.02^{a}

Note: Data (mean ± *SD*; *n* = 3) with different letters are significantly different among treatments according to ANOVA test ($p < .05$).

4 | DISCUSSION

The results of this study showed no adverse effects of BCS on growth performance of shrimp when supplemented with BCS5, BCS15 and BCS30 of the diet. Previous studies have reported that BCS supplementation had no adverse effects on FCR in laying hens (Akhtar, Nasir, & Abid, 2003; Yalçın et al., 2009), and body weight in broiler chickens (Ali, Suthama, & Mahfud, 2014) and human (Dehkordi & Kamkhah, 2008; Haque, Nasiruddin, & Najmi, 2011; Qidwai, Hamza, Qureshi, & Gilani, 2009). Our results also revealed that the inclusion of BCS in higher level (50 g/kg of the basal diet) retarded some growth parameters including FCR compared to the control group. Some previous

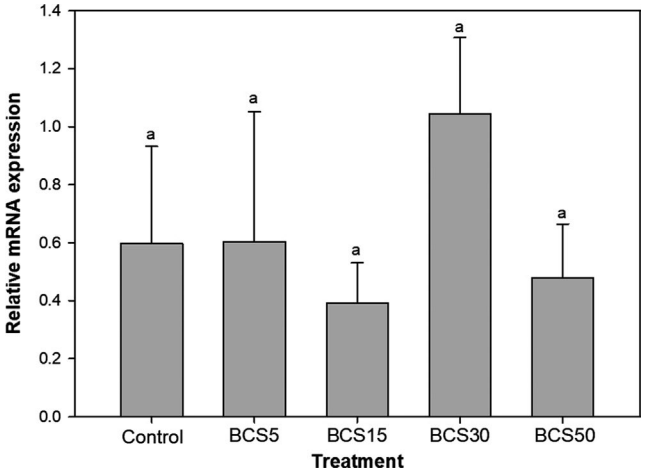

FIGURE 1 Expression of Δ6 fatty acyl desaturase gene in hepatopancreas of Litopenaeus vannamei fed the experimental diets for 12 weeks. Bars with different letters are significantly different among treatments according to ANOVA test ($p < .05$)

studies also reported negative effects of BCS on growth performance in animals (Hoseini et al., 2013). For example, BCS caused significantly lower body weight gain in birds and human (Abbas & Ahmed, 2010; Datau, Wardhana, Surachmanto, Pandelaki, & Langi, 2010). This may be attributed to the adverse effects of components such as saponin and alkaloids in BCS (Hermes, Faten, Attia, Ibrahim, & L-Nesr, 2009). Therefore, future research is needed to target the antinutritional compounds of BCS to reduce their adverse effects on animal's performance.

Despite the lower amounts of ALA and HUFAs including EPA, DHA and ARA in the diets supplemented with BCS compared to control diet, fillet of shrimp that fed BCS30 and BCS50 contains higher amounts of these essential FAs compared to the control group. Considering the higher levels of HUFA precursor FAs such as oleic acid, C20:3n-3 and LA in the BCS30 and BCS50 diets, it is likely that BCS is an appropriate source of these FAs for producing HUFAs in *L. vannamei*. Recent molecular studies have indicated that *L. vannamei* may have the ability to synthesize both DHA and EPA from either ALA or LA, particularly in lower salinities (Chen et al., 2015, 2017). Δ6 Fad is known as the first limiting enzyme involved in HUFA biosynthetic pathway (Tocher et al., 2006). The mRNA expression of the *Δ6 FAD* has been already reported in *L. vannamei* (Chen et al., 2015, 2017). Dietary LA and ALA are known to promote the expression of Δ6 desaturase, whereas HUFAs may suppress the expression of Δ6 desaturase (Chen et al., 2017; Zheng et al., 2004). Therefore, higher amounts of oleic acid, C20:3n-3 and LA along with lower levels of HUFAs in diets containing higher levels of BCS might promote HUFA biosynthesis in shrimp. Moreover, phytosterol, an active substance of BCS, is believed to increase the Δ9, Δ6 and Δ5 enzymes involved in converting C18 FAs to HUFAs (Leikin & Brenner, 1989). Phytosterol may stimulate the expression of the *Δ6 Fad* gene that facilitates transforming C18 FAs to DHA, EPA and ARA (Zheng et al., 2004; Zheng, Tocher, Dickson, Bell, & Teale, 2005). Although the mRNA expression of the *Δ6 FAD* was not statistically significant among treatments, the transcript levels of *Δ6 FAD* were relatively higher in shrimp that fed BCS30 compared to control group. This was consistent with the higher amounts of HUFA levels in shrimp's fillet fed BCS30.

Our data also showed a remarkable reduction (between 40% and 60%) in haemolymph cholesterol and triglyceride contents of shrimp that fed different levels of BCS compared to control group. Several mechanisms have been suggested to explain the cholesterol-lowering activity of BCS. The decrease in blood cholesterol is probably due to the presence of active substances in BCS, particularly phytosterol, and vitamin C that influences the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis (Al-Beitawi et al., 2009). Moreover, sterols or stanols in BCS might be displaced with cholesterol from mixed micelles (Child & Kuksis, 1986), as they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol concentrations and consequently lowers cholesterol absorption (Child & Kuksis, 1983). In addition, thymoquinone (the main component of BCS volatile oil) uptakes the low-density lipoprotein cholesterol (LDLC) via upregulation of low-density lipoprotein receptor (LDLR) gene and inhibits the synthesis of cholesterol via suppression of the HMG-COAR gene (Horton, Goldstein, & Brown, 2002). Furthermore, thymoquinone upregulates the apolipoprotein A-1 and downregulates the apolipoprotein B100 genes, which are linked to cholesterol metabolism in HepG2 cells (Al-Naqeeb & Ismail, 2009). The lowering effect of haemolymph triglyceride contents could be related to the presence of nigellamine in BCS. Nigellamine (a diterpene alkaloid) is known to decrease triglyceride levels in mouse hepatocytes (Morikawa, Xu, Ninomiya, Matsuda, & Yoshikawa, 2004). Moreover, high contents of LA (56.86%) in BCS can reduce the haemolymph triglyceride concentration. Previous studies showed that LA inhibited the liver mRNA expression and activity of stearoyl-CoA desaturase (Scd1) that are positively correlated with haemolymph triglyceride levels (Mutch et al., 2005; Ntambi, Sessler, & Takova, 1996; Sessler, Kaur, Palta, & Ntambi, 1996). In line with our results, the inclusion of BCS also decreased cholesterol and triglyceride concentrations in broiler, human, rat, rabbit and mouse cells (Bamosa, Ali, & Al-Hawsawi, 2002; Ibraheim, 2002; Le et al., 2004; Miraghaee et al., 2011; Morikawa et al., 2004; Zaoui et al., 2002).

It is known that the haemolymph biochemical parameters can be used to assess the shrimp nutritional status and physiological condition (Yu, Li, Lin, Wen, & Ma, 2008). It is also stated that fillet cholesterol concentration has a positive correlation with haemolymph cholesterol levels (Kanazawa, Chim, & Laubier, 1988). Therefore, it is likely that the lower cholesterol observed in haemolymph of shrimp that fed BCS compared to control group might reflect possible differences in fillet cholesterol. In this study, we did not measure the shrimp fillet cholesterol and triglycerides; however, we are undertaking further feeding trial to measure the effect of BCS on the cholesterol and triglycerides levels in shrimp fillet.

In addition, the levels of haemolymph total protein and cortisol showed associations with growth performance. No significant differences were observed in total protein in the haemolymph of shrimp that fed BCS compared to control treatment. In line with our results, BCS caused no changes in plasma proteins when supplemented to the diet of broiler chicks (Toghyani et al., 2010) and rabbit (Tousson et al., 2011). Consistent with the lower growth of shrimp fed diet containing BCS50, a significant increase in haemolymph cortisol was observed in this treatment, suggesting the association of haemolymph cortisol and growth rate in shrimp. It has been previously known that lower haemolymph cortisol is associated with higher growth in fish (Lankford & Weber, 2006). Therefore, haemolymph cortisol and total protein might be suitable indicators for the effect of BCS on shrimp growth.

5 | CONCLUSIONS

The present study provides the first evaluation of the impact of BCS in farmed Pacific white shrimp. Despite some adverse impacts of dietary BCS in on shrimp growth performance, it showed positive effects on body FA composition, consistent with insignificant increase in Δ6 FAD gene. Dietary BCS also lowered the haemolymph cholesterol and triglyceride concentrations, but enhanced the haemolymph cortisol of shrimp. These results suggest that up to 30 g/kg BCS might be suitable for supplementation in the diet of farmed shrimp.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The experiments were conducted in accordance with the Iranian Society for the Prevention of Cruelty to Animals and the Canadian Council on Animal Care.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in: https://www.researchgate.net/profile/Arash_Akbarzadeh.

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