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Utilization of date seed meal in the diet of Pacific white shrimp (*Penaeus vannamei*): growth performance, body and fatty acid composition, biochemical parameters, and tolerance of salinity stress

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Abstract
Current study aimed to examine the effectiveness of date seed meal (DSM) in the diet of Pacific white shrimp (*Penaeus vannamei*). Shrimp were fed on diets containing different amounts of DSM including 0, 50, 100, 150, and 230 g/kg for a period of 8 weeks. Growth parameters and body proximate composition showed no significant differences among treatments ($p > 0.05$). Similarly, no notable differences were observed in hemolymph biochemical parameters in shrimp fed with different amounts of DSM compared with the control group ($p > 0.05$). The contents of whole body polyunsaturated fatty acids (PUFAs) including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and total n-3 in *P. vannamei* fed diet containing 100 g/kg DSM were significantly greater than control treatment ($p < 0.05$). The survival of shrimp fed diets with different amounts of DSM exhibited no significant differences when exposed to high and low salinity stress (55 and 8 g/L, respectively) ($p > 0.05$). Altogether, the results of this study showed that DSM could be utilized as a cheapest source of carbohydrate in the shrimp diet without depressive effects on its performance.

Keywords Date seed meal · Shrimp · Growth · Fatty acids · Biochemical parameters · Stress

Introduction

The commercial production of farmed shrimp has been expanding steadily worldwide (Amaya et al. 2007). Shrimp farming is contributing a major income to several countries in tropical and subtropical areas, especially in Asia (Burford et al. 2004; Zhang et al. 2013). However, high cost of feed is one of the most important challenges in shrimp aquaculture. To decrease the

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high cost of shrimp production and to deliver inexpensive feeds for farmers, substitute organic-based plant by-products have been a prioritized field of study during the last few decades (Oduro-Boateng and Bart-Plange 1988; Yousif et al. 1996; Amisah et al. 2009; Abarike et al. 2012). The utilization of inexpensive alternative protein sources, particularly those that are not directly consumable by human, could ensure a remarkable economic benefit via cost reduction (Azaza et al. 2009). Besides, non-protein nutrients including lipids and carbohydrates have been effectively used in aquaculture in order to reduce the feed costs (Yousif 2012).

Lipids and carbohydrates are known as the main nutritional constituents of shrimp's diet, providing the required energy for optimal growth (Gaxiola et al. 2005). A proper quantity of lipids and carbohydrates in the diet can minimize the protein requirement with no adverse effects on growth performance. The level and source of carbohydrates affect the efficiency of its utilization (Lovell 1998). Ingredients rich in carbohydrate are the most inexpensive form of dietary energy (Azaza et al. 2009). Thus, preparing low-cost diets via locally accessible food ingredients, particularly carbohydrate ingredients, can significantly reduce the shrimp farming cost.

Low-quality dates and date by-products, including date seed meal (DSM), are known as favorable sources of cheap carbohydrate (Yousif et al. 1996). Date seed is discarded product of date processing and packing plants. Although, DSM is available at low or no cost, it contains many valuable contents including carbohydrates, oil, dietary fiber, protein, minerals, natural antioxidants, and bioactive poly phenols (Golshan Tafti et al. 2017). DSM contains 50–70 g/kg crude protein, 40–100 g/kg fat, 10–20 g/kg total ash, 120–270 g/kg crude fiber, and 550–737 g/kg nitrogen-free extract (NFE) (Aldhaheri et al. 2004). Thus, DSM is considered as a worth source of dietary carbohydrates. It is known that DSM meal can enhance growth performance and improve feed efficiency of animals, owing to its natural anabolic agents (Belal and Al-Owaifer Belal and Al-Owafeir 2004; Elgasim et al. 1995; Hussein et al. 1998; Al-Farsi and Lee 2011). However, some studies showed that fish performance was not significantly affected by DSM (Belal and Al-Owafeir 2004; Yousif et al. 1996). High indigestible carbohydrate contents of DSM might limit its incorporation in fish diet (Rahman et al. 2007).

Considering the nutritional value of DSM, particularly in terms of carbohydrate, fatty acid contents, and micronutrients including calcium, potassium, and phosphorus, more investigation is needed on the use of DSM in aquaculture. Majority of studies exploring the inclusion of DSM in aquatic organisms evaluate the growth performance. For better understanding of the suitability of DSM as a novel source of dietary carbohydrate in aquatic animals, the biological performance should be evaluated more broadly. For example, studying serum biochemical indices in response to diet changes is worth to assess nutritional and health status of animals (Lin et al. 2015; Jacobson-Kram and Keller 2001). Moreover, intensive and semi-intensive farming of shrimp may be exposed to environmental changes including fluctuations of salinity, temperature, dissolved oxygen, (Chiba et al. 2004; Joseph and Philip 2007; Pascual et al. 2003; Fotedar and Evans 2011), or toxic compounds such as ammonia (Pakravan et al. 2017; Pakravan et al. 2018). In intensive culture systems, salinity stress might disrupt shrimp homeostasis and adversely affect growth, survival, and consequently high economic loss in shrimp culture (Li et al. 2007). Given that carbohydrate is a readily available source of energy for most crustacean species, diets rich in carbohydrate can fulfill the high-energy requirement of aquatic animals under stress (Wang et al. 2017). Therefore, it is important to evaluate shrimp responses to stressors, while diet is changed.

It seems that there is no information about the inclusion of DSM in the diet of cultured shrimp. Therefore, this investigation was performed to examine the influence of DSM as an alternative source of inexpensive carbohydrate on growth performance, whole body composition, hemolymph biochemical indices, and resistance against salinity stress in *P. vannamei*.

Materials and methods

Diet preparation

The DSM was purchased from a date by-product Company, Isfahan, Iran. The proximate composition of DSM is presented in Table 1. Five diets were prepared with different levels of DSM replaced with wheat meal and rice meal comprising 0 (control diet), 50, 100, 150, and 230 g/kg (Akbarzadeh et al. 2019). The information related to the prepared diets are presented in Table 2. All trial diets were prepared on iso-nitrogenous and iso-energetic. The ingredients of the diets were mixed and pellet was made by an extruder (diameter of pellet was 2 mm), then diets were dried and preserved as previously described in Pakravan et al. (2017).

Experimental shrimp and feeding

Penaeus vannamei were purchased from a shrimp farm in North Tiab, Hormozgan, Iran, and transferred to Kolahi Shrimp Development and Training Center located at Hormozgan province, Iran. After 1 week of acclimation to laboratory condition and feeding with commercial diet, healthy shrimp with weight of 4.0 ± 0.1 g were distributed into five groups with three replicates. For each replicate, 50 shrimp were randomly placed in circular fiberglass tanks (300 L volumes with about 200 L water). Daily water replacements were about 50%. Shrimp were fed by experimental diets at a rate of 5% body weight per day at 8:00, 14:00, and 20:00 h during a period of 8 weeks. Every 2 weeks, the amount of daily feed was readjusted by defining the shrimp total weight in each replicate. During the experimental period, temperature, DO, salinity, and pH were about 32 ± 2 °C, 8 mg/L, 36.5 g/L, and 8.1 ± 0.1, respectively.

After 8 weeks of feeding trial, shrimp from each tank were sampled and weighted. Afterwards, eight of the weighted shrimp from each tank were used for analyses of body proximate composition, fatty acid profile, and serum biochemical contents. The hemolymph was taken to evaluate the biochemical parameters and the body was utilized for the purpose of analyzing the body proximate composition and fatty acid profile.

Table 1 Proximate compositions of date seed meal (DSM)

Item Proximate composition (dry basis)	Value (g/kg)
Protein	65
Lipid	55
Fiber	320
Ash	30
Energy	4400 (kcal/kg)

Table 2 The ingredient and chemical composition of the experimental diets with 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM)

	Diets				
	Control	50	100	150	230
Ingredients (g/kg)					
Fish meal	329.20	329.20	329.20	329.20	329.20
Soybean meal	200.00	200.00	200.00	200.00	200.00
Meat powder	96.00	96.00	96.00	96.00	96.00
Date seed meal	00.00	50.00	100.00	150.00	230.00
Rice flour	90.00	70.00	50.00	30.00	00.00
Wheat flour	184.80	154.80	124.80	94.80	44.80
Fish oil	40.00	40.00	40.00	40.00	40.00
Soybean oil	20.00	20.00	20.00	20.00	20.00
Vitamin mixture[a]	10.00	10.00	10.00	10.00	10.00
Mineral mixture[b]	10.00	10.00	10.00	10.00	10.00
Filler (cellulose)	20.00	20.00	20.00	20.00	20.00
Chemical composition (g/kg)					
Crud protein	385.00	398.80	385.80	389.70	397.80
Crud lipid	208.60	186.50	212.70	204.90	219.10
Carbohydrate	188.30	195.60	161.30	158.80	127.00
Fiber	29.80	30.40	32.10	31.60	32.90
Ash	87.20	87.70	94.90	98.70	114.40
NFE	259.70	266.20	242.40	243.50	202.80
Dry matter (%)	89.88	89.90	88.68	88.36	89.13
Moisture (%)	10.12	10.10	11.32	11.64	10.87
Energy (kcal kg^{-1})[c]	4170.60	4056.10	4102.70	4038.10	3999.10

[a] Supplied (IU or mg/kg diet): vitamin A, 1800 IU; vitamin D3, 1200 IU; vitamin E, 120 mg; vitamin B_{12}, 24 mg; riboflavin, 15 mg; niacin, 90 mg; D-pantothenic acid, 27 mg; menadione, 3 mg; folic acid, 4.8 mg; pyridoxine, 9 mg; thiamine, 9 mg; D-biotin, 0.48 mg; choline chloride 360 mg; cobalamin 24 mg; ascorbic acid 156 mg; nicotinic acid 90 mg; inositol 72; antioxidant 15 mg

[b] Supplied (mg kg^{-1} diet): Zn, 18 mg; I, 0.6 mg; Mg, 7.8 mg; Co, 0.15 mg; Se, 0.15 mg; CU, 1.8 mg; Fe, 12 mg

[c] Energy was calculated as 4, 4, and 9 $kcalkg^{-1}$ of protein, carbohydrate, and lipids, respectively (calculated from physiological fuel values)

Growth parameters

Growth parameters of shrimp were measured as follows:

$$WG(g) = FW(g) - IW(g)$$

$$SGR = 100 \times (LnFW - LnIW/\text{day})$$

$$FCR = \text{Total weight of feed used}(g)/WG(g)$$

$$\text{Survival}\% = 100 \times (\text{initial shrimp number} - \text{dead shrimp number})/(\text{initial shrimp number})$$

WG is weight gain, IW is initial body weight, FW is final body weight, SGR is specific growth rates, and FCR is feed conversion ratios.

Body proximate and fatty acid composition

The whole body proximate composition of shrimp (five samples from each tank) was evaluated using AOAC method (1995) as previously described in Pakravan et al. (2017).

The fatty acid profile of experimental shrimp and diets (three samples from each tank and diet) were evaluated using a modified method of Lepage and Roy (1984) as previously described in Pakravan et al. (2017).

Hemolymph biochemical analyses

Three shrimp in the beginning of the experiment (day 1) and three shrimps from each tank at the end of feeding period were sampled to assay the biochemical indices. Hemolymph was collected from each experimental shrimp as the method of Kakoolaki et al. (2013), using a sterile disposable 25 gauge needle attached to a 1-mL sterile plastic syringe containing 0.4 mL anticoagulant solution (10 mM Tris HCl, 100 mM sodium citrate, 250 mM sucrose, pH 7.6) (Huang et al. 2004). The sampled hemolymph was centrifuged at 4600*g* for 10 min at 4 °C. The prepared supernatant was collected in a sterile tube and used to measure the amount of total protein, albumin, globulin, creatinine, glucose, magnesium, phosphate, cholesterol, urea, uric acid, blood urea nitrogen, and alkaline phosphatase activity. These factors were tested using Roche Kits and an automatic blood analyzer (COBAS Integra 400 Plus, Germany).

Salinity stress

At the end of feeding period, shrimp were challenged to salinity stressor. For each test, 10 shrimp (14.16 ± 1.82 g) from each tank were taken and placed into the 300-L tanks. Shrimp were gradually exposed to low and high salinity stress at a rate of 8 and 55 g/L, respectively (Hurtado et al. 2006). The survival of the exposed shrimp was recorded up to 48 h and measured as follows:

$$\text{Survival}\% = 100 \times (\text{initial shrimp number} - \text{dead shrimp number})/(\text{initial shrimp number})$$

Statistical analysis

Data obtained from this experiment were analyzed using one-way ANOVA and multiple comparisons were performed by Turkey's post hoc based on significant effect at $p < 0.05$. All the statistical analyses were conducted by SPSS program (version16). Values are expressed as mean ± standard deviation. The figures were drew using Sigma Plot (version 11.0).

Results

Growth parameters

The growth parameters of *P. vannamei* fed diets containing different levels of DSM were evaluated every 2 weeks (Table 3). Almost all of the diets were accepted by the shrimp. There were no significant differences in IW, FW, WG, FCR, SGR, and percent survival among treatments ($p > 0.05$).

Table 3 IW, FW, WG, SGR, and survival of *Penaeus vannamei* fed diet with 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM)

	Treatments				
Items	Control	50	100	150	230
After 2 weeks					
I W (g)	4.00 ± 0.10	4.03 ± 0.05	4.00 ± 0.10	4.03 ± 0.11	3.96 ± 0.05
FW (g)	6.46 ± 0.52	6.56 ± 0.77	6.73 ± 0.20	7.54 ± 0.62	6.37 ± 0.75
WG (g)	2.46 ± 0.62	2.53 ± 0.83	2.73 ± 0.17	3.50 ± 0.74	2.40 ± 0.70
SGR (g/day)	3.35 ± 0.84	3.44 ± 0.96	3.72 ± 0.19	4.45 ± 0.78	3.34 ± 0.76
Survival (%)	98.00 ± 2.00	97.33 ± 2.30	97.33 ± 2.30	98.00 ± 2.00	98.00 ± 0.00
After 4 weeks					
FW (g)	8.16 ± 1.23	9.33 ± 2.08	9.00 ± 2.19	9.10 ± 1.35	7.93 ± 0.61
WG (g)	4.16 ± 1.20	5.30 ± 2.12	5.00 ± 2.09	5.06 ± 1.45	3.96 ± 0.56
SGR (g/day)	2.40 ± 0.49	2.83 ± 0.79	2.72 ± 0.81	2.78 ± 0.60	2.38 ± 0.22
Survival (%)	96.66 ± 1.15	95.33 ± 2.30	96.66 ± 1.15	96.66 ± 1.15	96.66 ± 1.15
After 6 weeks					
FW (g)	11.48 ± 1.39	11.73 ± 0.05	11.72 ± 2.41	11.89 ± 0.73	11.57 ± 0.87
WG (g)	7.48 ± 1.48	7.69 ± 1.11	7.72 ± 2.31	7.86 ± 0.84	7.61 ± 0.82
SGR (g/day)	2.37 ± 0.35	2.42 ± 0.23	2.41 ± 0.42	2.45 ± 0.20	2.43 ± 0.14
Survival (%)	95.33 ± 2.30	94.00 ± 0.00	95.33 ± 1.15	94.66 ± 1.15	94.66 ± 1.15
After 8 weeks					
FW (g)	14.43 ± 1.68	14.61 ± 2.10	13.86 ± 3.11	14.85 ± 1.71	13.06 ± 0.67
WG (g)	10.43 ± 1.78	10.58 ± 2.13	9.86 ± 3.01	10.82 ± 1.67	9.09 ± 0.64
SGR (g/day)	2.15 ± 0.24	2.17 ± 0.24	2.07 ± 0.34	2.20 ± 0.17	2.01 ± 0.07
Survival (%)	92.00 ± 2.00	91.33 ± 2.30	92.66 ± 3.05	91.33 ± 1.15	91.33 ± 1.15

IW initial weight, *FW* final weight, *WG* weight gain, *SGR* specific growth ratio

Data (mean ± SD) with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$)

Body proximate composition

The body proximate compositions (wet weight basis) of *P. vannamei* fed diets with different amounts of DSM are shown in Table 4. The protein, lipid, and ash contents of shrimp did not show any significant differences among experimental treatments ($p > 0.05$).

Hemolymph biochemical parameters

The results of hemolymph biochemical parameters are presented in Fig. 1. There were no significant differences in hemolymph biochemical parameters of shrimp fed diets containing different amounts of DSM and the control group ($p > 0.05$). The levels of serum proteins such as total protein, albumin, and globulin showed a decreasing trend in shrimp fed diets with higher amounts of DSM (Fig. 1a–c). Albumin concentration was significantly lower in shrimp fed with 230 g/kg DSM compared with shrimp sampled at day1 (Fig. 1b). Similarly, the levels of creatinine were significantly higher in shrimp sampled at day 1 compared with shrimp fed with different levels of DSM (Fig. 1d). Glucose and magnesium levels were significantly higher in shrimp fed with 230 g/kg DSM compared with 50 g/kg treatment ($p < 0.05$, Fig. 1f, g). The amount of hemolymph cholesterol showed a decreasing trend in shrimp fed with different levels of DSM (Fig. 1i). Shrimp fed diet containing 100–230 g/kg DSM meal showed a significant increase in hemolymph

Table 4 Body proximate composition (wet weight basis) of *Penaeus vannamei* fed diet with 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM)

	Treatments				
Items (g/kg)	Control	50	100	150	230
Moisture	734.80 ± 6.34	732.03 ± 7.65	738.57 ± 10.64	738.67 ± 14.51	734.73 ± 6.75
Protein	227.48 ± 7.56	226.02 ± 10.99	229.32 ± 10.13	225.64 ± 12.85	231.47 ± 7.93
Lipid	15.35 ± 0.99	15.87 ± 0.37	14.24 ± 0.80	15.61 ± 1.57	12.64 ± 2.47
Ash	60.26 ± 2.57	61.66 ± 0.32	56.70 ± 0.90	62.16 ± 2.81	49.80 ± 10.73

Data (mean ± SD) with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$)

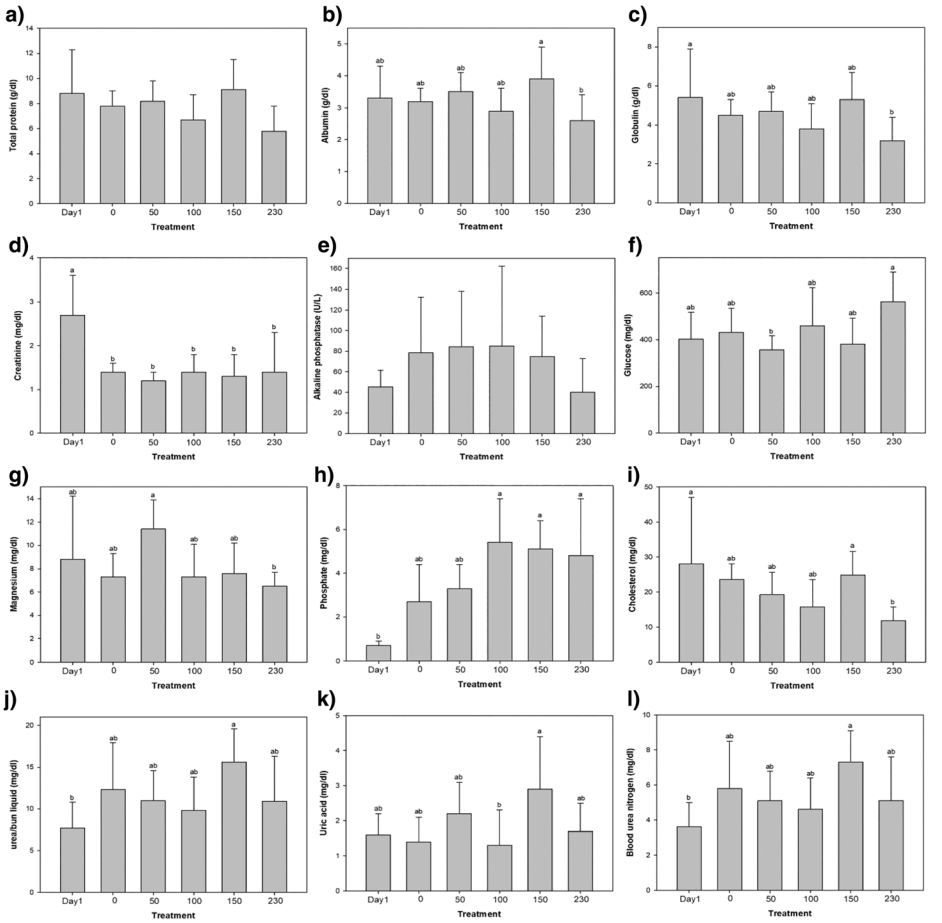

Fig. 1 The concentrations of total protein (**a**), albumin (**b**), globulin (**c**), creatinine (**d**), glucose (**f**), magnesium (**g**), phosphate (**h**), cholesterol (**i**), urea (**j**), uric acid (**k**), blood urea nitrogen (**l**), and alkaline phosphates activity (**e**) in hemolymph of Pacific white shrimp (*P. vannamei*) fed diet containing 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM). Data (mean ± SD) with different letters are significantly different ($p < 0.05$) among treatments according to ANOVA test ($p < 0.05$)

phosphate compared with other groups ($p < 0.05$, Fig. 1h). Inclusion of 150 g/kg DSM caused a significant increase in uric acid, blood urea nitrogen, and urea/burn nitrogen levels (Fig. 1j–l).

Fatty acid profile

Fatty acid composition for trial diets and body of *P. vannamei* fed with diets included different amounts of DSM are presented in Tables 5 and 6. Diet containing 50 g/kg DSM had significantly higher amounts of polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA), arachidonic acid (ARA), and total n-3 compared with control diet ($p < 0.05$, Table 5). The contents of PUFAs including DHA, eicosapentaenoic acid (EPA), and total n-3 in the body of *P. vannamei* fed diet containing 100 g/kg DSM were significantly higher than those of control treatment ($p < 0.05$, Table 6).

Salinity stress

The survival (%) of *P. vannamei* fed diets containing different amounts of DSM after exposure to 8 and 55 g/L salinity is presented in Table 7. After 48-h exposure to low and high salinity stress, survival did not show any significant differences among all treatments ($p > 0.05$).

Table 5 Fatty acid profile of experimental diets with 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM) (% total fatty acid)

	Treatments				
Items	Control	50	100	150	230
C14:0	3.87^{cd}	3.61^{d}	4.11^{bc}	4.40^{ab}	4.57^{a}
C15:0	0.73^{a}	0.71^{ab}	0.71^{ab}	0.65^{bc}	0.61^{c}
C16:0	25.73^{a}	24.60^{b}	24.94^{ab}	23.17^{c}	23.92^{bc}
C17:0	0.91^{a}	0.87^{ab}	0.85^{ab}	0.82^{ab}	0.79^{b}
C18:0	6.28^{ab}	6.38^{a}	5.94^{c}	5.98^{bc}	5.90^{c}
SFA	37.54^{a}	36.18^{bc}	36.57^{ab}	35.05^{c}	35.81^{bc}
C16:1n7	5.94	5.70	5.82	5.68	5.48
C17:1	0.31	0.30	0.34	0.38	0.25
C18:1n9	32.11	32.28	32.14	32.73	32.97
MUFA	38.37	38.28	38.31	38.80	38.71
C18:2n6 (LA)	6.69^{a}	6.72^{a}	6.41^{ab}	6.00^{c}	6.05^{bc}
C18:3n3 (ALA)	1.47^{a}	1.46^{a}	1.45^{a}	1.36^{b}	1.43^{a}
C20:3n3	0.21	0.19	0.17	0.19	0.18
C20:4n6(ARA)	0.42^{b}	0.50^{a}	0.42^{b}	0.43^{b}	0.45^{ab}
C20:5n3 (EPA)	3.81	3.93	3.83	3.89	3.86
C22:6n3(DHA)	8.27^{c}	8.89^{ab}	8.69^{ab}	8.93^{a}	8.50^{bc}
PUFA	20.89^{b}	21.71^{a}	20.99^{b}	20.83^{b}	20.50^{b}
Total n-3	13.77^{b}	14.48^{a}	14.15^{ab}	14.40^{a}	13.98^{ab}
Total n-6	7.11^{a}	7.22^{a}	6.83^{ab}	6.43^{b}	6.51^{b}
n-3/n-6	1.93^{c}	2.00^{bc}	2.07^{abc}	2.23^{a}	2.14^{ab}

LA linoleic acid, *ALA* alpha linolenic acid, *ARA* arashidonic acid, *EPA* eicosapentaenoeic acid, *DHA* docosahexaenoic acid, *SFA* saturated fatty acid, *MUFA* mono unsaturated fatty acid, *PUFA* polyunsaturated fatty acid

Data (mean) with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$)

Table 6 Fatty acid profile of whole body of *Penaeus vannamei* fed diet with 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM) (% total fatty acid)

	Treatments				
Items	Control	50	100	150	230
C14:0	1.09^{ab}	0.90^{b}	1.07^{ab}	1.30^{a}	1.19^{a}
C15:0	0.82^{a}	0.79^{ab}	0.70^{b}	0.78^{ab}	0.82^{a}
C16:0	22.78^{a}	22.30^{ab}	22.06^{abc}	21.98^{bc}	21.53^{c}
C17:0	1.33^{a}	1.45^{ab}	1.41^{ab}	1.52^{a}	1.49^{a}
C18:0	12.92^{ab}	12.79^{ab}	12.41^{b}	13.30^{a}	12.83^{ab}
SFA	38.96^{a}	38.24^{bc}	37.66^{c}	38.89^{ab}	37.87^{c}
C16:1n7	1.79	1.77	1.85	1.71	1.86
C17:1	0.23	0.30	0.15	0.30	0.23
C18:1n9	25.34	25.00	24.83	24.64	24.75
MUFA	27.36	27.08	26.83	26.65	26.85
C18:2n6 (LA)	5.73^{bc}	5.41^{c}	5.57^{bc}	5.74^{b}	6.25^{a}
C18:3n3 (ALA)	0.74	0.68	0.65	0.59	0.84
C20:3n3	0.74	0.68	0.65	0.59	0.84
C20:4n6(ARA)	3.49	3.40	3.38	3.21	3.38
C20:5n3 (EPA)	11.91^{b}	12.93^{ab}	13.18^{a}	12.32^{ab}	12.49^{ab}
C22:6n3(DHA)	11.51^{b}	11.93^{ab}	12.38^{a}	12.16^{ab}	11.90^{ab}
PUFA	34.13^{b}	35.05^{ab}	35.84^{a}	34.62^{ab}	35.71^{ab}
Total n-3	24.90^{b}	26.23^{ab}	26.88^{a}	25.67^{ab}	26.07^{ab}
Total n-6	9.22^{b}	8.81^{c}	8.96^{bc}	8.95^{bc}	9.64^{a}
n-3/n-6	2.69^{c}	2.97^{a}	2.99^{a}	2.86^{ab}	2.70^{bc}

LA linoleic acid, *ALA* alpha linolenic acid, *ARA* arashidonic acid, *EPA* eicosapentaenoeic acid, *DHA* docosahexaenoic acid, *SFA* saturated fatty acid, *MUFA* mono unsaturated fatty acid, *PUFA* polyunsaturated fatty acid

Data (mean) with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$)

Discussion

Date by-products have been used as animal feed for a long time and efficiently utilized by sheep (AL-Suwaiegh 2016), poultry (Hussein et al. 1998), and calf (El Hag and Ekhanjari 2000). Moreover, in recent years, researchers investigated the utilization of the date by-products in the diet of farmed fish including gray mullet (*Valamugil seheli*) (Yousif 2012), Nile tilapia (*Oreochromis niloticus*) (Belal 2008; Gaber et al. 2012), and African catfish (*Clarias gareiepinus*) (Sotolu et al. 2014(. However, little information is available on the use

Table 7 Survival (%) of *Penaeus vannamei* fed diet with 0 (control), 50, 100, 150, and 230 g/kg of date seed meal (DSM) at the end of 48 h after 8 and 55 g/L salinity stress

	Treatments				
Items	Control	50	100	150	230
Survival (%) after 8 g/L salinity stress	96.7 ± 5.8	80.0 ± 10.0	90.0 ± 10.0	90.0 ± 00.0	90.0 ± 00.0
Survival (%) after 55 g/L salinity stress	66.7 ± 15.3	56.7 ± 15.3	60.0 ± 0.0	70.0 ± 10.0	70.0 ± 10.0

Data (mean ± SD) with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$)

of DSM as crustacean feed ingredient. The present article reports the first use of DSM in the diet of *P. vannamei*. Our results showed that DSM could comprise up to 230 g/kg of the *P. vannamei* diet without any depressing effect on shrimp performance, and therefore reduce the expenses of cultured shrimp food.

In our study, percent survival and growth parameters of shrimp fed diet containing DSM did not reveal any significant differences compared with those of control treatment. In line with these results, no significant differences in shrimp body proximate composition were found among the experimental treatments. Thus, substituting the rice and wheat flour with DSM did not have a negative effect on the growth performance of *P. vannamei*. These results are in line with previous findings that utilized date by-products in the diet of farmed fish. For example, in blue spot gray mullet fry (*V. seheli*), diets substituted with 100, 200, and 300 g/kg DSM achieved the best survival rate (Yousif 2012). Moreover, up to 300 g/kg replacement of corn meal with fungi-degraded date seed caused better growth performance in Nile tilapia (*O. niloticus*) (Belal 2008). The efficiency of carbohydrate utilization as energy sources by shrimp varies depending on the level and source of carbohydrates (Lovell 1998). It is believed that treating of DSM using specific materials or methods to transform the fibers to simpler forms of carbohydrate molecules might cause better utilization of DSM by animals, and consequently, their better growth (Gaber et al. 2012; Belal 2008; Osman et al. 2001). Although DSM processing might increase the price of diet, given the lower price of DSM compared with wheat or rice flour, and having no negative effects on shrimp performance, DSM can be applied as an inexpensive source of carbohydrate in the diet of cultured shrimp.

This study provides the first evidence on the effect of diets containing DSM on biochemical parameters of shrimp hemolymph. Our results showed no notable differences in hemolymph biochemical parameters in shrimp fed with different amounts of DSM in comparison with the control group, and therefore DSM caused no adverse effects on hemolymph parameters in *P. vannamei*. The hemolymph biochemical parameters can reflect the nutritional status, the health status, and the adaptability to environment in fish and shrimp (Chen et al. 2016; Yu et al. 2008). Numerous metabolic variables including hemolymph glucose, proteins, and cholesterol can be used to assess the shrimp physiological condition (Yu et al. 2008). The nature of diet is a prevailing agent affecting shrimp hemolymph metabolites. Blood glucose and proteins are very sensitive to dietary carbohydrate and protein in shrimp and can be indicators of their nutritional status (Pascual et al. 2003). There were no significant differences in total protein, albumin, and globulin in the serum of shrimp fed diet included different levels of DSM in comparison with control treatment, demonstrating that the immune response of shrimp was not influenced by substitution of carbohydrate source. However, shrimp fed with 230 g/kg DSM showed higher amount of glucose compared with the other experimental groups. DSM is known as a rich source of carbohydrates (Hamada et al. 2002) and might increase glucose circulation in hemolymph (AL-Suwaiegh 2016). The produced glucose in digestive gland can be directed to glucose-6-phosphate, which is then distributed to the blood as an energy source in tissues (Rosas et al. 2002). Blood glucose concentration is known as an important indicator of dietary energy intake; therefore, the values of glucose concentration obtained in this study confirmed that the experimental diets containing DSM supplied the shrimp with their requirement for energy. Cholesterol is a lipid class in crustacean's hemolymph that controls many aspects of crustacean physiology, and can be used as an indicator of diet quality (Pascual et al. 2003). In crustaceans, cholesterol serves as a constituent of

cellular membranes, sub-cellular structures, and as a precursor of steroid hormones and molting hormones such as ecdysone (Bonilla-Gómez et al. 2012). The higher amounts of DSM inclusion in the diet caused a decreasing trend of plasma cholesterol in shrimp, which may be considered as a positive nutritional aspect. Since shrimp is rich in cholesterol naturally, and excess cholesterol in food is a risk factor for developing cardiovascular disease in consumers (Pires et al. 2018). Our results provide evidence for the effects of dietary DSM on lipid metabolism.

Given that rice and wheat flour were replaced with DSM, it is better to ensure that essential fatty acid necessities of shrimp are met. The results of the present work showed that the majority of fatty acids in the whole body of shrimp, especially n-3 and n-6 PUFAs (i.e., ALA, LA, ARA, DHA, and EPA) were similar or even higher in shrimp fed with DSM diets in comparison with those of control treatment, consistent with the fatty acid composition of experimental diets. As an essential component of cell membranes and precursors of eicosanoids, fatty acids, particularly n-3 PUFAs, are necessary for routine metabolic functions of most organisms (Hurtado et al. 2007; Palacios et al. 2004). Marine shrimp have a restricted ability to synthesize n-3 PUFAs (Suprayudi et al. 2004). It is known that DHA and EPA are essential for farmed shrimp comprising *P. vannamei* (Gonzalez-Felix et al. 2002). Because of this, long-chain PUFAs are usually added to the diets of marine shrimp (Chen et al. 2014). The present results confirmed that DSM serve as an acceptable source of fatty acids in shrimp feed.

In this experiment, high and low salinity were selected as common environmental stressors that are prevalent in the shrimp culture systems. Ambient salinity changes impact metabolism, growth, oxygen consumption, feeding rate, molting, survival, and tolerance to toxic metabolites of *P. vannamei* (Li et al. 2007), although this shrimp species is a euryhaline decapod species and is generally considered an osmoconformer (Chen et al. 2014). To retain homeostasis by osmoregulation, shrimp need energy from nutrients via a compensatory process, and lipids and carbohydrate play important roles in this process (Chen et al. 2014). Carbohydrate is known as the most inexpensive and primary and immediate source of energy that can directly fulfill the higher energy demand of crustacean species in a stress condition, particularly at low salinity (Tseng and Hwang 2008). Our results showed that the survival of shrimp fed with diets containing different levels of DSM after 48-h exposure to high or low levels of salinity stress was not significantly different compared with that of the control group. This result demonstrated that diets containing DSM could provide acceptable amounts of energy to help shrimp successfully cope with salinity stress. Moreover, our results showed that in all treatment groups, shrimp could survive more successfully in lower salinity stress compared with higher one. This phenomenon has been attributed to the fact that juveniles of *P. vannamei* live in low salinity lagoons in their natural habitat for 6–12 weeks before returning to oceanic waters (Jayasankar et al. 2009).

Conclusion

In conclusion, this study suggested that the inclusion of DSM (up to 230 g/kg) as a carbohydrate source in diet had no adverse effects on the performance of *P. vannamei*. Therefore, DSM can be used as a low-cost carbohydrate source and a functional feed ingredient in the diet of farmed shrimp. The potential uses of DSM in further studies on fish and shrimp are promising.

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Compliance with ethical standards

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.

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