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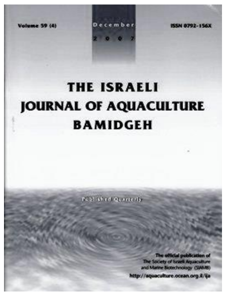

Dietary Methionine Requirements of Pacific White Shrimp *Litopenaeus vannamei,* of Three Different Sizes

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Key words: *Litopenaeus vannamei*, methionine requirements, growth performance, body composition

Abstract

Three feeding trials were conducted to determine the dietary methionine requirements for *Litopenaeus vannamei* of three different sizes, small (0.55±0.01g), medium (4.18±0.05g), and large (9.77±0.08g). Seven diets were formulated with seven different levels of methionine (0, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or 0.6%). The greatest weight gain (WG) and specific growth rate (SGR) of small and large shrimp were observed when dietary methionine levels were 0.8%, and 0.71%, respectively (P<0.05). The highest survival rate of small and large shrimp was observed when dietary methionine levels were 0.97%, and 1.07%, respectively (P<0.05). For small and medium shrimp, the optimal dietary methionine level for maximum weight gain estimated by quadratic regression analysis was 0.91% and 0.67% of dry diet, respectively. For large shrimp, the optimal dietary methionine requirement estimated by broken-line model based on weight gain was 0.66% of dry diet.

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Introduction

Methionine is one of the ten essential amino acids for nutrition included in fish and shrimp feeds, particularly those formulated with high levels of plant protein (Millamena *et al*., 1996; Sookying *et al*., 2013). Appropriate methionine content could reduce other amino acid oxidation (NRC, 2011), thus promoting greater growth rate. Many studies have been conducted to determine the level of this amino acid required in several shrimp species (Huang *et al*., 2003; Millamena *et al*., 1996; Huai *et al*., 2009; Zeng, 2012).The reported values range from 1.83% to 2.52% of dietary protein.

Litopenaeus vannamei has become a popular crustacean species for aquaculture worldwide because of its economic value, rapid growth rate, and tolerance to a wide range of salinity and temperatures (Wickins and Lee, 2007). Determination of essential amino acid requirements is considered important in shrimp nutrition research (Akiyama, 1986; Shiau S.Y., 1998). Studies have been carried out on requirements of other essential amino acids (EAA) such as lysine, threonine, arginine for Pacific white shrimp (Akiyama, 1986; Fox et al., 1995; Huai et al., 2009; Xie et al., 2012; Zhou et al., 2012, 2013), however to our knowledge, no research has been published on the methionine requirements of Pacific white shrimp at different growth stages. The objective of the present study was to evaluate the effect of dietary methionine on growth performance, feed utilization, and body composition for three different sizes of *L. vannamei*.

Materials and methods

Experimental diets. For experiment 1 (small shrimp), seven diets (D1, D2, D3, D4, D5, D6 and D7) were formulated to contain 40% crude protein; for experiment 2 (medium shrimp) seven diets (D1, D2, D3, D4, D5, D6 and D7) were formulated to contain 38% crude protein; for Experiment 3 (large shrimp) seven diets (D1, D2, D3, D4, D5, D6 and D7) were formulated to contain 34% crude protein (Chen *et al*., 2011). The DL-methionine was provided by Evonik Degussa Co., Ltd (Shanghai, China) and added to the basal diet at seven different levels ranging from 0% to 0.6%. The diets were adjusted to be isonitrogenous by decreasing the levels of L-aspartic acid and L-glycine acid. The dietary amino acids were analyzed by the China National Analytical center, Guangzhou (NACC) using high performance liquid chromatography (HPLC, HP1100, USA). Ingredients in the experimental diets are presented in Table 1 and Table 2, proximate compositions of the experimental diets are presented in Table 3, and amino acid compositions of experimental diets are shown in Table 4. Fish meal and soybean meal were used as the main protein sources in the basal diet.

Table 1. Composition of experimental diets

Ingredients	*40%*	*38%*	*34%*
Fish meal	20.00	20.00	20.00
Soybean meal	30.00	25.00	15.00
Soy protein concentrate	7.00	7.00	5.00
Peanut meal	6.00	6.00	6.00
Shrimp head meal	5.00	5.00	5.00
Squid viscera powder	3.00	3.00	3.00
Wheat flour	18.40	23.40	35.40
Soya lecithin	1.00	1.00	1.00
Fish oil	3.00	3.00	3.00
Choline chloride	0.50	0.50	0.50
Stay- C	0.20	0.20	0.20
Vitamin premix[1]	1.00	1.00	1.00
Mineral premix[2]	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	0.70	0.70	0.70
Sodium alginate	2.00	2.00	2.00
CMC	0.60	0.60	0.60

[1] Vitamin premix (g/kg): V_A 2.5; V_D 6.25; V_E 75; V_K 2.5; V_{B1} 0.25; V_{B2} 1.0; V_{B3} 5.0; V_{B6} 0.75g; V_{B12} 2.5; V_{B5} 2.5; Folic acis 0.25; Biotin 2.5; Inositol 379 ; Cellulose 500 ;

[2] Mineral premix (g/kg): KCl, 90; KI, 0.04; NaCl, 40;$CuSO_4$-$5H_2O$, 3; $ZnSO_4$-7H2O, 4 $CoSO_4$-$7H_2O$, 0.02; $FeSO_4$-$7H_2O$, 20; $MnSO_4$-H_2O, 3 ; $MgSO_4$-$7H_2O$, 124; $Ca(HPO_4)2$-$2H_2O$, 500; $CaCO_3$, 215

Table 2 Dietary methionine levels in Experiment 1, 2 and 3 (%)

	D1	*D2*	*D3*	*D4*	*D5*	*D6*	*D7*
D-form methionine	0.00	0.10	0.20	0.30	0.40	0.50	0.60
ASP:GLY(1:1)	0.60	0.50	0.40	0.30	0.20	0.10	0.00

Table 3. Nutritional level of experimental diets (% dry matter)

		D1	*D2*	*D3*	*D4*	*D5*	*D6*	*D7*
	Moisture	7.49	9.53	7.35	7.05	7.43	7.10	7.17
	Crude protein	40.55	39.74	40.67	41.29	40.72	39.17	40.58
	Crude lipid	6.74	6.67	6.65	6.70	6.09	5.80	5.93
Experiment 1	Ash	11.89	11.72	11.30	11.74	11.57	11.79	11.85
	Methionine	0.7	0.8	0.84	0.97	1.07	1.12	1.23
	Moisture	10.18	7.90	8.85	7.14	8.54	8.74	6.49
	Crude protein	38.79	38.09	39.12	38.38	37.91	39.23	37.84
	Crude lipid	5.62	5.70	5.50	5.39	5.35	6.97	6.77
Experiment 2	Ash	10.89	11.32	11.13	11.28	10.96	11.43	11.42
	Methionine	0.67	0.77	0.86	0.95	1.01	1.12	1.22
	Moisture	10.52	9.23	9.18	9.00	10.25	9.54	9.96
	Crude protein	34.00	33.72	34.99	35.56	33.84	34.92	35.18
	Crude lipid	6.05	5.67	6.15	6.09	6.31	6.14	5.98
Experiment 3	Ash	10.15	10.17	10.20	10.12	9.95	10.10	10.24
	Methionine	0.63	0.71	0.80	0.90	0.98	1.07	1.15

Crude protein, crude fat, ash and methionine were tested as dry matter.

Table 4 Amino acid composition of experimental diet (% dry matter)

Treatment		*Met*	*Cys*	*Lys*	*Thr*	*Arg*	*Ile*	*Leu*	*Val*	*His*	*Phe*
	D1	0.7	0.50	2.29	1.47	2.72	1.64	2.89	1.74	0.97	1.87
Experiment 1	D2	0.8	0.49	2.30	1.42	2.74	1.73	2.91	1.83	0.98	1.85
	D3	0.84	0.47	2.24	1.41	2.69	1.64	2.84	1.73	0.95	1.82
	D4	0.97	0.48	2.27	1.44	2.69	1.66	2.89	1.76	0.96	1.87
	D5	1.07	0.49	2.29	1.44	2.73	1.72	2.91	1.83	0.97	1.86
	D6	1.12	0.47	2.35	1.45	2.67	1.61	2.86	1.70	0.96	1.85
	D7	1.23	0.50	2.32	1.44	2.73	1.72	2.91	1.82	0.98	1.89
	D1	0.67	0.47	2.11	1.35	2.52	1.56	2.70	1.68	0.91	1.73
Experiment 2	D2	0.77	0.48	2.15	1.37	2.57	1.60	2.76	1.71	0.93	1.76
	D3	0.86	0.48	2.13	1.38	2.55	1.60	2.97	1.67	0.91	1.77
	D4	0.95	0.49	2.14	1.39	2.57	1.57	2.76	1.68	0.93	1.78
	D5	1.01	0.47	2.14	1.34	2.53	1.60	2.72	1.70	0.92	1.79
	D6	1.12	0.47	2.15	1.37	2.58	1.63	2.76	1.87	0.93	1.75
	D7	1.22	0.48	2.13	1.39	2.55	1.57	2.76	1.66	0.93	1.77
	D1	0.63	0.44	1.76	1.17	2.35	1.35	2.37	1.46	0.79	1.51
Experiment 3	D2	0.71	0.44	1.80	1.17	2.35	1.36	2.38	1.48	0.79	1.54
	D3	0.80	0.44	1.79	1.18	2.38	1.37	2.39	1.48	0.79	1.53
	D4	0.90	0.44	1.80	1.18	2.39	1.39	2.40	1.51	0.8	1.54
	D5	0.98	0.44	1.80	1.17	2.38	1.38	2.39	1.50	0.80	1.53
	D6	1.07	0.44	1.80	1.18	2.40	1.39	2.40	1.50	0.79	1.54
	D7	1.15	0.44	1.79	1.17	2.37	1.41	2.40	1.51	0.80	1.54
1		0.77	—	1.94	1.01	2.44	1.06	1.88	1.24	0.65	1.12
2		0.96	0.35	2.12	1.44	2.32	1.40	2.16	1.60	0.84	1.60

Met, methionine; Cys, cystine; Lys, Lysine; Thr, threonine; Arg, Arginine; Ile, Isoleucine; Leu, leucine; Val, valine; His, histidtine; Phe, phenylalanine.

[1] amount in 40% shrimp tissue crude protein (Forster et al., 2002);

[2] amount in 40% shrimp tissue crude ptotein (Akiyama et al., 1991)

All dry ingredients except for the methionine and CMC, were finely ground, weighed, and hand mixed for 5 min. DL-methionine was pre-coated with CMC in water at 60 °C to prevent leaching loss and then blended with the other mixed dry ingredients (Alam *et al*., 2004). The mixed dry ingredients were then transferred to a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment Ltd., Ottawa, Canada) and mixed for 15-min. The oil mix was then slowly added and all ingredients were further mixed for another 10 min. The distilled water (~30-35%, v/w) was added to the mixture to form dough of even consistency which was pelletized with a 1.0-mm-diameter die (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) for Experiment 1, and a 1.5-mm-diameter die for Experiments 2 and 3. The diets were dried until the moisture was reduced to <10%. The dry pellets were placed in plastic bags and stored -20 °C until use.

Experimental procedures. The feeding trials were carried out in Shenzhen Experimental Base, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. Healthy post larval shrimp obtained from a commercial hatchery were cultured and fed commercial feed in a cement pond until the commencement of the trial. Prior to each feeding trial, the selected shrimp of required size were acclimated to experimental conditions for 10 days and then fed the experimental diets. The three experiments were carried out consecutively.

Experiment 1: 840 small shrimp, (average initial body weight 0.55±0.01g) were distributed randomly into 21 round fiberglass tanks (500 L, 3 tanks for each diet/40 shrimp per tank).

Experiment 2: 630 medium shrimp (average initial body weight 4.18±0.05g) were distributed randomly into 21 round fiberglass tanks (500 L, 3 tanks for each diet/30 shrimp per tank).

Experiment 3: 525 large shrimp (average initial body 9.77±0.08g) were distributed randomly into 21 round fiberglass tanks (500 L, 3 tanks for each diet/25 shrimp per tank).

Shrimp were fed the respective diets at about 4-8% body weight (BW) three times a day at 8:00, 15:00 and 22:00. There was a continuous flow of sand-filtered seawater and continuous aeration. Water quality parameters were monitored daily. The feeding trial lasted 40 days for Experiment 1, 26 days for Experiment 2, and 29 days for Experiment 3. During the test period, the water quality was as follows: water dissolved oxygen was higher than 5 mg/L, temperature 28-33 °C, salinity 30-33‰, and ammonia nitrogen less than 0.02 mg/L. All values are averages for the duration of the trials.

Measurement and analysis

Sample collection. At the end of each experiment, the shrimp were counted and weighed. Five shrimp were randomly selected from each tank to determine proximate carcass composition (samples were kept at -20 °C). Another five shrimp were randomly selected; the hepatopancreas and gut were removed to assess digestive enzyme activity and were kept at -80 °C.

Proximate analysis. Diets and whole shrimp triplicate samples were analyzed and their proximate composition was assessed. Moisture, crude protein, crude lipid and ash were determined by standard methods (AOAC, 1995). Moisture was determined by drying in an oven at 105 °C for 24 h; crude protein (N × 6.25) was analyzed by the Kjeldahl method after acid digestion (1030-Auto-analyzer, Tecator, Höganäs, Sweden); crude lipid was determined by ether-extraction method by Soxtec System HT (Soxtec System HT6, Tecator, Sweden); crude ash by incineration in a muffle furnace at 550 °C for 24 h.

Calculations and statistical analysis. Results are presented as means ± SD and subjected to one-way analysis of variance (ANOVA) to test the effects of experimental diets using the software of the SPSS for windows (Ver. 17.0, U.S.A). Duncan's new multiple range test was used to resolve the differences between treatment means. Statistical significance was determined at $P < 0.05$ unless otherwise noted. Second-degree polynomial regression analysis ($Y=a+bX+cX^2$) (Zeitoun *et al*., 1976) or broken line model ($Y= a + bX$) (Robbins *et al*., 1979) was employed to estimate the optimum methionine requirement.

Results

Growth performance. Growth performance of *L. vannamei* in three experiments was significantly ($P < 0.05$) affected by the dietary methionine levels (Table 5).

Table 5 Effects of dietary methionine level on growth of *Litopenaeus vannamei* of different sizes

Treatment		*IW[1] (g)*	*FW[2] (g)*	*WG/%[3]*	*SGR/%[4]*	*FCR[5]*	*Survival[6]*
Experiment 1	D1	0.55±0.01	4.55±0.12^{ab}	693.20±2.53^{a}	5.26±0.03^{ab}	1.14±0.04	96.67±0.83^{ab}
	D2	0.55±0.01	5.03±0.08^{d}	788.88±18.48	5.52±0.02^{c}	1.07±0.05	97.50±1.44^{ab}
	D3	0.54±0.02	4.75±0.21^{bcd}	757.55±24.44	5.43±0.09^{bc}	1.04±0.04	97.50±1.44^{ab}
	D4	0.54±0.01	4.63±0.12^{bc}	752.30±14.21	5.38±0.03^{bc}	1.14±0.05	99.17±0.83^{b}
	D5	0.55±0.01	4.90±0.25^{cd}	743.30±11.56	5.48±0.07^{c}	1.03±0.04	94.17±2.20^{a}
	D6	0.55±0.00	4.52±0.19^{ab}	698.78±19.43	5.26±0.06^{ab}	1.17±0.02	97.50±0.00^{ab}
	D7	0.55±0.00	4.28±0.17^{a}	649.18±14.04	5.12±0.06^{a}	1.16±0.08	96.67±0.83^{ab}
Experiment 2	D1	4.22±0.01	10.30±0.25	143.83±3.59	3.43±0.06	1.34±0.04	97.78±1.11^{ab}
	D2	4.22±0.05	10.39±0.24	146.22±2.52	3.46±0.04	1.32±0.01	98.89±1.11^{ab}
	D3	4.17±0.10	10.23±0.11	145.61±4.50	3.45±0.07	1.31±0.03	98.89±1.11^{b}
	D4	4.13±0.05	10.18±0.15	146.42±1.37	3.47±0.02	1.30±0.02	98.89±1.11^{b}
	D5	4.18±0.06	10.36±0.34	147.81±6.54	3.49±0.10	1.33±0.02	96.67±1.93^{ab}
	D6	4.14±0.01	10.01±0.17	141.73±2.55	3.39±0.04	1.35±0.05	98.89±1.11^{b}
	D7	4.17±0.03	10.22±0.08	144.86±0.73	3.44±0.01	1.40±0.09	95.56±2.94^{a}
Experiment 3	D1	9.80±0.14	17.12±0.14	74.67±2.14^{ab}	1.92±0.04^{ab}	1.56±0.04	98.67±1.33^{ab}
	D2	9.72±0.09	17.66±0.32	81.65±2.76^{b}	2.06±0.05^{b}	1.61±0.03	94.67±1.33^{a}
	D3	9.71±0.06	17.06±0.20	75.78±1.69^{ab}	1.95±0.03^{ab}	1.63±0.03	96.00±0.00^{ab}
	D4	9.75±0.02	17.05±0.32	74.98±2.02^{ab}	1.93±0.04^{ab}	1.66±0.09	96.00±2.31^{ab}
	D5	9.84±0.03	17.23±0.65	75.17±3.57^{ab}	1.93±0.07^{ab}	1.54±0.06	98.67±1.33^{ab}
	D6	9.80±0.08	16.92±0.30	72.64±1.06^{a}	1.88±0.02^{a}	1.56±0.03	100.00±0.00^{b}
	D7	9.79±0.07	16.90±0.59	72.56±3.61^{a}	1.88±0.07^{a}	1.60±0.06	97.33±1.33^{ab}

Values are means ± SEM of three replicates and values with different letters (a, b, c, d) in the same column are significantly different ($P < 0.05$) in each experiment.

[1] IW: Initial weight

[2] FW: Final weight

[3] WG (%): weight gain=100×(final body weight- initial body weight)/initial body weight.

[4] SGR (% /day): specific growth rate=100×(Ln final weight - Ln initial weight)/total number of experimental days.

[5] FCR: feed conversion ratio=dry feed intake/wet weight gain.

[6] Survival (%)=100×final number of fish/initial number of shrimp.

In Experiment 1, survival ranged between 94.17%-99.17%. Survival rate of shrimp was not significantly affected by the different diets ($P>0.05$). Weight gain (WG) increased as dietary methionine levels increased up to 0.97% (D4), and then declined ($P<0.05$). Shrimp fed diets with methionine levels from 0.8% to 1.07% (D2–D5) showed comparable SGR and were significantly higher than those fed other diets (D1, D6-D7) ($P < 0.05$). The lowest WG and SGR were observed in group D7, when dietary methionine level was 1.23% ($P<0.05$). The secondary curve equation between weight gain and dietary methionine level was $Y = -1287.9 x^2 +2351.7 x-304.13$ ($R^2 = 0.8424$) (Fig. 1). The optimum dietary methionine level was 0.91% for maximum weight gain, corresponding to 2.28% in dietary protein. The total sulfur amino acids (Met+Cys) were estimated to be 1.39% in diet. The feed conversion ratio (FCR) decreased with increasing dietary methionine up to 1.07%, and thereafter increased (*P*>0.05).

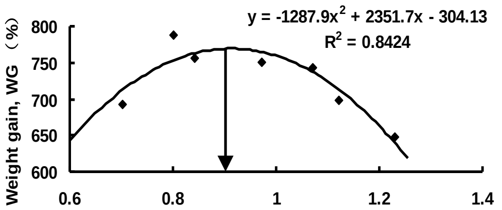

Fig. 1 Relationship between dietary methionine level and weight gain of *L. vannamei* in Experiment 1.

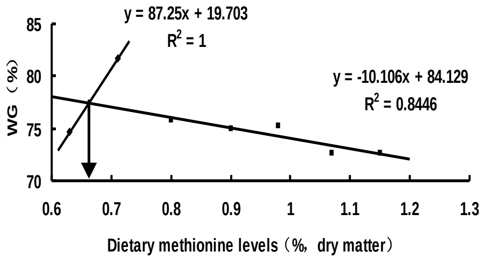

Fig. 2 Relationship between dietary methionine level and weight gain of *L. vannamei* in Experiment 3.

In Experiment 2, survival ranged from 95.56% to 98.89%. Shrimp fed diets with 0.86%, 0.95%, and 1.12% methionine levels (D3, D4 and D6) showed significantly higher survival values than D7 shrimp fed diet with 1.22% methionine (*P* < 0.05), and no significant differences were found in survival of D1-D6 shrimp fed diets with methionine levels from 0.67% to 1.12% (*P*>0.05). WG increased with increased dietary methionine up to 1.01% (D5), and thereafter declined (*P*>0.05). The same tendency was found for SGR. Compared to shrimp fed the diet with 0.67% methionine level, FCR in diets containing 0.77%, 0.86%, and 0.95% methionine levels groups had a tendency to decrease, however in the other groups supplemented with 1.12% and 1.22% methionine FCR tended to increase (*P*>0.05). No significant differences were found in WG, SGR, and FCR of shrimp fed diets with methionine from 0.67%-1.22 (D1-D7), respectively (*P* > 0.05).

In Experiment 3, survival ranged from 94.67% to 100.00%. Survival rate of shrimp fed 1.07% methionine (D6) was significantly higher rate than D2 shrimp fed a 0.71% methionine (*P*<0.05), and no significant differences were found in survival of shrimp in other dietary treatments (*P*>0.05). Weight gain in shrimp fed D2 diets with 0.71% methionine was significantly higher (*P*<0.05) than that of shrimp fed diets with methionine from 1.07% to 1.15% (D6-D7), and no significant difference was found between the other groups (*P* > 0.05). Shrimp fed diets with 0.71% methionine (D2) had significantly higher (*P*<0.05) SGR than those fed diets with methionine that ranged from 1.07% to 1.15% (D6-D7). There were no significant differences in SGR between the other groups. The broken-line regression model between weight gain and dietary methionine level was used for estimating the requirement of dietary methionine. The optimal methionine level for maximal growth of shrimp was 0.66%, corresponding to 1.94% of dietary protein (Fig. 2). The total sulfur amino acids (Met+Cys) were estimated to be 1.10% of diet. No significant difference was found in FCR of shrimp fed diets with methionine levels from 0.63% to 1.15% (D1-D7) (*P*>0.05).

Whole body composition. The proximate composition of whole body of *L. vannamei* (three sizes: small, medium and large) fed the diets containing grade methionine levels are shown in Table 6.

Table 6 Effects of dietary methionine level on whole body composition of *Litopenaeus vannamei* of different sizes

Treatment		*Moisture (%)*	*Crude protein(%)*	*Crude lipid (%)*	*Ash (%)*
Experiment 1	D1	77.67±0.2	71.38±0.43	5.08±0.21^{cd}	13.16±0.27
	D2	76.50±0.1	71.25±0.57	4.72±0.51^{bc}	13.21±0.22
	D3	76.16±0.3	71.56±0.27	4.23±0.24^{abc}	13.30±0.07
	D4	76.29±0.1	71.17±0.91	5.83±0.34^{d}	13.16±0.33
	D5	76.08±0.4	70.86±0.15	5.96±0.44^{d}	13.71±0.43
	D6	76.13±0.3	71.67±0.58	4.01±0.12^{ab}	13.17±0.24
	D7	76.57±0.1	71.74±0.49	3.23±0.18^{a}	13.26±0.28
Experiment 2	D1	75.14±0.3	73.39±0.13^{a}	4.55±0.56^{a}	12.55±0.19
	D2	75.38±0.3	74.00±0.39^{ab}	4.69±0.30^{ab}	12.26±0.60
	D3	74.69±0.4	74.32±0.28^{ab}	6.16±0.30^{c}	12.14±0.17
	D4	75.37±0.2	74.56±0.54^{ab}	5.68±0.56^{bc}	12.05±0.08
	D5	76.08±0.5	75.05±0.44^{b}	5.99±0.21^{c}	11.92±0.37
	D6	75.24±0.4	74.82±0.34^{b}	5.64±0.35^{bc}	11.79±0.34
	D7	76.18±0.5	75.27±0.52^{b}	7.42±0.33^{d}	11.64±0.66
Experiment 3	D1	72.73±0.9	77.23±0.46^{c}	6.23±0.18^{b}	10.67±0.64
	D2	74.36±0.3	75.99±0.67^{abc}	5.73±0.14^{ab}	12.03±0.40
	D3	71.74±1.8	76.74±0.26^{bc}	5.69±0.14^{ab}	10.59±1.12
	D4	74.32±0.2	74.68±0.54^{a}	5.15±0.44^{a}	12.11±0.56
	D5	73.92±0.2	75.24±0.58^{ab}	5.14±0.06^{a}	11.50±0.25
	D6	74.00±0.8	74.39±0.63^{a}	5.51±0.23^{ab}	11.73±0.39
	D7	73.36±0.5	74.98±0.71^{a}	5.27±0.20^{a}	11.49±0.47

Values are means ± SEM of three replicates and values with different letters (a, b, c, d) in the same column are significantly different (P < 0.05) in each experiment.
Crude protein, crude lipid and ash tested as dry matter.

In Experiment 1, whole body lipid content of shrimp fed diets D4 and D5 were significantly higher (P<0.05) than those of shrimp fed diets D2, D3, D6 and D7. Whole body protein content increased with increasing methionine levels, though there were no statistical differences between all the treatments. No significant differences were observed in whole body moisture and ash contents among all diet treatments (P > 0.05).

In Experiment 2, whole body protein content of shrimp fed diets D5, D6 and D7 was significantly higher (P<0.05) than in shrimp fed D1 diet. Whole body lipid content of shrimp fed D3, D4, D5, D6 and D7 diets was significantly higher (P<0.05) than in shrimp fed D1 and D2 diets. Shrimp fed with 1.22% methionine (diet D7) showed comparable whole body lipid and was significantly higher (P<0.05) than in shrimp fed other diets (D1-D6). No significant differences were observed in whole body moisture content among all diet treatments (P>0.05). Whole body ash content decreased with increasing methionine levels, but there were no statistical differences between all treatments.

In Experiment 3, whole body protein content of shrimp fed D1 diet was significantly higher (P<0.05) than in shrimp fed D4, D5, D6 and D7 diets. Whole body lipid content of shrimp fed D1 diet was significantly higher (P<0.05) than in shrimp fed D4, D5 and D7 diets. No significant differences were observed in whole body moisture and ash contents among all diet treatments (P>0.05).

Discussion

In the present study, optimal growth performance and feed utilization were observed in small shrimp (wt:0.5g) fed the 0.97% methionine diet, corresponding to 2.43% of dietary protein. Quadratic regression analysis of weight gain against dietary methionine levels indicated that the optimal dietary methionine level for maximum weight gain was 0.91% of dry diet, corresponding to 2.28% of dietary protein. In medium shrimp (4.18g), the optimal dietary methionine level for maximum weight gain was 0.67% of dry diet, corresponding to 1.76% of dietary protein. For the large shrimp (9.77g), a broken-line regression analysis between WG and dietary methionine levels indicated that the optimum dietary methionine level for maximum growth was estimated to be 0.66% of dry diet, corresponding to 1.94% of dietary protein. The results in this study correspond to other reports, (2.16% of dietary protein for *L. vannamei* ,Zeng, 2012, and 2.4% of dietary protein for black tiger shrimp, *P. monodon*, Millamena *et al*., 1996). However, the current results were lower than the reported 2.52% of dietary protein for *L. vannamei* (Huai, 2009). The dietary methionine requirements for shrimp species ranged from 2-3% of dietary protein (NRC, 2011). This wide variability between different aquaculture species may be due to differences in size, age, experimental conditions, such as water temperature, water salinity, feeding regime, feed allowance, stock density, flow rate, and the use of different ingredients for basal diets such as commercial or purified ingredients.

The growth performance of all three sizes of Pacific white shrimp improved with methionine supplementation in diets, confirming the importance of methionine to this species. WG and SGR increased and thereafter declined with increasing dietary methionine. Similar results were found in other studies (Millamena *et al*., 1996; Zhu *et al*., 2006; Yan *et al*., 2007; Zeng, 2012). The highest WG for small and large shrimp was observed when dietary methionine levels were 0.97% and 0.71%, respectively. With further increases in dietary methionine levels, WG and SGR significantly decrease. The decreased growth rate in shrimp fed diets with higher methionine contents may be due to a toxic effect (Choo *et al*., 1991) and stress caused by excess methionine in the body which may lead to extra energy expenditure, deamination, and nitrogen excretion (Walton, 1985; Chen *et al*., 1994). For the medium sized shrimp, no significant differences were found in WG, SGR, and FCR of shrimp fed diets with methionine levels from 0.67-1.22%, which may indicate that 0.67% methionine level could meet the requirements of this sized shrimp. In general, SGR of shrimp was highest in small *L. vannamei*, followed by medium *L. vannamei,* and large *L. vannamei* in decreasing order. FCR and SGR showed the opposite trend however the difference was not statistically significant among all dietary treatments. This tendency in *L. vannamei* may be due to differences in size, age, and the use of different protein contents for basal diets (Zhu *et al*., 2006).

In conclusion, results of this experiment indicated that methionine is essential for the three sizes of *L. Vannamei* tested, and this species is able to efficiently utilize crystal methionine. For small shrimp, the optimal dietary methionine requirement estimated by quadratic regression analysis of weight gain against dietary methionine level was 0.91% of dry diet (2.28% of dietary protein). For the medium and large shrimp groups, the optimal dietary methionine levels for maximum weight gain ranges between 0.66 to 0.67% of dry diet (1.76% of dietary protein).

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