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Determination of Methionine Requirement of Juvenile Marine Black Giant Tiger Shrimp (*Penaeus monodon*)

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ABSTRACT: This study investigated the methionine (Met) requirement of marine black giant tiger shrimp (*Penaeus monodon)* using the growth and nutrient utilization indices. DL-Met was supplemented in the basal diet at (0.0, 0.2, 0.4, 0.6, 0.8%) making treatments (T1-T5). Fifteen shrimps (7.87±0.04g) were randomly stocked in each experimental glass tank and fed at 2.5% body weight thrice daily for 42 days. Results indicated that growth performance of the shrimp improved consistently from the group fed diet deficient in Met up to the maximum value of 22.8% in group fed diet supplemented with 6g Met per kg diet and then declined with further increase in the level of supplemental Met, suggesting 6g/kg diet as the optimum requirement for the shrimp. However, broken line regression analyses indicated the Met requirement of the shrimp as 6.80g/kg.

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Shrimps are highly valued seafood that commands billions of Dollars in the international market. According to FAO (2016) shrimp farming is one of the most profitable segments of the aquaculture sector, and it is a big business with yearly growth rate of 6% (FAO, 2017) and accounting for US$23.58 billion Dollars in 2014 (Tacon (2017).

The main shrimps cultivated are the giant tiger shrimp (*Penaeus monodon*); and the White-leg shrimp *(Litopenaeus vannamei*) (Chakravarty *et al*. 2015) which accounted for US$18.46 billion Dollars in 2014 (Tacon, 2017). Amino acids (AA) play important and versatile roles in fish nutrition and metabolism in terms of signaling and stimulation of appetite (NRC, 2011).

Li *et al* (2008) stated that dietary supplementation of AA or derivatives, or a combination of the two may provide new strategies to develop AA-balanced feeds that can offset environmental impacts on aquaculture animals, improve growth performance, and profitability of the aquaculture industry. However, excess AA in diets causes feed toxicity, reduced growth performance, water quality problems and increased costs of production. Therefore this study investigated the methionine requirement of the giant black marine tiger shrimp *P. monodon*, which is one of the most widely cultivated shrimps across the globe.

MATERIALS AND METHODS

Feed preparation: Ingredients (Table 1) were thoroughly mixed and pelletized using California industrial Laboratory Pelleting Mill (CPM) No. NH 396165. Pelleting was done at 75°C. After that the diets were steamed at 90°C for 5 minutes, sieved to remove clogged ones and then oven dried at 90°C for 1 hour. The dried diets were kept at 4°C until used.

Animal feeding and measurements: The shrimp were purchased from a private shrimp farm at Lam Luka outside Bangkok, acclimatized for 2 weeks, before stocking into 140 l glass tanks. They were randomly grouped into 15 sets each per tank. Each treatment was replicated thrice making a total of 15 tanks for the treatments. Feeding was done at 2.5% body weight daily which was administered in three equal installments between 0800 and 0900, 1300 and 14000 and 1700-1800 h. The experiment lasted for 6 weeks. At the end of the experiment, fish were counted and weighed. The growth parameters and feed utilization indices were calculated as follows: Weight gain = Final wt. – initial wt. Specific growth rate (SGR) = 100 (Ln W_2 – Ln W_1)/T; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period; Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g); Protein efficiency ratio (PER) = Weight gain

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(g)/Protein intake (g). Feed efficiency (FE) = 1/FCR * 100.
Water quality parameters were measured weekly. Temperature and dissolved oxygen were measured with combined digital Oxygen-Temperature Meter (YSI No. Rs232, USA). pH was measured by a pH meter. Salinity was measured with Hand–held Refractometer (ATAGO) S/Mill-E-Japan while the nitrite, ammonia and alkalinity were measured with AQUA VBC Kits (Chulalongkon University Thailand) based on colour separation.

Data Analyses: The proximate analyses of the feed samples were carried out following the methods of AOAC (1995). The methionine requirement was estimated based on broken line analysis methods (Robbins et al. 2006) by regressing the mean weight gain values against the methionine levels in the diets and by regressing the feed efficiency values against the dietary methionine levels using the 4th polynomial regression analysis of the Microsoft Excel. Data were subjected to one way analysis of variance (ANOVA) using SAS/SAT Institute Software (1998). Duncan multiple range test was used to separate means among treatments at (P=0.05) (Duncan, 1955).

RESULTS AND DISCUSSIONS

The result of growth performance of the shrimp is presented in Table 3 which suggests the methionine requirement of the shrimp to be 6g/kg diet. Growth was maximized at this point and showed a decline with further increase in DL-methionine supplementation. However, the broken line regression analyses based on mean weigh gain (Fig. 1) indicated methionine requirement of the shrimp as 6.80g/kg. Similarly, 4th polynomial regression analysis using feed efficiency (Fig. 2) indicated the Methionine requirement as 6.2g/kg. This also shows that different parameters may have different optimum nutrient requirement for maximum growth performance. Therefore the methionine requirement of *P monodon* may safely be reported to range between 6.2 and 6.8 g/kg. Nwanna *et al* (2010) reported different optimal levels of phosphorus as supporting the growth and mineralization in common carp. The difference between the values obtained from the present study and 8.6g/kg methionine recommended for commercial shrimp feeds (Velasco *et al* 2000) may be ascribed to the bigger size of shrimp used in the present study. This is supported by the assertion of Davies (2005) that, in general, the smaller the animal, the faster the percentage of weight gain, resulting in a daily requirement higher than that of a larger shrimp growing at a slower rate. The weight gain and specific growth rate of the fish fed on 6g/kg methionine diet were significantly better than that of the fish fed diets 1-3, but the same with that of the fish fed diet 5. This findings support the assertion of Sarder *et al* (2009) that increasing dietary Met+Cys in the right quantity significantly improved weight gain and SGR in Rohu (*Labeo rohita*) fingerlings. Similarly, the feed conversion and protein efficiency ratios of the fish fed on 6g/kg methionine diet were significantly better than those of the fish fed methionine deficient diet (diet 1) Fish in all the different treatments were characterised with high feed conversion ratio/low feed efficiency and low protein efficiency ratios. Co´rdova-Murueta (2002) and Türkmen (2007) also reported high FCR of 3.2 and 3.9 in *P. semisulcatus* and 4.3 in *Marsupenaeus japonicas* fed formulated diets. Similarly, Türkmen (2007) reported FCR value of 4.3 in the culture of *M. japonicas*.

Table 1. Gross composition of experimental diets (g/100g) (35% CP)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Shrimp head meal (42% CP)	2.00	2.00	2.00	2.00	2.00
Gelatin (95% CP)	10.0	10.0	10.0	10.0	10.0
Isolated soy bean meal (86.9% CP)	20.0	20.0	20.0	20.0	20.0
Wheat gluten meal (65% CP)	4.00	4.00	4.00	4.00	4.00
Peas (40% CP)	11.8	11.8	11.8	11.8	11.8
Wheat starch	41.7	41.7	41.7	41.7	41.7
Fish oil	6.00	6.00	6.00	6.00	6.00
Vitamin-mineral premix	4.50	4.50	4.50	4.50	4.50
DL-Methionine	0.00	0.20	0.40	0.60	0.80

Kg^{-1} Vit A 10,000000IU, Vit. D3 1000000IU, Vit. E 10,000 mg, Vit. K3 1000 mg
Vit. B1 500mg, Vit. B2 5000mg, Vit. B6 1500mg, Vit. C 10,000mg
Folic acid 1000mg,
Kg^{-1} Fe 2010mg, Cu 3621mg, Zn 6424mg, Mn 10062 mg, Co 105mg
I 1000mg, Selenium 60mg

Table. 2 Proximate composition of experimental diets

	D1	D2	D3	D4	D5
Protein	34.8	35.4	35.1	35.9	35.1
Lipid	9.50	8.50	9.00	8.50	8.50
Ash	15.3	16.5	16.5	16.2	16.0
Moisture	9.70	8.10	7.60	7.60	8.00

The FCR obtained from the present study is also higher than the values from carp fed diets supplemented with DL-Methionine (Nwanna *et al* 2012).

Table 3. Growth and nutrient utilization of Tiger shrimp fed experimental diets

	D1	D2	D3	D4	D5
Initial Wt.	7.85 ±0.02	7.89 ±0.04	7.84 ±0.06	7.87 ±0.00	7.88 ±0.07
Final Wt.	12.4[b] ±0.71	12.5[b] ±0.32	12.6[b] ±0.75	13.8[a] ±0.25	12.9[ab] ±0.03
Mean Wt. gain	4.58[b] ±0.72	4.61[b] ±0.30	4.65[b] ±0.72	5.93[a] ±0.24	5.05[ab] ±0.08
SGR	1.09[b] ±0.16	1.11[b] ±0.06	1.13[b] ±0.14	1.34[a] ±0.05	1.17[ab] ±0.03
FCR	3.51[a] ±1.81	3.47[ab] ±0.40	3.21[ab] ±0.78	3.11[b] ±0.30	3.18[ab] ±0.10
Protein efficiency ratio	5.60[b] ±0.20	5.80[b] ±0.22	6.50[ab] ±0.14	7.50[a] ±0.40	6.50[ab] ±0.12

Table 4. Water Quality Parameters

	Temperature (oC)	Oxygen (mg/l)	pH	Salinity (mg/l)	Alkalinity (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)
Treatment 1	27.9 ±0.40	10.5 ±0.32	6.92 ±0.08	9.57 ±0.23	103.3 ±5.77	0.12 ±0.00	0.28 ±0.20
Treatment 2	27.7 ±0.46	11.3 ±1.12	6.93 ±0.06	9.93 ±0.32	103.3 ±5.77	0.12 ±0.00	0.25 ±0.00
Treatment 3	27.4 ±0.45	11.5 ±0.82	6.99 ±0.05	9.97 ±0.41	103.3 ±5.77	0.12 ±0.00	0.27 ±0.22
Treatment 4	27.8 ±0.26	10.3 ±0.23	6.95 ±0.04	9.37 ±0.59	104 ±5.29	0.12 ±0.00	0.28 ±0.20
Treatment 5	27.8 ±0.42	10.6 ±0.71	6.96 ±0.06	9.97 ±0.21	105 ±5.00	0.12 ±0.00	0.25 ±0.00

This suggests lower feed digestibility, conversion and assimilation by the shrimp. At week 4, the weight gain of the shrimp fed diet with 6g/kg was already significantly better than that of the group fed diet deficient in methionine. The water quality parameters measured showed that temperature, pH, and alkalinity were in the same range used in culturing warm water finfishes (Boyd and Tucker 2014), while other values clearly indicate that culturing of this shrimp is very sensitive to salinity, ammonia, nitrite, and dissolved oxygen concentration. Apart from needing high level of oxygen, the supply must be constant for 24 hours of the day.

Unlike the tropical finfish that can thrive well in oxygen level of 5-6 mg/l, the shrimp needs dissolved oxygen level of about 10 mg/l, for good production. However, the different concentrations of supplemental DL-Methionine did not affect the water quality parameters.

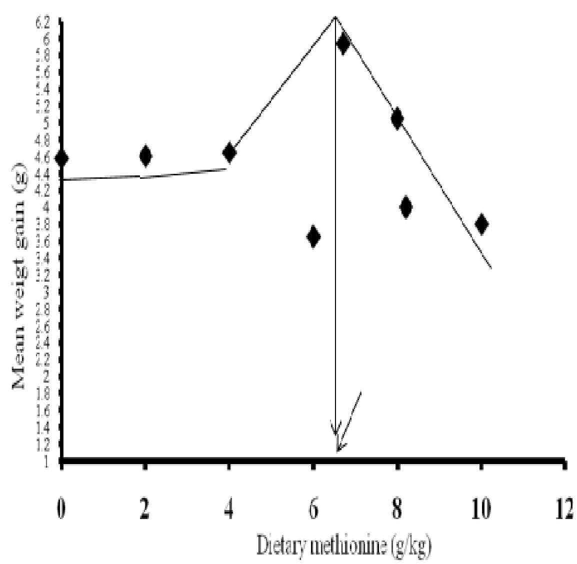

Fig 1. Total methionine requirement for growth of P. monodon (quadratic – linear method)

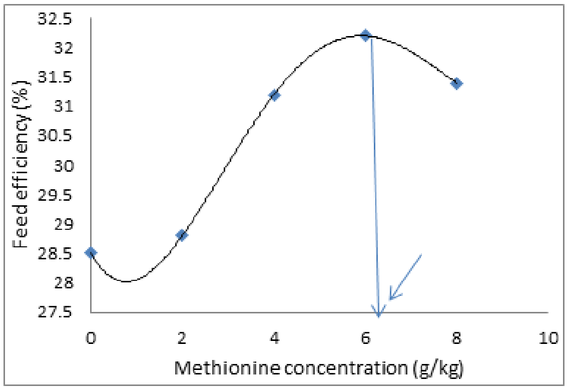

Fig. 2. Methionine requirement of *P monodon* based on 4th degree polynomial regression analysis

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