Aquaculture Nutrition

Aquaculture Nutrition 2013 **19**; 39–46 doi: 10.1111/anu.12089

Effects of dietary crystalline methionine or oligo-methionine on growth performance and feed utilization of white shrimp (*Litopenaeus vannamei*) fed plant protein-enriched diets

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Abstract

An eight-week feeding experiment was conducted to investigate the effects of dietary crystalline methionine (CMet) or oligo-methionine (OMet) on growth performance and feed utilization of white shrimp, *Litopenaeus vannamei*. A practical diet was used as control diet. The other four diets replacing 30% and 60% fish meal by plant meal were formulated. To balance the methionine content, 1 g kg^{-1} CMet (SPP30-CMet) or OMet (SPP30-OMet) was added in 30% fish meal replacing diets, and 2 g kg^{-1} CMet (SPP60-CMet) or OMet (SPP60-OMet) was added in 60% fish meal replacing diets. Results showed that methionine source significantly affected growth, body compositions and hepatosomatic indices (HSI) of white shrimp ($P < 0.05$). Shrimps in SPP60-CMet treatment showed significantly lower weight gain, body crude protein content and higher HSI than those in the control ($P < 0.05$). However, no significant difference in these indices was observed between the control and OMet supplemented treatments ($P > 0.05$). Shrimps in SPP30-OMet treatment showed significantly higher feed efficiency ratio and protein efficiency ratio than those in SPP30-CMet treatment ($P < 0.05$). This study indicated that compared with the CMet, dietary OMet resulted in better growth and feed efficiency of *L. vannamei* fed with plant protein-enriched diets.

KEY WORDS: feed utilization, growth, methionine, plant protein, shrimp

Received 12 August 2012; accepted 15 April 2013

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Introduction

At present, despite the growth of aquaculture, the global production of fish meal has remained fairly static. Consumption of fish meal is increasingly being concentrated in Asia with China continuing as by far the single largest market (IFFO, 2011). Commercial shrimp feed contains about 25% fish meal (Tacon & Barg 1998). The global use of fish meal for commercial shrimp feed was more than 800 thousand tons in recent years. Due to the limited production and increasing price of fish meal, the amount of fish meal can be used in shrimp feed has to be decreased (Tacon & Metian 2008). Alternative dietary protein sources to replace fish meal in shrimp feed are important for the future of the shrimp farming industry. Plant protein sources hold promise because of their secure supply and lower price. However, they are less suitable for aquatic animal feed than fish meal because of the presence of antinutrients and suboptimal essential amino acid profile (Tacon 1994).

Addition of crystalline amino acids (CAAs) to formulated aquatic feed is used to balance the amino acid composition and is likely to become an increasingly common practice in aquatic industry (Yuan *et al.* 2011). Several studies have shown that CAAs were well utilized in meeting amino acid requirements of fish (Espe & Lied 1994; Rodehutscord *et al.*1995; Williams *et al.* 2001; Rollin *et al.* 2003; Espe *et al.* 2006). However, there are still some obstacles to be overcome when using CAAs in shrimp feed. CAAs represent high potential for leaching from feed pellets and may have different adsorption rate in the host compared with intact protein. Consequently, it could reduce the efficiency of protein synthesis (Yuan *et al.* 2011). Several studies in different fish and shrimp species have indicated that CAAs appear to be utilized with a lower efficiency than amino acid supplied by intact protein (Espe & Njaa 1991; Schuhmacher & Gropp 1997; Zarate &

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Lovell 1997; Refstie *et al*. 2001; Liu *et al*. 2002; Dabrowski *et al*. 2003, 2010; Peres & Oliva-Teles 2005; Hauler *et al*. 2007). Therefore, it is necessary to find substitute with low leaching loss and high utilization efficiency to replace CAAs.

The enzymatic oligomerization of α-amino acid esters has been studied by many researchers for a wide array of proteases and amino acid ester over the years (Schwab *et al*. 2012). Jost *et al*. (1980) synthesized water-insoluble oligo-methionine from methionine ethyl ester using papain as a catalyser. High availability of this synthesized oligo-methionine (OMet) has been reported in rats (Chiji *et al*. 1990; Hara & Kiriyama 1991; Kasai *et al*. 1996). The absorbability and bioavailability of oligo-methionine have been also studied in fish (Masumoto *et al*. 1999). However, no data on using this enzymatic synthesized methionine in shrimp feed have been published. This synthesized OMet has the potential to be used as substitute for crystalline methionine (CMet) in shrimp feed because of its form as the combined amino acid, and the water-insoluble character, which may reduce leaching loss in water. Thus, the objective of this study was to compare the effects of two different dietary methionine sources (CMet and OMet) on growth performance and feed utilization of white shrimp (*Litopenaeus vannamei*) fed with plant protein-enriched diets.

Materials and methods

Diet preparation

The oligo-methionine was prepared by ourselves in laboratory scale from methionine ethyl ester (Sigma-Aldrich, St. Louis, MO, USA) using papain (A3824, Applichem) as a catalyser according to the method of Jost *et al*. (1980). The product was a mixture of peptides from dipeptide to octapeptide confirmed by mass spectrometry analysis. The average peptide chain length of the oligo-methionine is 5.02 calculated based on the method of Schwab *et al*. (2012).

A practical diet was formulated to contain 250 g kg^{-1} fish meal, which used as control diet (Table 1). Based on the control diet, the other four isonitrogenous and isolipid diets were formulated. Thirty per cent or sixty per cent fish meal in the control diet was replaced by a mixture of soybean meal protein and peanut meal protein (SPP). In 30% fish meal replacing diets, 1 g kg^{-1} crystalline methionine (L-methionine, MET, A1340, Applichem) (named as SPP30-CMet) or 1 g kg^{-1} oligo-methionine (named as SPP30-OMet) was added. In 60% fish meal replacing diets, 2 g kg^{-1} crystalline methionine (named as SPP60-CMet) or 2 g kg^{-1} oligo-methionine (named as SPP60-OMet) was added. The five experimental diets had the same concentration of methionine. Dietary cysteine concentration was also adjusted to the similar level with that of control diet. Dietary lysine was adjusted to meet the requirement of white shrimp. Even in the SPP60-CMet and SPP60-OMet diets, the lysine concentration achieved to 18 g kg^{-1} in total diet which is still higher than lysine requirement (16 g kg^{-1}) of *L. vannamei* reported by Fox *et al*. (1995).

Ingredients were ground into fine powder through a 246-μm mesh. Then, all the ingredients were thoroughly mixed with the fish oil, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26; South China University of Technology, Guangdong, China) and dried for about 12 h in a ventilated oven at 45 °C and then kept in freezer at −20 °C.

Leaching test of the diets

Leaching loss of methionine from diets of SPP30-CMet, SPP30-OMet, SPP60-CMet and SPP60-OMet was measured after immersion into seawater (salinity 30 g L^{-1}) for 30 min according to Smith *et al*. (2007) with some modifications. Briefly, about 1 g diet was put into the 500-mL beaker containing 400 mL of sea water and mechanically stirred at 25 °C. Then, diets were removed from the water and rinsed gently and briefly in distilled water, then dried at 40 °C and analysed for methionine content.

Experimental design and feeding trial

White shrimps *L. vannamei* were obtained from a commercial hatchery and acclimated to the system for 2 weeks before trials. During this period, the shrimps were fed the control diet.

Shrimps (initial body weight: 0.62 ± 0.01 g) were randomly distributed into 5 treatments, each of which has 3 replicates. Each replicate has 30 shrimps in a 300-l tank (filled with 200 L seawater). The shrimps were fed with experimental diets to apparent satiation four times daily (06 : 00, 12 : 00, 18 : 00 and 24 : 00) for 8 weeks. Seawater in each tank was changed for 2/3 every day. Photoperiod was provided using a 12-h light: 12-h dark regime. The uneaten feed was collected 60 min after each meal, dried to constant weight at 60 °C and reweighed to calculate feed consumption. During the experimental period, water temperature was 28–30 °C, pH 7.6–8.3 and salinity 30–32 g L^{-1}.

Table 1 Ingredient and chemical composition of experimental diets

	Experimental diets				
Ingredients (g kg^{-1})	Control	SPP30-CMet	SPP30-OMet	SPP60-CMet	SPP60-OMet
Fish meal[1]	250	175	175	100	100
Shrimp shell meal[2]	50	50	50	50	50
Wheat meal[3]	348.8	309.8	309.8	268.8	268.8
Squid visceral meal[2]	60	60	60	60	60
Soybean meal[2]	120	200	200	280	280
Peanut meal[2]	100	130	130	160	160
Fish oil[2]	22	25	25	30	30
Soybean lecithin[2]	11	11	11	11	11
Choline chloride	3	3	3	3	3
Ethoxy quinoline[4]	0.5	0.5	0.5	0.5	0.5
Antimycin [5]	1	1	1	1	1
$Ca(H_2PO_4)$ $2H_2O$	3.7	3.7	3.7	3.7	3.7
Vitamin premix [6]	10	10	10	10	10
Mineral premix [7]	20	20	20	20	20
Crystalline methionine [8]	0	1	0	2	0
Oligo-methionine	0	0	1	0	2
Proximate composition (g kg^{-1})					
Dry matter	931	936	933	938	936
Methionine	8.1	8.0	8.1	8.1	8.0
Cysteine	5.5	5.6	5.6	5.6	5.6
Methionine + Cysteine	13.6	13.6	13.7	13.7	13.6
Crude protein	386	382	389	385	382
Crude lipid	78.8	79.5	74.9	78.9	76.2
Ash	101	105	105	103	107

[1] Fish meal: steam-dried fish meal, (COPENCA Group, Lima, Peru).
[2] Purchased from Qingdao Great Seven Bio-tech, Co. Ltd., Qingdao, China.
[3] Purchased from Qingdao White Cherryflower Industry and Commerce Co. Ltd, Shandong, China.
[4] Ethoxy quinoline: Kindly provided by Qingdao Master Biotechnology Co. Ltd, Qingdao, China.
[5] Antimycin: Contained 50% calcium propionic acid and 50% fumaric acid. Kindly provided by Qingdao Master Biotechnology Co. Ltd, Qingdao, China.
[6] Vitamin premix: Vitamin premix (mg or IU kg^{-1} diet): thiamin 25, riboflavin (80%) 45, pyridoxine hydrochloride 20, vitamin B_{12} (1%) 10, niacin 200, Ca-pantotenate 60, inositol 800, biotine (2%) 60, folic acid 20, vitamin K_3 10, retinyl acetate 16,000, vitamin D_3 2500, DL-α-tocopherol acetate (50%) 240, L-ascorbyl-2-monophosphate-Na (35%) 2000, microcrystalline cellulose 2473.
[7] Mineral premix: $MgSO_4$ $7H_2O$ 1200, $FeSO_4$ H_2O 80, $CuSO_4$ $5H_2O$ 10, $ZnSO_4$ H_2O 50, $MnSO_4$ H_2O 45, $CoCl_2$ (1%) 50, Na_2SeO_3 (1%) 20, $CaIO_3$ (1%) 60, Zeolite 485.
[8] Crystalline methionine: $_L$-methionine, MET, A1340, Applichem.

Sampling

At the end of the feeding trial, all the shrimps were counted and weighed. Sixteen shrimps were randomly collected from each tank. Eight of them were measured for the body length (from postorbital edge to tip of telson) and dissected to get hepatopancreas. Hepatopancreas was carefully removed and weighed. The other eight shrimps were frozen at −20 °C for the analysis of body compositions.

Chemical analysis

Standard method AOAC (1995) was used for analysing ingredients, experimental diets and shrimp body compositions. Moisture and ash content were determined gravimetrically to constant weight in an oven at 105 °C and 550 °C, respectively. Crude lipid was determined gravimetrically after extraction with ethyl ether (Extraction System B-811, BUCHI, Switzerland). Crude protein was determined by Kjeldahl method with a FOSS Kjeltec System (2300, Sweden) using boric acid to trap released ammonia. Amino acids compositions of feed ingredients (Table 2), experimental diets (Table 3) and methionine content in diets before and after immersion were determined by amino acid analyser (Biochrom 30; GE Health care Co. Ltd, Cambridge, UK).

Calculations and statistical methods

Growth performances and feed utilization were expressed as the following formulae:

Table 2 Essential amino acids composition of feed ingredients (g kg^{-1} dry matter)

Amino acids	Fish meal	Soybean meal	Peanut meal	Squid visceral meal	Wheat meal
Threonine	29.3	18.3	13.4	10.1	2.8
Valine	21.7	23.9	20.2	22.0	4.5
Methionine	21.6	7.8	7.2	5.7	2.3
Isoleucine	24.3	20.7	15.5	11.2	3.4
Leucine	51.9	38.3	32.6	26.8	10.2
Phenylalanine	29.5	24.9	26.1	20.0	7.0
Lysine	49.1	28.9	16.7	13.1	2.5
Histidine	13.5	12.2	11.6	8.1	2.7
Arginine	43.5	32.9	56.1	39.4	4.3

Tryptophan was not analysed.

Table 3 Amino acids composition of the experimental diets (g kg^{-1} dry matter)

	Experimental diets				
Amino acids (g kg^{-1})	Control	SPP30-CMet	SPP30-OMet	SPP60-CMet	SPP60-OMet
Aspartic acid	34.2	35.4	35.2	36.6	36.5
Threonine	12.8	12.4	12.6	11.9	11.9
Serine	16.9	16.8	16.3	16.6	16.4
Glutamic acid	72.0	72.8	72.4	73.5	73.6
Glycine	18.0	17.5	17.7	17.0	17.2
Cysteine	5.5	5.6	5.6	5.6	5.6
Alanine	14.1	13.5	13.4	12.8	12.9
Valine	13.8	14.5	14.6	15.2	15.3
Methionine	8.1	8.0	8.1	8.1	8.0
Isoleucine	12.4	12.6	12.8	12.7	12.8
Leucine	26.8	26.5	26.4	26.3	26.2
Tyrosine	10.9	10.9	10.7	10.8	10.6
Phenylalanine	17.8	18.1	18.4	18.4	18.2
Histidine	7.7	7.9	8.1	8.1	8.0
Lysine	19.6	18.6	18.4	17.7	17.8
Arginine	24.9	25.8	25.5	26.6	26.4

Tryptophan was not analysed.

Weight gain rate, WG (%) = 100 × [(final body weight−initial body weight)/initial body weight].

Feed intake, FI (%/d) = 100 × total amount of the feed consumed (g)/[(initial body weight + final body weight)/2]/days.

Feed efficiency ratio, FER = weight gained (g)/total amount of the feed consumed (g).

Protein efficiency ratio, PER = weight gained (g)/protein consumed (g).

Survival rate (SR) (%) = 100 × (final fish number/initial fish number).

Condition factor, CF = 100 × fish weight/$(\text{body length})^3$.

Hepatosomatic index, HSI (%) = 100 × (hepatopancreas weight/body weight).

Leaching rate of methionine (%) = 100 × (methionine content in diet (dry matter) before immersion–methionine content in diet (dry matter) after immersion)/methionine content in diet (dry matter) before immersion.

All statistical analyses were performed using the SPSS 13.0 for Windows. Methionine source and fish meal replacing level were evaluated as class variables in a two-way ANOVA with interaction. The one-way ANOVA was used to compare the control and the other four treatments that were excluded from the two-way ANOVA. The level of significance was set at $P < 0.05$, and Tukey's test was used to compare the mean values between individual treatments.

Table 4 Growth performances and feed utilization of white shrimp fed the experimental diets for 56 days

	FBW (g)	WG (%)	FI (% day^{-1})	FER	PER	Survival (%)
One-way ANOVA model						
Control	8.0 ± 0.1^{ab}	1187 ± 6^{ab}	4.11 ± 0.05	0.74 ± 0.01^{ab}	2.13 ± 0.03^{ab}	100 ± 0
SPP30-CMet	7.8 ± 0.0^{b}	1129 ± 8^{b}	4.34 ± 0.05	0.68 ± 0.01^{b}	1.94 ± 0.03^{b}	96 ± 1
SPP30-OMet	8.1 ± 0.1^{a}	1215 ± 16^{a}	4.08 ± 0.08	0.75 ± 0.02^{a}	2.15 ± 0.04^{a}	100 ± 0
SPP60-CMet	7.3 ± 0.1^{c}	1062 ± 15^{c}	4.29 ± 0.08	0.69 ± 0.02^{ab}	1.99 ± 0.05^{ab}	99 ± 1
SPP60-OMet	7.7 ± 0.0^{b}	1140 ± 7^{b}	4.27 ± 0.06	0.70 ± 0.02^{ab}	2.00 ± 0.05^{ab}	98 ± 2
P values	<0.001	<0.001	0.061	0.026	0.026	0.126
P values in the two-way ANOVA model						
Methionine source	<0.001	<0.001	0.078	0.045	0.045	0.256
Replacement level	<0.001	<0.001	0.345	0.356	0.326	0.694
Interaction	0.683	0.758	0.112	0.068	0.068	0.076

Values represent mean ± SEM (n = 3).
Values in the same column with different superscript letters are significantly different ($P < 0.05$) as determined by Tukey's test.
FBW, final body weight; WG, weight gain; FI, feed intake; FER, feed efficiency ratio; PER, protein efficiency ratio.

Results

Growth performance

As we can see from Table 4, weight gain of shrimps was significantly affected by both methionine source and fish meal replacing level ($P < 0.05$). In the treatments supplemented with CMet, weight gain of shrimps decreased with the increasing of dietary plant protein content and was significantly lower than that in the control when 60% fish meal was replaced ($P < 0.05$). However, no significant difference was observed between control and treatments supplemented with OMet ($P > 0.05$). Weight gain of shrimps fed dietary OMet was significantly higher than those fed dietary CMet, regardless of dietary fish meal replacing level ($P < 0.05$).

Feed efficiency rate (FER) and protein efficiency rate (PER) were significantly affected by methionine source ($P < 0.05$). Shrimps fed the SPP30-OMet diet had significantly higher FER and PER than those fed the SPP30-CMet diet ($P < 0.05$).

No significant difference was observed in FI or survival rate among the treatments ($P > 0.05$).

Body composition

Supplementation of the two different methionine sources in plant protein-enriched diets significantly affected the moisture and crude protein contents ($P < 0.05$), not the crude lipid content ($P > 0.05$) in the whole body of white shrimps (Table 5). The crude protein content in whole body of shrimps fed with dietary CMet decreased with the increasing of plant protein sources content and was significantly lower than that in the control when 60% fish meal was replaced ($P < 0.05$). However, no significant difference was

Table 5 Compositions of the whole body of white shrimp fed the experimental diets for 56 days (g kg^{-1} wet matter basis)

	Whole body			
	Moisture	Protein	Lipid	Ash
One-way ANOVA model				
Control	752 ± 2.2^{b}	177 ± 1.9^{a}	28.9 ± 0.7^{a}	41.6 ± 1.0
SPP30-CMet	771 ± 5.4^{ab}	162 ± 3.5^{ab}	25.1 ± 0.7^{b}	42.7 ± 0.5
SPP30-OMet	756 ± 5.7^{ab}	176 ± 4.6^{a}	26.3 ± 0.2^{ab}	41.8 ± 0.8
SPP60-CMet	775 ± 4.9^{a}	157 ± 4.9^{b}	24.2 ± 0.5^{b}	43.7 ± 1.1
SPP60-OMet	762 ± 2.2^{ab}	166 ± 1.4^{ab}	25.3 ± 0.7^{b}	43.5 ± 0.7
P values	0.020	0.002	0.002	0.344
P values in the two-way ANOVA model				
Methionine source	0.021	0.006	0.073	0.532
Replacement level	0.391	0.119	0.122	0.135
Interaction	0.763	0.155	0.922	0.699

Values represent mean ± SEM (n = 3).
Values in the same column with different superscript letters are significantly different ($P < 0.05$) as determined by Tukey's test.

observed between control and treatments supplemented with OMet ($P > 0.05$). Similar significant differences were also observed in moisture content in whole body of white shrimps among the treatments.

Condition factor and hepatosomatic indices

Supplementation of the two different methionine sources in diets enriched with plant meal significantly affected CF ($P < 0.05$) and very significantly affected HSI of white shrimps ($P < 0.01$) (Table 6). Shrimps fed with dietary CMet had lower CF than those fed with dietary OMet, although no statistic difference was observed. The HSI of shrimps fed with dietary CMet increased with the increasing of plant protein sources content and was significantly higher than that in the control when 60% fish meal was replaced ($P < 0.05$). Shrimps fed with plant protein-enriched diets had similar HSI compared with control by supplying OMet. Shrimps fed with dietary OMet had significantly lower HSI than those fed with dietary CMet, regardless of dietary fish meal replacing level ($P < 0.05$).

Table 6 Effects of experimental diets on condition factor (CF) and hepatosomatic index (HSI) of white shrimp after 56 days feeding

	CF	HSI
One-way ANOVA model		
Control	0.92 ± 0.01	4.43 ± 0.05^{bc}
SPP30-CMet	0.91 ± 0.02	4.61 ± 0.03^{ab}
SPP30-OMet	0.94 ± 0.01	4.33 ± 0.06^{c}
SPP60-CMet	0.92 ± 0.01	4.70 ± 0.08^{a}
SPP60-OMet	0.96 ± 0.01	4.24 ± 0.06^{c}
P values	0.078	<0.001
P values in the two-way ANOVA model		
Methionine source	0.025	<0.001
Replacement level	0.329	0.923
Interaction	0.503	0.152

Values represent mean ± SEM ($n = 3$).
Values in the same column with different superscript letters are significantly different ($P < 0.05$) as determined by Tukey's test.

Table 7 Methionine leaching rate of the test diets after immersion into seawater for 30 min (g kg^{-1} dry matter)

Diets	SPP30-CMet	SPP30-OMet	SPP60-CMet	SPP60-OMet	*P* values
30 min	99 ± 2.9^{b}	19 ± 3.6^{c}	171 ± 2.9^{a}	67 ± 2.0^{d}	<0.001

Values represent mean ± SEM ($n = 3$).
Values in the same row with different superscript letters are significantly different ($P < 0.05$) as determined by Tukey's test.

Leaching rate of methionine

Table 7 shows the leaching rate of methionine in the groups of SPP30-CMet, SPP30-OMet, SPP60-CMet and SPP60-OMet after immersion into seawater for 30 min. The leaching rate of methionine in diets supplemented with OMet was significantly lower than that in diets supplemented with CMet, regardless of dietary fish meal replacing level ($P < 0.05$).

Discussion

The results in the present study showed that dietary plant protein inclusion significantly affected the growth performance of white shrimp. The weight gain of shrimps fed dietary CMet significantly decreased when 60% fish meal was replaced. Lower digestibility, reduction in palatability and feed intake and imbalance of amino acid could be the main reasons for the reduced growth performance of shrimps with the dietary plant protein source substitution of fish meal increasing (Forster *et al.* 2003; Amaya *et al.* 2007; Hernández *et al.* 2008). As no significant difference was observed in feed intake among the treatments, in the present study, feeding rate and palatability could not explain the reduced growth of white shrimps with increasing of dietary soybean meal and peanut meal (SPP) level. Therefore, the effect of dietary plant protein inclusion on shrimp growth performance was probably due to the imbalance of amino acid and lower digestibility in the present study.

In soybean meal and peanut meal, the two most widely used plant protein sources in shrimp feed, methionine is considered as the first limiting amino acid. In the present study, two sources of methionine were used to balance the methionine content in high SPP inclusion diets. Compared with CMet, OMet showed a quite higher efficiency to improve the growth and feed utilization of white shrimps fed with methionine deficient diets. The main reason of the higher efficiency of OMet could be explained by its low leaching loss as supported by the leaching test data (Table 7). Because shrimps have slow feeding behaviour and are benthos feeders, the leaching loss of CAAs is more severe in shrimp feeds. This suggests that a large part of CMet could be lost by leaching before consumption by shrimp. Similar to the result of the present study, it was reported that crystalline arginine supplementation was not effectively utilized by kuruma shrimp (*Marsupenaeus japonicus*) due to the leaching of loss of arginine (Teshima *et al.* 2004). In the present study, PER had similar changing trend with WG among the treatments, which is to be

expected as growth is largely driven by protein deposition. It is generally accepted that CAAs may be absorbed slightly more rapidly and/or earlier in the gastrointestinal tract than protein-bound amino acids. This faster and/or earlier absorption may result in impaired protein synthesis and deposition (Cowey 1995). For the rats fed the low protein diets supplemented OMet or CMet, OMet seemed to alleviate the plasma threonine and serine decreasing for body protein synthesis due to a slower absorption rate (Chiji *et al*. 1990). These suggest that enzymatic oligomerization technique may improve the dietary value of supplemented methionine not only by reducing leaching but also by increasing the utilization of absorbed AAs for protein synthesis. However, whether the absorption rate of oligo-methionine was lower than crystalline methionine still needs to confirm in further studies. The results of the present study indicated that with the OMet supplementation, 60% fish meal could be replaced by a mixture of soybean meal and peanut meal without significantly negative effects on growth and feed utilization.

The whole body compositions of white shrimps were significantly affected by the different sources of methionine after 56 days feeding. Compared with CMet, OMet represents an encouraging potential to increase crude protein content in the whole body of white shrimps. No significant difference in crude protein composition was observed between control and OMet treatments. This suggests that dietary methionine in the SPP-enriched diets could be effectively supplemented up to the requirement level in the form of OMet. The increases of protein content in body composition of shrimps with optimal methionine inclusion were also observed in previous studies (Teshima *et al*. 2002; Chi *et al*. 2011; Yuan *et al*. 2011). In shrimp *Penaeus monodon*, 30% Met deficiency diminished protein accretion and increased deamination (Richard *et al*. 2010), suggesting a change in amino acid metabolism in shrimp receiving an imbalanced dietary methionine supplementation. The body composition results further confirmed that OMet could be utilized with higher efficiency than CMet by white shrimp, which may result in higher protein deposition and finally better growth performance.

Besides being used for protein synthesis, the main utilization of methionine is as the donor of methyl groups. Sulphur metabolism mainly occurs in hepatic tissue (Mato *et al*. 1997). Espe *et al*. (2008) pointed out that a daily methionine intake of 0.05–0.06 g seems to be required in Atlantic salmon growing from 0.5 kg to secure high methylation capacity in hepatic tissue and to avoid increased liver size. In the present study, the HSI of shrimps fed with OMet was significantly lower than those fed with CMet both in the 30% and 60% fish meal replacing level treatments. This result suggests that OMet could ensure a healthy shrimp by not developing increased liver size. Chiji *et al*. (1990) reported that rats fed low protein diets supplemented with OMet had significantly lower fat accumulation in liver than those fed with CMet. Methionine deficiency could cause liver fat accumulation in animals, possibly a result of methionine-induced threonine imbalance (reviewed by Harper *et al*. 1970). In the present study, whether the increased HSI of white shrimps fed with CMet was induced by disorder of sulphur metabolism or by fat accumulation still needs further studies. Analysis should also include evaluation of metabolic product involved in sulphur metabolism and histological examination of hepatopancreas of shrimp fed the experimental diets.

In summary, compared with CMet, dietary OMet resulted in better growth and feed efficiency of *L. vannamei*. The supplementation of OMet was effective in improving the nutritive value of plant protein source, which is deficient in methionine for *L. vannamei*. However, based on the studies on rats, effects of dietary OMet supplementation on growth performance depend on the length of peptide chain of OMet (Kasai *et al*. 1996). To find the most suitable chains of peptide used in shrimp feeds, further study is needed to isolate and separately study different peptides with a same peptide chain length from OMet.

Acknowledgement

This study was financially supported by grant No. 201103034 from the National Public Sector Funding for Special Research Project.

References

Amaya, E.A., Davis, D.A. & Rouse, D.B. (2007) Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. *Aquaculture*, **262**, 393–401.

Chi, S.Y., Tan, B.P., Lin, H.Z., Mai, K.S., Ai, Q.H., Wang, X.J., Zhang, W.B., Xu, W. & Liufu, Z.G. (2011) Effects of supplementation of crystalline or coated methionine on growth performance and feed utilization of the pacific white shrimp, *Litopenaeus vannamei*. *Aquacult. Nutr*., **17**, e1–e9.

Chiji, H., Harayama, K. & Kiriyama, S. (1990) Effects of feeding rats low protein dies containing casein or soy protein isolate supplemented with methionine or oligo-$_L$-methionine. *J. Nutr*., **120**, 166–171.

Cowey, C.B. (1995) Protein and amino acid requirements: a critique of methods. *J. Appl. Ichthyol*., **11**, 199–204.

Dabrowski, K., Lee, K.J. & Rinchard, J. (2003) The smallest vertebrate, teleost fish, can utilize synthetic dipeptide based diets. *J. Nutr*., **133**, 4225–4229.

Dabrowski, K.Y., Zhang, Y.F., Kwasek, K., Hliwa, P. & Ostaszewska, T. (2010) Effects of protein-, peptide- and free amino acid-based diets in fish nutrition. *Aquacult. Res*., **41**, 668–683.

Espe, M. & Lied, E. (1994) Do Atlantic salmon (*Salmo salar*) utilize mixtures of free amino acids to the same extent as intact protein sources for muscle protein synthesis? *Comp. Biochem. Physiol*., **107**, 249–254.

Espe, M. & Njaa, L.R. (1991) Growth and chemical composition of Atlantic salmon (*Salmo salar*) given a fish meal diet or a corresponding free amino acid diet. *Fisk. Dir. Skr. Ser. Ernæring*, **6**, 260–266.

Espe, M., Lemme, A., Petri, A. & El-Mowafi, A. (2006) Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? *Aquaculture*, **255**, 255–262.

Espe, M., HevrØy, E.M., Liaset, B., Lemme, A. & El-Mowafi, A. (2008) Methionine intake affect hepatic sulphur metabolism in Atlantic salmon, *Salmo salar*. *Aquaculture*, **274**, 132–141.

Forster, I., Dominy, W., Obaldo, L. & Tacon, A. (2003) Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931). *Aquaculture*, **219**, 655–670.

Fox, J.M.A., Lawrance, A.L. & Chan, L.E. (1995) Dietary requirements for lysine by juvenile *Penaeus vannamei* using intact and free amino acid sources. *Aquaculture*, **131**, 279–290.

Hara, H. & Kiriyama, S. (1991) Absorptive behavior of oligo-$_{L}$-methionine and dietary proteins in a casein or soybean protein diet: porto-venous differences in amino acid concentrations in unrestrained rats. *J. Nutr*., **121**, 638–645.

Harper, A.E., Benevenga, N.J. & Wholehueter, R.M. (1970) Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev*., **50**, 428–558.

Hauler, R.C., Carter, C.G. & Edwards, S.J. (2007) Feeding regime does not influnce lysine utilization by Atlantic salmon, *Salmo salar* L., parr. *Aquaculture*, **273**, 545–555.

Hernández, C., Olvera-Novoa, M.A., Aguilar-Vejar, K., González- Rodríguez, B. & Parra, I.A. (2008) Partial replacement of fish meal by porcine meat meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*, **277**, 244–250.

IFFO (International fishmeal and fish oil organization) (2011) What are fishmeal and fish oil? http://www.iffo.net/default.asp?contentID = 716.

Jost, R., Brambilla, E. & Monti, J.C. (1980) Papain catalyzed oligomerization of α-amino acids. Synthesis and characterization of water-insoluble oligomers of L-Methionine. *Helv. Chim. Acta*, **63**, 375–384.

Kasai, T., Tanaka, T. & Kiriyama, S. (1996) Polymerization degree of oligomethionine to determine its bioavailability when added to a low-protein diets. *Biosci. Biotech. Biochem*., **60**, 828–834.

Liu, Y.J., Tian, L.X. & Liu, D.H. (2002) Influence of practical diet supplemented with free or coated lysine on the growth, plasma free amino acids and protein synthesis rate in the muscle of *Ctenopharyngodon idellus* (in Chinese with English Abstract). *J. Fish China*, **26**, 252–258.

Masumoto, T., Bista, J.D., Itoh, Y., Hosokawa, H. & Shimeno, S. (1999) Bioavailability of modified forms of methionine in yellowtail and rainbow trout. *Bull. Mar. Sci. Fish. Kochi Univ*., **19**, 43–48.

Mato, J.M., Alvarez, L., Ortiz, P. & Pajares, M.A. (1997) S-Adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol. Ther*., **73**, 265–280.

Peres, H. & Oliva-Teles, A. (2005) The effect of dietary protein repalcement by crystalline amino acid on growth and nitrogen utilization of turbot *Scophthalmus maximus* juveniles. *Aquaculture*, **250**, 755–764.

Refstie, S., Storebakken, T., Baeverfjord, G. & Roem, A.J. (2001) Long term protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. *Aquaculture*, **193**, 91–106.

Richard, L., Blanc, P.P., Rigolet, V., Kaushik, S.J. & Geurden, I. (2010) Maintenance and growth requirements for nitrogen, lysine and methionine and their utilisation efficiencies in juvenile black tiger shrimp, *Penaeus monodon*, using a factorial approach. *Brit. J. Nutr*., **103**, 984–995.

Rodehutscord, M., Jacobs, S., Pack, M. & Pfeffer, E. (1995) Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 170 g to supplements of either l-arginine or l-threonine in a semipurified diet. *J. Nutr*., **125**, 249–254.

Rollin, X., Mambrini, T., Abboudi, T., Larondelle, Y. & Kaushik, S.J. (2003) The optimum dietary indispensable amino acid pattern for growing Atlantic salmon (*Salmo salar* L.) fry. *Brit. J. Nutr*., **90**, 865–876.

Schuhmacher, A.C.W. & Gropp, J.M. (1997) Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or crystalline amino acid diet. *Aquaculture*, **151**, 15–28.

Schwab, L.W., Kloosterman, W.M.J., Konieczny, J. & Loos, K. (2012) Papain catalyzed (co)Oligomerization of α-amino acids. *Polymers*, **4**, 710–740.

Smith, D.M., Tabrett, S.J., Irvin, S.J., Wakeling, J., Glencross, B.D. & Harris, D. (2007) Response of the black tiger shrimp, *Penaeus monodon* to feed containing the lupin alkaloid, gramine. *Aquaculture*, **272**, 556–563.

Tacon, A.G.J. (1994) Feed ingredients for carnivorous fish species: alternatives to fishmeal and other fishery resources. *FAO Fish Circ*., **881**, 35.

Tacon, A.G.J. & Barg, U.C. (1998) Major challenges to feed development for marine and diadromous finfish and crustacean species. In: Tropical Mariculture (De Silva, S.S.), pp. 171–208. Academic press, San Diego.

Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded. *Aquaculture*, **285**, 146–158.

Teshima, S., Alam, M.S., Ishikawa, M. & Kanazawa, A. (2002) Assessment of requirement values for essential amino acids in the prawn, *Marsupenaeus japonicus* (Bate). *Aquacult. Res*., **33**, 395–402.

Teshima, S., Ishikawa, M., Alam, M.S., Koshio, S. & Michael, F.R. (2004) Supplemental effects and metabolic fate of crystalline arginine in juvenile shrimp *Marsupenaeus japonicus*. *Comp. Biochem. Physiol*., **137**, 209–217.

Williams, K., Barlow, C. & Rodgers, L. (2001) Efficacy of crystalline and protein-bound amino acid enrichment of diets for barramundi/Asian sea bass (*Lates calcarifer* Bloch). *Aquacult. Res*., **32**, 415–429.

Yuan, Y.C., Gong, S.Y., Yang, H.J., Lin, Y.C., Yu, D.H. & Luo, Z. (2011) Effects of supplementation of crystalline or coated lysine and/or methionine on growth performance and feed utilization of the Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture*, **316**, 31–36.

Zarate, D.D. & Lovell, R.T. (1997) Free lysine (L-lysine.HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (*Ictalurus punctatus*). *Aquaculture*, **159**, 87–100.