

Optimal content and ratio of lysine to arginine in the diet of Pacific white shrimp, *Litopenaeus vannamei**

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Abstract The optimal quantity of dietary lysine (Lys) and arginine (Arg), and the optimal ratio of dietary Lys to Arg for Pacific white shrimp *Litopenaeus vannamei* were investigated. Coated Lys and Arg were added to a basal diet (37.99% crude protein and 7.28% crude lipid) to provide graded levels of Lys and Arg. The experimental diets contained three Lys levels (2.51%, 2.11%, and 1.70% of total diet), and three Arg levels (1.41%, 1.80%, and 2.21% of total diet) and all combinations of these levels were tested. Pacific white shrimp, with a mean weight of 3.62 ± 0.1 g, were randomly distributed in 36 fiberglass tanks with 30 shrimp per tank and reared on the experimental diets for 50 days. After the feeding trial, the growth performance, survival, feed conversion rate (FCR), body composition and protease and lipase activities in the hepatopancreases of the experimental shrimps were determined. The results show that weight gain (WG), specific growth rate (SGR), FCR, body protein, body Lys and Arg content were significantly affected by dietary Lys and Arg ($P < 0.05$) and improved when dietary Lys and Arg levels were 2.11%–2.51% and 1.80%–2.21%, respectively. Protease and lipase activities in the hepatopancreases of the shrimps appeared higher when dietary Lys and Arg quantities were 2.11%–2.51% and 1.80%–2.21%, although the difference was not statistically significant ($P > 0.05$). Therefore, according to our results, the optimal Lys and Arg quantities in the diet of Pacific white shrimp, *L. vannamei*, were considered to be 2.11%–2.51% and 1.80%–2.21%, respectively, and the optimal ratio to be 1:0.88–1:1.05.

Keyword: Pacific white shrimp; *Litopenaeus vannamei*; lysine; arginine; digestive enzyme

1 INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei*, is commonly farmed for human food and is one of the three highest production shrimp species in the world. The amino acid balance in the diet of aquacultured species can have a major impact on body protein deposition. Lysine (Lys) and arginine (Arg) are two essential amino acids and thus are an important component of shrimp feed.

There have been many reports on the Lys and Arg requirements of aquatic animals, including rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) (Cowey, 1994), cobia (*Rachycentron canadum*) (Zhou et al., 2005; Zhao et al., 2007), and *Penaeus monodon* (Millamena et al., 1998). Huang et al. (2003) and Fox et al. (1995) reported the Lys requirements of *Penaeus vannamei*.

Zhou et al. (2012) and Xie et al. (2012) also reported the Lys and Arg requirements of *L. vannamei*, respectively.

Studies on the relationship between Lys and Arg in animal nutrition have found contrasting results. Some studies have shown antagonism between Lys and Arg, such as in rats (Jones et al., 1966), pigs (Aherne and Nielsen, 1983), and chicks (Carew et al., 1998). Other studies on aquatic animals including fingerling channel catfish (Robinson et al., 1981), *Dicentrarchus labrax* (Tibaldi et al., 1994), *Pelteobagrus vachelli* (Feng et al., 2011), juvenile hybrid striped bass (*Morone saxatilis* × *M. chrysops*) (Griffin et al., 1994)

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and *Penaeus japonicus* (Hew and Cuzon, 1982) did not find evidence of antagonism between Lys and Arg. However, some reports found some evidences for antagonism between Lys and Arg in soft turtles (Zhou et al., 2003) and rainbow trout (Kaushik and Fauconneau, 1984; Kim et al., 1992).

The nutritional relationship between Lys and Arg in *L. vannamei* has not been reported to the best of the authors' knowledge.

As other protein sources have been used to replace fishmeal in recent years, free amino acids are often added to feed to meet the nutritional needs of animals. For example, free amino acids were used to balance the amino acids in the feed of juvenile rainbow trout (Yamamoto et al., 2002). However, some researchers showed that crystalline amino acid (CAA) was less useful for carp (Aoe et al., 1970; Nose et al., 1974) and channel catfish (Dupree and Halver, 1970). Possible reasons were that high CAA dissolution rates led to a reduction in efficiency (Chhom, 1993) and CAA was absorbed faster than the amino acid in protein. The lack of synchronicity in absorption may have resulted in the reduction of amino acids for synthetic protein (Cowey and Lequet, 1983).

Coated amino acids have been used to improve the effect of CAA. Murai et al. (1980, 1982a, b) used essential amino acids coated with casein in carp diets and obtained similar growth rates as a control group. Chen et al. (1992) fed *P. monodon* with feed that contained coated Arg, and found that experimental shrimps have the potential to grow very quickly. Alam et al. (2004) reported that diets containing coated CAA yielded higher feed conversion efficiencies, protein efficiency ratios, and specific growth rates in juvenile kuruma shrimp, *Marsupenaeus japonicus*, than diets containing non-coated CAA.

2 MATERIAL AND METHOD

2.1 Experimental diets

Fish meal and corn gluten meal were used in the basal shrimp diet as the main source of protein, fish oil as a fat source, and corn starch and sucrose as a sugar source (Table 1). In this study, coated Lys and Arg were added to a basal diet to provide graded levels of Lys and Arg. Nine diets were formulated with three Lys levels (2.51%, 2.11%, and 1.70% of the diet), and each with three Arg levels (1.41%, 1.80%, and 2.21% of the diet). The level of dietary protein in the basal diet was 37.99% and dietary lipid level was 7.28% to support optimum growth. The

dietary Lys, Arg and other important amino acids were determined and are shown in Table 2.

Prior to diet preparation, all the raw materials were ground through an 80-mesh sieve, weighed, and mixed. Afterwards, fish oil (with the soybean lecithin dissolved in it) was added gradually and mixed constantly, and then 40 mL of water was slowly added into the mixture for each 100 g of dry matter, forming a dough that was then processed into long thin strips (diameter: 1.5 mm) using a twin-screw cold extruder. The diets were then dried (50°C, 12 h), pelletized, and sieved. The dry pellets were placed in covered plastic bags and stored in a refrigerator at -20°C for use.

2.2 Shrimp feeding trial

The feeding experiment was conducted in a closed re-circulating system in the Research and Development Center of Liuhe Feed Co., Ltd., Qingdao, China. Before the start of the experiment, shrimp (average weight of 3.62±0.1 g) were acclimated to experimental conditions in a large tank for 2 weeks during which they were fed a commercial diet. After the acclimation period, shrimp were pooled and randomly distributed between 36 fiberglass tanks to form groups of 30 shrimps. Four tanks were fed with same experimental diet for each treatment. The experimental shrimps were fed with their respective diets four times a day at 6:30, 11:30, 16:30, and 21:30. Uneaten food and feces were collected 30 min after feeding, then they were dried and weighed to calculate feed efficiency. The daily amount of the diet was adjusted according to the amount of the uneaten diet.

An air pump provided aeration via porous stones. Water temperature was measured daily and ranged from 25–27°C. Dissolved oxygen was >5 mg/L, salinity 2–3, pH 8.0±0.1. The photoperiod was natural and all tanks had similar light conditions. The feeding trial lasted for 50 days.

2.3 Sampling and analytical methods

At the end of the experiment, the shrimps were starved for 24 h prior to sampling. Total number and weight of the shrimps in each tank were determined. Eight shrimps from each tank were randomly collected for analysis the content of crude protein and amino acids. Five shrimps were sampled randomly for analysis of protease and lipase. Others were analyzed in duplicate for moisture (105°C, to constant weight), crude lipid (Soxhlet extraction method) and ash (550°C, to constant weight) (AOAC, 1995). Amino acid composition of the raw material, experimental

Table 1 Ingredients and chemical proximate composition of the experimental diets (% dry matter, DM)

Ingredient (g/kg diet, 100% DM)	Lys/Arg								
	2.51/1.41	2.51/1.80	2.51/2.21	2.11/1.41	2.11/1.80	2.11/2.21	1.70/1.41	1.70/1.80	1.70/2.21
Fish meal	10	10	10	10	10	10	10	10	10
Corn gluten meal	45	45	45	45	45	45	45	45	45
Corn starch	25.4	24.42	23.42	26.2	25.22	24.22	27	26.02	25.02
Soybean lecithin	2	2	2	2	2	2	2	2	2
Fish oil	3	3	3	3	3	3	3	3	3
Sucrose	4	4	4	4	4	4	4	4	4
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ascorbic phosphate ester	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chloride chorine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	1	1	1	1	1	1	1	1	1
Mineral premix	1	1	1	1	1	1	1	1	1
Monocalcium phosphate	2	2	2	2	2	2	2	2	2
Sodium alginate	2	2	2	2	2	2	2	2	2
Coated Lys	3.1	3.1	3.1	2.3	2.3	2.3	1.5	1.5	1.5
Coated Arg	0.4	1.38	2.38	0.4	1.38	2.38	0.4	1.38	2.38
Total	100	100	100	100	100	100	100	100	100
Chemical proximate analysis									
Crude protein (DM%)	38.05	38.69	37.86	38.16	37.50	37.64	38.14	38.52	37.32
Crude lipid (DM%)	7.34	7.37	7.23	7.29	7.28	7.21	7.32	7.27	7.25
Ash (DM%)	4.85	4.81	4.87	4.89	4.95	4.88	4.91	4.89	4.91

Coated Lys and Arg were provided by Hangzhou King Techina Feed Co., Ltd. Lys-HCl content in the product was 50%, and Arg-HCl was 40%.

Table 2 Amino acids compositions of the experimental diets (%dry matter, DM)

Amino acid	Lys/Arg								
	2.51/1.41	2.51/1.80	2.51/2.21	2.11/1.41	2.11/1.80	2.11/2.21	1.70/1.41	1.70/1.80	1.70/2.21
Lysine	2.51	2.55	2.59	2.09	2.07	2.11	1.73	1.76	1.80
Arginine	1.40	1.81	2.20	1.40	1.81	2.20	1.40	1.81	2.20
Methionine	0.75	0.78	0.78	0.84	0.81	0.81	0.77	0.85	0.83
Threonine	1.15	1.21	1.16	1.25	1.20	1.25	1.16	1.26	1.24
Valine	1.34	1.61	1.51	1.57	1.58	1.63	1.51	1.62	1.66
Isoleucine	1.08	1.16	1.12	1.19	1.15	1.18	1.10	1.20	1.21
Leucine	4.51	4.91	4.79	5.05	4.92	5.02	4.75	5.12	5.14
Histidine	0.76	0.83	0.81	0.84	0.84	0.82	0.78	0.86	1.06
Phenylalanine	1.75	1.85	1.79	1.89	1.85	1.89	1.80	1.92	1.33

diet and whole shrimp were determined using standard methods. Samples were hydrolyzed at 110–120°C for 24 h in a digestion block with 6 mol/L HCl, then the hydrolysates were diluted with 30% NaOH, the pH was adjusted to 2.20 and amino acids were determined with an amino acid analyzer (Model Biochrom30+,

Biochrom Ltd., Cambridge, UK).

The hepatopancreases of experimental shrimps were sampled carefully and frozen immediately at -70°C until the enzyme assays were conducted. A 1.0 g of each hepatopancreas was homogenized in 9.0 mL cold (4°C) physiological saline. Enzyme

Table 3 Weight gain (WG), specific growth ratio (SGR), feed conversion rate (FCR) and survival of the shrimp fed with the diets containing different levels of Lys and Arg (mean±SE, n=4)

Diet No.	Dietary Lys (%)	Dietary Arg (%)	Initial weight (g)	Final weight (g)	WG (%)	SGR (%)	FCR	Survival (%)
1	2.51	1.41	3.62±0.03	8.52±0.31 ^a	135.5±10.6 ^a	1.71±0.09 ^a	1.31±0.10 ^{ab}	83.3±5.77
2	2.51	1.80	3.63±0.03	9.45±0.12 ^b	160.7±5.42 ^{bc}	1.92±0.04 ^{bc}	1.19±0.05 ^{ab}	85.6±4.01
3	2.51	2.21	3.62±0.01	9.74±0.34 ^b	168.9±8.72 ^{bc}	1.98±0.07 ^c	1.11±0.09 ^a	85.6±2.22
4	2.11	1.41	3.61±0.04	9.10±0.03 ^{ab}	152.2±3.05 ^{abc}	1.85±0.02 ^{abc}	1.25±0.01 ^{ab}	87.8±7.28
5	2.11	1.80	3.61±0.02	9.35±0.15 ^b	159.2±3.26 ^{bc}	1.90±0.03 ^{bc}	1.21±0.04 ^{ab}	90.0±1.92
6	2.11	2.21	3.62±0.02	9.37±0.02 ^b	158.6±0.88 ^{bc}	1.90±0.01 ^{bc}	1.10±0.13 ^a	87.8±2.93
7	1.70	1.41	3.61±0.06	8.81±0.27 ^{ab}	144.2±8.06 ^{ab}	1.78±0.07 ^{ab}	1.42±0.08 ^b	88.9±4.01
8	1.70	1.80	3.62±0.03	8.90±0.08 ^{ab}	145.9±2.62 ^{abc}	1.80±0.02 ^{abc}	1.21±0.09 ^{ab}	82.2±4.01
9	1.70	2.21	3.61±0.04	8.49±0.15 ^a	134.9±5.58 ^a	1.71±0.05 ^a	1.25±0.10 ^{ab}	83.3±3.85
ANOVA								
<i>F</i> value			0.037	4.828	3.644	3.572	1.393	0.402
<i>P</i> value			1.000	0.003	0.011	0.012	0.265	0.905

Means in each column not sharing a common superscript are significantly different according to Duncan's test ($P<0.05$)

extracts were obtained after centrifugation at 10 000 r/min for 20 min at 4°C. The supernatants were taken to obtain protease and lipase.

Total activities of protease and lipase were measured following methods described by Liu and Zhu (1984); one unit of protease activity was defined as 1 µg tyrosine released per min at 40°C. One unit of lipase activity was defined as 1 µg aliphatic acid released per minute. Enzyme activities were expressed as specific activities, i.e. U/mg soluble protein.

2.4 Calculations and statistical analyses

Weight gain (WG) was calculated as: (final mean weight–initial mean weight)/initial mean weight×100%; special growth rate (SGR) as: (Ln final mean body weight–Ln initial mean body weight)/days×100%; feed conversion rate (FCR) as: feed intake/(final mean weight–initial mean weight).

All data are presented as means ± standard errors. After the homogeneity of variances was confirmed by way of Levene's test, the data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test to determine the effects of experimental diets using SPSS Ver. 17.0. Differences were considered significant at $P<0.05$.

3 RESULT

3.1 Growth performance and survival

Initial weight, final mean weight, WG, survival, SGR, and FCR of the experimental shrimps are shown

in Table 2. The WG, SGR, and FCR were affected significantly by the amount of dietary Lys and Arg ($P<0.05$). The WG and SGR were the lowest in the group fed with Lys/Arg proportions of 2.51/1.41 and 1.71/2.21, and highest in the treatments with Lys/Arg proportion of 2.51/2.21. For dietary Lys/Arg quantities of 2.11/1.80 and 2.11/2.21, the WG was 159.2% and 158.6%, SGR were 1.90 for both; there was no statistical difference from the treatment that contained Lys/Arg proportions of 2.51/2.21. Shrimps in other treatments showed poor growth performance. These results showed that the optimal Lys content was 2.11%–2.51%, Arg was 1.80%–2.21%, and the optimal Lys/Arg ratio was 1: 0.88–1.05 in the diet of *L. vannamei*. The FCR was the lowest in the treatments of 2.51/2.21 and 2.11/2.21, showing that an inappropriate proportion of Lys and Arg may result in lower feed conversion efficiency. In addition, no significant difference was found in the survival of shrimp fed with experimental diets, which ranged from 82.2%–90.0%.

3.2 Shrimp body composition

The crude protein, lipid, ash, moisture, and amino acid content of the experimental shrimps are shown in Table 4. The crude proteins, Lys and Arg were affected by dietary Lys and Arg. The Lys content in the experimental shrimp was higher in the treatments containing Lys/Arg proportions of 2.51/1.80, 2.51/2.21, and 2.11/2.21 than that in the treatments with Lys/Arg proportions of 2.11/1.40, 1.70/1.40,

Table 4 Amino acid contents (%DM) and chemical proximate composition (%DM) of the experimental shrimp (mean±SE, n=4)

Amino acid	Lys/Arg								
	2.51/1.41	2.51/1.80	2.51/2.21	2.11/1.41	2.11/1.80	2.11/2.21	1.70/1.41	1.70/1.80	1.70/2.21
Lys	4.14±0.05 ^{ab}	4.24±0.04 ^{bc}	4.34±0.03 ^c	4.07±0.02 ^a	4.15±0.05 ^{ab}	4.25±0.04 ^{bc}	4.05±0.02 ^a	4.00±0.07 ^a	4.00±0.08 ^a
Arg	4.22±0.04 ^a	4.34±0.04 ^a	4.56±0.08 ^c	4.22±0.08 ^a	4.51±0.07 ^{bc}	4.53±0.05 ^{bc}	4.25±0.03 ^a	4.47±0.03 ^{bc}	4.47±0.10 ^{bc}
Cys	0.51±0.02	0.51±0.01	0.61±0.10	0.55±0.03	0.58±0.10	0.50±0.07	0.58±0.05	0.57±0.03	0.49±0.03
Met	1.19±0.01	1.20±0.03	1.31±0.01	1.27±0.01	1.20±0.05	1.18±0.09	1.29±0.02	1.26±0.03	1.21±0.09
Asp	6.60±0.12	6.50±0.03	6.65±0.10	6.58±0.04	6.54±0.01	6.32±0.22	6.44±0.10	6.60±0.06	6.45±0.15
Thr	2.46±0.06	2.43±0.04	2.48±0.04	2.40±0.02	2.42±0.03	2.39±0.07	2.36±0.08	2.42±0.03	2.44±0.25
Ser	2.50±0.04	2.26±0.02	2.45±0.07	2.35±0.08	2.49±0.10	2.46±0.02	2.36±0.14	2.46±0.08	2.49±0.02
Glu	9.62±0.32	9.56±0.11	9.76±0.08	9.73±0.07	9.53±0.02	9.33±0.20	9.49±0.17	9.58±0.04	9.40±0.27
Gly	5.20±0.17	5.65±0.26	5.46±0.21	5.10±0.04	4.95±0.05	5.06±0.01	4.66±0.36	5.26±0.33	5.61±0.16
Ala	6.125±0.04	6.56±0.33	6.62±0.50	6.28±0.25	5.86±0.08	6.09±0.17	6.04±0.18	6.05±0.21	6.22±0.05
Val	2.71±0.12	2.44±0.15	2.78±0.03	2.64±0.03	2.50±0.09	2.57±0.16	2.64±0.04	2.70±0.02	2.66±0.17
Ile	2.38±0.13	2.12±0.16	2.45±0.04	2.31±0.02	2.16±0.09	2.23±0.18	2.27±0.04	2.36±0.01	2.34±0.16
Leu	4.45±0.15	4.31±0.07	4.57±0.04	4.48±0.04	4.38±0.06	4.32±0.12	4.33±0.05	4.45±0.01	4.33±0.14
Phe	2.75±0.09	2.57±0.04	2.81±0.09	2.91±0.12	2.65±0.11	2.63±0.13	2.75±0.05	2.73±0.01	2.71±0.06
His	1.48±0.04	1.42±0.05	1.46±0.02	1.52±0.02	1.49±0.03	1.38±0.07	1.44±0.05	1.49±0.02	1.36±0.08
Pro	4.19±0.14	3.97±0.06	4.15±0.12	3.94±0.29	4.11±0.04	4.20±0.04	4.22±0.04	3.97±0.16	3.97±0.21
Crude protein	70.97±0.21 ^a	71.23±0.73 ^a	72.92±0.21 ^b	70.34±0.28 ^a	73.09±0.05 ^b	73.04±0.73 ^b	70.31±0.26 ^a	71.27±0.71 ^a	71.36±0.06 ^a
Crude lipid	3.56±0.27	3.09±0.24	3.40±0.19	3.26±0.57	3.58±0.13	3.60±0.21	3.48±0.30	3.35±0.52	3.25±0.16
Ash	14.25±0.31	14.76±0.26	14.59±0.23	14.72±0.27	14.51±0.52	14.10±0.17	13.96±0.09	14.77±0.68	14.83±0.13
Moisture	77.84±0.92	78.95±0.43	78.50±0.19	78.48±0.61	77.88±1.14	77.21±0.38	77.79±0.68	78.32±0.53	78.65±0.45

Means in each line not sharing a common superscript are significantly different according to Duncan's test ($P<0.05$).

Table 5 Protease and lipase activities in the hepatopancreas of the experimental shrimp (mean±SE, n=4)

Dietary treatments		Protease (U/mg·pro)	Lipase (U/mg·pro)
Lys	Arg		
2.51	1.41	348.32±15.50	13.38±3.02
2.51	1.80	351.5±11.69	13.77±1.83
2.51	2.21	373.14±5.53	16.45±6.21
2.11	1.41	329.23±10.12	14.39±3.76
2.11	1.80	353.38±20.91	16.68±1.37
2.11	2.21	353.18±17.52	15.60±1.39
1.70	1.41	327.65±8.07	12.85±0.83
1.70	1.80	335.49±61.38	14.58±1.41
1.70	2.21	331.17±4.93	10.66±4.28

1.70/1.80 and 1.70/2.21. The Arg content in the experimental shrimp was higher in the treatments with Lys/Arg proportions of 2.51/2.21, 2.11/1.80, 2.11/2.21, 1.70/1.80, and 1.70/2.21 than that in other treatments, and was the lowest in the treatments with

Lys/Arg proportions of 2.11/1.41 and 1.71/1.41. The crude protein in the shrimp was high in the treatments with Lys/Arg proportions of 2.51/2.21, 2.11/1.80 and 2.11/2.21. These results showed that the deposition of body protein increased with an optimal ratio of Lys to Arg, as did body Lys and Arg levels. No significant difference was found in body moisture, crude lipid, and ash among all the treatments ($P>0.05$).

3.3 Digestive enzyme activity

The protease and lipase activities of the experimental shrimps are shown in Table 5. Examination of mean values indicate that protease and lipase activities in the hepatopancreas were relatively higher in the treatments with proportions of Lys/Arg of 2.51/2.21, 2.51/1.80, 2.11/1.80, and 2.11/2.21 than in other treatments ($P<0.05$). These results indicate that protease and lipase activities increased at the optimal ratio of Lys to Arg, although there was no statistical difference between these values ($P>0.05$).

4 DISCUSSION

Some experiments have found that coated CAA can be used in the diet of fish (Murai et al., 1980, 1982a, b, 1987; Alam et al., 2004) and also of shrimp that feed slowly (Chen et al., 1992; Alam et al., 2004, 2005) to improve the balance of dietary amino acids. In this study, coated Lys and Arg were used to adjust the content of Lys and Arg in the experimental diets.

In the present experiment, to maintain lower Lys and Arg levels in the basal diet, only 10% fishmeal was used in the diets, while corn gluten meal was 45%, and was the main protein raw material. As a result, the experimental diet may have been less attractive to shrimp to a certain extent, which may explain the lower growth performance we observed, e.g. the WG among all treatments were not up to 170%.

Huang et al. (2003) used casein and gelatin as protein sources to determine the essential amino acids requirements of *P. vannamei* and considered the minimum quantity of Lys to be 2.15%. Fox et al. (1995) reported that the Lys requirement of *P. vannamei* was 2.05% of diet. Xie et al. (2012) reported that the optimal Lys requirement of *P. vannamei* was 2.05% of a dry diet. Zhou et al. (2012) reported that the optimal Arg requirement was 2.32% of a dry diet. Millamena et al. (1998) reported that the requirements of juvenile *P. monodon* for Lys and Arg were 2.08% and 1.85% of the diet, respectively. In the present experiment, to determine the optimal ratio of dietary Lys to Arg, dietary Lys was designed at 1.70%, 2.10%, and 2.50%; Arg was 1.40%, 1.80% and 2.20%, respectively. Actual Lys and Arg content were determined. The values of Lys were 1.70%, 2.11% and 2.51%, and that of Arg were 1.41%, 1.80% and 2.21%.

According to the results of this study, growth performance and body composition of the experimental shrimps improved in the treatments with proportions of Lys/Arg of 2.51/1.80, 2.51/2.21, 2.11/1.80 and 2.11/2.21. The activities of protease and lipase in the hepatopancreas were also higher in the treatments mentioned above, although they were not significantly different from other treatments. These results were similar to the reports mentioned previously, indicating that the minimum dietary Lys and Arg requirements of Pacific white shrimp, *L. vannamei*, were 2.11% and 1.80%, respectively.

In the present study, poor growth was observed in the treatments with proportions of Lys/Arg of 2.51/1.70 and 1.70/2.21. Lower protein deposition

also appeared in the treatments with proportions of Lys/Arg of 2.51/1.41, 2.51/1.80, 2.11/1.41 and all the treatments of Lys with levels of 1.70. These results indicate that lower Lys content and inappropriate ratios of these two amino acids in the diet are likely to result in poor growth and protein deposition.

Some studies have shown antagonism between Lys and Arg in the diet of some animals (Jones et al., 1966; Aherne and Neilsen, 1983; Carew et al., 1998, Zhou et al., 2003). It has been hypothesized that the reason for this is that Lys and Arg share a common carrier and thus they have an inhibitory effect on each other (Berge et al., 1999). However, no antagonism was found in other studies on fingerling channel catfish (Robinson et al., 1981), *Dicentrarchus labrax* (Tibaldi et al., 1994), *Pelteobagrus vachelli* (Feng et al., 2011), and *Penaeus japonicus* (Hew and Cuzon, 1982). Zhou et al. (2003) believed that this difference was due to the index used. For example, antagonism would appear between Lys and Arg when using Arg deposition in the body as an index, while no antagonism would occur when growth performance is used as an index such as in the experiment on soft turtles (Zhou et al., 2003). In the present study, the growth performance in the treatments with Lys/Arg proportions of 2.51/1.70 and 1.41/2.21 was poorer than those with proportions of 1.70/1.40 and 1.70/1.80 Lys/Arg, in which both Lys and Arg were lower. This result may indicate antagonism between the two amino acids in *L. vannamei*. Further studies that add more Lys and Arg to experimental diets may help to elucidate if this is the case.

According to the results of this study, the optimal ratio of the two amino acids was 1:0.88–1:1.05.

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