Aquaculture 392–395 (2013) 142–147

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Dietary threonine requirements of juvenile Pacific white shrimp, *Litopenaeus vannamei*

Qi-Cun Zhou [a,*], Yong-Li Wang [b], Hua-Lang Wang [c], Bei-Ping Tan [b]

[a] School of Marine Sciences, Ningbo University, Ningbo 315211, China
[b] College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, China
[c] Aqua-feed Research and Development Center, Guangdong Evergreen Group Corp. Zhanjiang 524094, China

ARTICLE INFO

Article history:
Received 22 November 2012
Received in revised form 21 January 2013
Accepted 23 January 2013
Available online 18 February 2013

Keywords:
Pacific white shrimp
Threonine
Growth performance
Enzyme activity

ABSTRACT

An 8-week feeding trial was conducted to determine the dietary threonine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Six isonitrogenous and isolipidic practical diets (43% crude protein and 7.5% crude lipid) were formulated to contain graded dietary threonine levels ranging from 1.07% to 2.30% (dry weight). In all of the diets, the nitrogen content of the amino acids was kept the same by replacing threonine with a non-essential amino acid mixture. Each diet was randomly assigned to triplicate groups of 40 juvenile shrimp (approximately 0.53 g) that were fed 4 times daily to apparent satiation. The results indicated that significant difference was observed in the weight gain among all treatments. Maximum weight gain was obtained at 1.67% dietary threonine; however, weight gain did not significantly increase with further dietary threonine increases. The survival of the shrimps showed no significant differences among all treatments. Feed efficiency, protein efficiency ratio and protein productive value were also significantly influenced by the dietary threonine levels, and the trends were similar to those of growth performance. There were no significant differences among dry matter, crude protein, crude lipid or ash content in the whole body and muscle composition. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD) and phenoloxidase (PO) activities in hemolymph were significantly affected by dietary threonine levels. Shrimp fed the diet containing 2.30% threonine had higher AST and ALT values than those fed the other diets; however, the highest SOD and PO activities were observed at 2.05% dietary threonine. The optimal dietary threonine requirement, estimated by a broken-line model based on SGR, was 1.51% of the dry diet (corresponding to 3.53% of dietary protein on a dry-weight basis). Considering the threonine leaching loss in seawater within 30 min (duration of feeding each time), the threonine requirement for *L. vannamei* is 1.18% of dry diet (2.81% of the dietary protein).

1. Introduction

Proteins and amino acids play an important role in the structure and metabolism of all living organisms. Shrimp cannot synthesize all amino acids and must acquire several through their diet, and it has been shown that threonine is one of the essential amino acids (EAAs) in shrimp (National Research Council, NRC, 2011). As with many other essential amino acids, threonine is involved in immune function, as well as in maintaining adequate feed intake, growth and feed efficiency (Bodin et al., 2008; Helland and Grisdale-Helland, 2011; Li et al., 2007; Rodehutscord et al., 1995; Tibaldi and Tulli, 1999). Threonine, together with serine and tyrosine, is one of three primary amino acids bearing an alcohol group, and when a threonine residue undergoes phosphorylation through the action of a threonine kinase, it can be referred to as phosphothreonine (National Research Council, NRC, 2011). Moreover, threonine, lysine and methionine are the most common limiting indispensable amino acids (IAAs) in plant protein sources (Gatlin et al., 2007). Essential amino acids deficiencies will result in reduced growth performance and feed utilization; therefore, it is important to satisfy the shrimps' need for EAAs by formulating feeds containing balanced nutrients (Fox et al., 1995; Millamena et al., 1998; Teshima et al., 2002). The dietary threonine requirements have been estimated to be 1.3% of the diet for *Penaeus monodon* (Millamena et al., 1998) and 1.4% of the diet for *Marsupenaeus japonicus* (Teshima et al., 2002). For Pacific white shrimp reared in low-salinity water, the dietary threonine requirement has been estimated to be 1.36% of the dry diet (corresponding to 3.78% of dietary protein) (Huai et al., 2009).

The Pacific white shrimp, *Litopenaeus vannamei*, has become a worldwide species for aquaculture because of its great economic value, rapid growth rate, and tolerance to a wide range of salinity and temperature (Bray et al., 1994; Clifford, 1994; Saoud et al., 2003; Wickins and Lee, 2007). Determination of the essential amino acids requirements is considered to be the highest priority area in shrimp nutrition research (Akiyama, 1986). However, there have

* Corresponding author. Tel./fax: +86 574 876 09581.
E-mail address: zhouqicun@nbu.edu.cn (Q.-C. Zhou).

0044-8486/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.01.026

been few studies examining the EAAs requirements of Pacific white shrimp (Akiyama, 1986; Fox et al., 1995; Huai et al., 2009; Xie et al., 2012; Zhou et al., 2012). To our knowledge, there has been no previous report on the threonine requirement of Pacific white shrimp reared in sea-water. Therefore, the objective of the present study was to determine the optimal dietary threonine requirements for juvenile *L. vannamei* and to evaluate the effects of dietary threonine levels on growth performance, feed utilization and key enzyme activities of threonine metabolism and immune response.

2. Materials and methods

2.1. Diet preparation

Six isonitrogenous and isolipidic diets (43% protein, 7.5% lipid) were formulated to make diets with graded levels of L-threonine (1.07–2.30% of dry diet) (Table 1). Fish meal, wheat gluten, shrimp head meal and wheat flour were used as intact protein sources and were supplemented with a mixture of crystalline amino acids (CAAs) (L-form, purity ≥ 99%, Kayon Biological Technology, Shanghai, China) to simulate the amino acid profile of shrimp tissue (Huai et al., 2009). Fish oil and soybean oil (1:1) were used as the lipid sources. All diets were kept isonitrogenous by decreasing the levels of non-essential amino acids (aspartate and glycine) as the threonine levels increased. The targeted dietary threonine concentrations were 1.07, 1.28, 1.67, 1.89, 2.05 and 2.30%. The total amino acid compositions of each diet are shown in Table 2. Dietary ingredients were ground through 80-mesh and weighed. All the dry ingredients were thoroughly mixed until they were homogenous in a Hobart-type mixer. Lipids and water were then added and thoroughly mixed. Cold-extruded pellets (1.0 mm and 1.5 mm in diameter) were produced and air-dried to approximately 10% moisture, sealed in vacuum-packed bags, and stored frozen (−20 °C) prior to use in the feeding trial.

2.2. Shrimp and experimental conditions

Juvenile shrimp (*L. vannamei*) were obtained from the Guangdong Evergreen Group (Zhanjiang, China). Prior to the experiment, the shrimp were acclimated and fed with a commercial diet (the threonine level in the commercial diet was 1.4% of the dry diet, 40% crude protein, 8% crude lipid, Guangdong Evergreen Group, Zhanjiang, China) for 2 weeks. At the beginning of the experiment, 40 shrimp of the same size were weighed in two groups and stocked randomly and sorted into a 500-l cylindrical fiberglass tank. The total weight of 40 shrimp is 21.02 ± 0.02 g, the individual weight is about approximately 0.53 g. Each diet was randomly assigned to three replicate groups of shrimps. All groups of shrimp were fed at the same fixed rate. Diets were fed by hand to an apparent satiation, all uneaten feed after feeding 1 hour were siphoned from tanks before feeding on a daily basis. The amount of feed was 6–10% of body weight divided into four equal feedings daily at 7:00, 12:00, 17:00 and 22:00 h, and the daily amount of each diet was adjusted every two weeks by weighing the total weight of shrimps in each tank. These feeding rates were designed to assure apparent satiation without overfeeding.

All tanks were provided with continuous aeration through air stones to maintain dissolved oxygen levels at or near saturation. During the experimental period, temperatures ranged from 26 to 31 °C, salinity was approximately 26–29 g l^{-1}, pH was 7.6–7.8, ammonia nitrogen levels were lower than 0.05 mg l^{-1}, and dissolved oxygen levels were not less than 6.0 mg l^{-1}. The feeding trial lasted for 8 weeks.

Table 1
Formulation and proximate composition of experimental diets (% dry matter).

Ingredient (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Wheat gluten meal	17.00	17.00	17.00	17.00	17.00	17.00
Fish meal	24.00	24.00	24.00	24.00	24.00	24.00
Shrimp head meal	5.00	5.00	5.00	5.00	5.00	5.00
Dextrin	32.00	32.00	32.00	32.00	32.00	32.00
Fish oil	0.95	0.95	0.95	0.95	0.95	0.95
Soybean oil	0.95	0.95	0.95	0.95	0.95	0.95
Essential amino acid premix[a]	2.75	2.75	2.75	2.75	2.75	2.75
Non-essential amino acid mixture[b]	5.15	4.99	4.82	4.66	4.50	4.34
Vitamin premix[c]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[c]	1.00	1.00	1.00	1.00	1.00	1.00
Calcium biphosphate	1.50	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Carboxymethy cellulose	3.00	3.00	3.00	3.00	3.00	3.00
Cellulose	3.87	3.83	3.79	3.75	3.71	3.67
Soy lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin C-phosphate	0.03	0.03	0.03	0.03	0.03	0.03
Threonine	0.00	0.20	0.40	0.60	0.80	1.00
Approximate composition (%)						
Crude protein	42.48	42.66	42.69	42.56	43.28	43.11
Crude lipid	7.44	7.42	7.46	7.64	7.43	7.48
Ash	8.33	8.36	8.55	8.32	8.42	8.21

[a] Essential amino acid mixture (g 100 g^{-1} dry diet): Arginine, 1.0180; Histidine, 0.1821; Isoleucine, 0.1624; Methionine, 0.2910; Phenylalanine, 0.2921; Lysine, 0.7111; Tryptophan, 0.0673; Leucine, 0.1216.
[b] Non-essential amino acid mixture (g 100 g^{-1} dry diet): L-aspartic acid: glycine = 1:1.
[c] Vitamin and mineral premix were based on Zhou et al. (2012).

Table 2
Amino acid composition of the experimental diets (% dry matter)[a].

Amino acids	Dietary threonine levels (%)					
	1.07	1.28	1.67	1.89	2.05	2.30
Essential amino acids						
Threonine	1.07	1.28	1.67	1.89	2.05	2.30
Lysine	1.95	2.03	1.99	2.00	2.13	2.03
Phenylalanine	1.75	1.41	1.38	1.40	1.50	1.49
Arginine	2.71	2.59	2.51	2.61	2.58	2.60
Methionine	1.23	1.15	1.13	1.13	1.18	1.16
Cystine	0.47	0.48	0.48	0.59	0.49	0.48
Leucine	2.91	2.63	2.57	2.60	2.42	2.65
Isoleucine	1.64	1.50	1.44	1.46	1.44	1.51
Histidine	0.77	0.77	0.88	0.77	0.78	0.80
Valine	1.55	1.59	1.56	1.59	1.60	1.60
Non-essential amino acids						
Aspartic acid	4.91	4.72	4.74	4.67	4.59	4.53
Serine	1.44	1.17	1.44	1.50	1.53	1.48
Glutamic acid	9.45	9.12	9.42	9.53	9.76	9.62
Glycine	3.63	3.59	3.49	3.57	3.48	3.30
Lactamine	1.45	1.46	1.45	1.27	1.58	1.46
Tyrosine	1.13	0.75	0.72	0.72	0.83	0.52
Proline	3.07	3.00	2.94	3.55	3.52	3.06

[a] Amino acid content of the dry diet.

2.3. Sample collection techniques and chemical analyses

At the termination of the 8-week feeding trial, the shrimp in each tank were sampled for analyses 24 h after the last feeding. Six shrimp per tank were used to analyze the approximate whole body composition. Another six shrimp in each tank were used to analyze the approximate muscle composition, and the muscle samples were collected by scalpel on ice and then stored at −20 °C until analysis. Blood samples from ten shrimp in each tank were taken from the pericardial cavity using a 1-ml syringe, sorted into 1.5-ml Eppendorf tubes and centrifuged at 4 °C, 5000 r min^{-1} for 10 min. Then, the supernatant was collected, packaged and stored at −80 °C until analysis of enzymatic activity.

Crude protein, crude lipid, moisture and ash content in the diet, the muscles and the whole body were determined following the procedures of the Association of Official Analytical Chemists, AOAC (1995). Briefly, the moisture content was determined by drying to a constant weight at 105 °C, and the crude protein content (N × 6.25)