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Dietary threonine requirement of juvenile large yellow croaker, *Larmichthys crocea*

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Abstract

A feeding trial was conducted to determine the dietary threonine requirement of juvenile large yellow croaker (*Larmichthys crocea*). Six diets were formulated containing 45% crude protein with six graded levels of threonine (0.71–2.46% in about 0.35% increment). Each diet was randomly assigned to triplicate groups of 60 juvenile fish (initial body weight 6.00 ± 0.10 g). Fish were fed twice daily (05:00 and 16:30) to apparent satiation for 8 weeks. The result indicated that significant difference was observed in the weight gain among all treatments. Specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and nitrogen retention (NR) increased with increasing levels of threonine up to 1.75% diet ($P < 0.05$), and thereafter, declined. No significant differences in body dry matter, crude protein, crude lipid or ash content were found among dietary treatments. Theronine contents of fish muscle were significantly affected by dietary threonine levels ($P < 0.05$). Fish fed the diet with 0.71% threonine showed the lowest threonine content (2.94%) in fish muscle, while fish fed the diet with 1.75% threonine had the highest value (3.16%). Other essential amino acid contents of muscle were not significantly different among the dietary treatments. On the basis of SGR, FE or NR, the optimum dietary threonine requirements of juvenile *L. crocea* were estimated to be 1.86% of diet (4.13% of dietary protein), 1.90% of diet (4.22% of dietary protein) and 2.06% of diet (4.58% of dietary protein), respectively, using second-order polynomial regression analysis.

Keywords: threonine, growth, juvenile, *Larmichthys crocea*

Introduction

Proteins and amino acids play an important role in the structure and metabolism of all living organisms. Threonine is one of the 10 essential amino acids (EAAs) required for normal growth of various fish (NRC 2011). After methonine and lysine, threonine is usually considered to be the third limiting indispensable amino acid in plant protein sources (Gatlin, Barrows, Brown, Dabrowski, Gaylord, Hardy, Herman, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Skonberg, Souza, Stone, Wilson & Wurtele 2007; Nunes, Sa, Browdy & Vazquez-Anon 2014). Threonine could be potentially marginal or limiting in practical diet formulations, especially when plant protein sources are used to replace substantial amounts of dietary fishmeal protein (Tibaldi & Tulli 1999). Threonine, together with other EAAs, is involved in physiological function such as protein synthesis and uric acid formation, as well as in maintaining adequated feed intake, growth and feed efficiency (Helland & Grisdale-Helland 2011; Zhou, Wang, Wang & Tan 2013). Threonine deficiencies result in reduced growth performance and feed utilization were reported in Indian major carp *Labeo rohita* (*Hamilton*) (Fatma Abidi & Khan 2008) and Indian catfish *Heteropneustes fossilis* (Bloch) (Ahmed 2007). Therefore, it is important for elaborating balanced plant protein-based aquafeeds that ensure cost-effectiveness, rapid growth and minimal environmental impact (Bodin, Mambrini, Wauters, Abboudi, Ooghe, Boulenge, Larondelle & Rollin 2008).

Dietary threonine requirements have been quantified by the ration level technique in several species of fish, varying from 1.8% to 5.1% of dietary protein (NRC, 2011), including Atlantic salmon smolts, *Salmo salar* 2.5% (Helland & Grisdale-Helland 2011),

Channel catfish, *Ictalurus punctatus* 2.2% (Wilson, Allen, Robinson & Poe 1978), Chinook salmon, *Oncorhynchus tshawytscha* 2.2% (Delong, Halver & Mertz 1962), Chum salmon, *Oncorhynchus keta* 3.0% (Akiyama, Arai & Murai 1985), European sea bass, *Dicentrarchus labrax* 2.3–2.6% (Tibaldi & Tulli 1999), Grass carp, *Ctenopharyngodon idella* 3.6% (Gao, Yang, Liu, Chen, Guo, Yu & Tian 2014), Hybrid striped bass, *Morone chrysops* × *M. saxatilis* 3.0% (Keembiyehetty & Gatlin 1997), Indian major carp, *Cirrhinus mrigala* (Hamilton) 4.5% (Ahmed, Khan & Jafri 2004), Indian major carp, *L. rohita* 3.8–4.2% (Fatma Abidi & Khan 2008), Indian catfish, *H. fossilis* 3.2% (Ahmed 2007), Japanese Flounder, *Paralichthys olivaceus* 3.2% (Alam, Teshima, Koshio, Yokoyama & Ishikawa 2003), Jian carp, *Cyprinus carpio* var. Jian 5.13% (Feng, Peng, Wu, Hu, Jiang, Liu, Jiang, Li & Zhou 2013), Milkfish, *Chanos chanos* 4.5% (Borlongan 1991), Nile tilapia, *Oreochromis niloticus* 3.18% (Liebert & Benkendorff 2007), Rainbow trout, *Oncorhynchus mykiss* 2.6% (Bodin *et al.* 2008), Red drum, *Sciaenops ocellatus* 2.3% (Boren & Gatlin 1995) and Striped bass, *Morone saxatilis* 2.5% (Small & Soares 1999).

Large yellow croaker (*Larmichthys crocea*) is an important economically marine fish species that has been popularly cultured in southeast China (Shen & Heino 2014). One of the prerequisites for developing high efficiency diet for *L. crocea* requires complete knowledge of its nutritional requirement (Zhang, Ai, Mai, Tan, Li & Zhang 2008). Several studies have investigated the nutritional requirements and physiological characteristics of the *L. crocea* (Ai, Xu, Mai, Xu, Wang & Zhang 2011; Zhao, Ai, Mai, Zuo & Luo 2013; Zuo, Ai, Mai & Xu 2013; Wang, Ai, Mai, Xu & Zuo 2014). However, except for lysine and methionine (Mai, Wan, Ai, Xu, Liufu, Zhang, Zhang & Li 2006; Zhang *et al.* 2008), no information is available on EAAs requirement of this fish. Hence, the purpose of the present study was to quantify the optimum dietary threonine requirement for maximum growth of the juvenile *L. crocea* and to examine the effects of dietary threonine on growth performance, feed utilization and body composition.

Materials and methods

Experimental diets

The ingredient composition of the experimental diets is presented in Table 1. The basal diet was formulated to contain a combination of fish meal, soybean meal, yeast meal and gelatin as the intact protein sources. Fish oil (Gaolong Industrial, Fujian, China), corn oil (Shangdong Liuhe Group, Shangdong, China) and lecithin (Shangdong Liuhe Group) were used as lipid sources, and dextrin (Guangzhou Pharmaceuticals, China) was used as a carbohydrate source. The protein source was supplemented with crystalline amino acids (CAA, L-form, purity ≥99%; Kayon Biological Technology, Shanghai, China) premix to simulate the whole body amino acid pattern of fish tissue (Mai *et al.* 2006). The basal diet contained about 45.0% crude protein and 11.7% crude lipid, which have been evidenced optimal for growth of *L. crocea* (Duan, Mai, Zhong, Si & Wang 2001). All diets were kept isonitrogenous (20 MJ kg^{-1}, gross energy) by decreasing the levels of non-EAAs (glutamic acid) as the threonine levels increased with a 0.35% increment. The threonine contents of six experimental diets were 0.71%, 1.03%, 1.42%, 1.75%, 2.13% and 2.46% respectively (Table 2). The diets were prepared as described by Mai *et al.* (2006) with some adjustment. Dietary ingredients were ground through a 320-μm screen. All the dry ingredients were blended thoroughly, and stirred with oil and water until homogenous. The pH of diets was adjusted to 7.0–7.5 with sodium hydroxide (Wilson, Harding & Garling 1977). The pellets (1.5 × 2.0 mm and 2.5 × 3.0 mm) were obtained using a pelletizer (F-26(II); South China University of Technology, Guangzhou, China) and dried for about 12 h in a ventilated oven at 45°C. After drying, the diets were sealed in plastic bags and stored frozen (−20°C) prior to use in the feeding trial.

Experimental procedure

The experiment was conducted at Xiangshan Bay of Ningbo, Zhejiang Province, Southern China. Juvenile *L. crocea* were obtained from a local commercial hatchery. Upon arrival, they were reared in floating sea cages (3.0 × 3.0 × 3.0 m), and acclimated to the control diet (Diet 1) for 2 weeks. At the end of the acclimation, the fish were fasted for 24 h and weighed after being anesthetized with eugenol (1: 10 000; Shanghai Reagent, China). Uniform size healthy ones (6.00 ± 0.10 g) were randomly segregated to 18 cages (1.0 × 1.0 × 1.5 m), distributing 60 numbers in each cage. Each diet was randomly assigned to triplicate

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

	Diets					
Ingredients (%)	**D1 (0.71)**	**D2 (1.03)**	**D3 (1.42)**	**D4 (1.75)**	**D5 (2.03)**	**D6 (2.46)**
Fish meal*	13.00	13.00	13.00	13.00	13.00	13.00
Soybean meal†	13.00	13.00	13.00	13.00	13.00	13.00
Yeast meal‡	3.00	3.00	3.00	3.00	3.00	3.00
Gelatin§	10.00	10.00	10.00	10.00	10.00	10.00
Premix¶	37.74	37.74	37.74	37.74	37.74	37.74
Mineral premix‖	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix**	2.00	2.00	2.00	2.00	2.00	2.00
Amino acid premix††	17.51	17.51	17.51	17.51	17.51	17.51
L-threonine	0.00	0.35	0.70	1.05	1.40	1.75
Glutamic acid	1.75	1.40	1.05	0.70	0.35	0.00
Approximate composition (%)						
Moisture	10.2	9.9	9.9	10.5	10.4	10.2
Crude protein	45.0	45.5	45.7	45.0	45.8	45.5
Crude lipid	11.7	11.8	12.1	11.7	12.5	12.2
Ash	7.0	7.4	7.9	7.0	6.9	7.3
Gross energy (kJ g^{-1})	19.9	20.1	19.8	20.4	20.5	19.9

*Cishan Fisheries, Qingdao, China; protein 67.5; lipid 7.76% (% dry matter).

†Liulu Oil Lit., Haerbin, China; protein 46.2; lipid 1.7% (% dry matter).

‡Cishan Fisheries, Qingdao, China; protein 57.1; lipid 3.5 (% dry matter).

§Rousselot Gelatin Lit., Guangzhou, China; protein 95.3; lipid 1.9 (% dry matter).

¶Premix contained (%): dextrin, 23; menhaden fish oil, 6; soybean oil, 2.5; lecithin, 2.5; attractant (glycine and betaine), 0.3; mould inhibitor (contained 50% calcium propionic acid and 50% fumaric acid), 0.1; ethoxyquine, 0.05; microcrystalline cellulose, 3.29.

‖Mineral premix (mg or g kg^{-1} diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2{\cdot}6H_2O$ (1%), 50 mg; $CuSO_4{\cdot}5H_2O$, 10 mg; $FeSO_4{\cdot}H_2O$, 80 mg; $ZnSO_4{\cdot}H_2O$, 50 mg; $MnSO_4{\cdot}H_2O$, 60 mg; $MgSO_4{\cdot}7H_2O$, 1200 mg; Ca $(H_2PO_4)_2{\cdot}H_2O$, 8000 mg; NaCl, 100 mg; Zoelite, 10.447 g.

**Vitamin premix were based on Mai *et al.* (2006).

††Amino acid premix (g kg^{-1} dry diet): arginine 17.5; histidine 3.9; isoleucine 9.1; leucine 21.1; lysine 19.9; methionine 9.6; phenylalanine 8.8; valine 9; aspartic acid 18.8; serine 7.8; alanine 12.2; cystine 0.9; tyrosine 7.2; glutamic acid 29.3.

cages. The fish were slowly hand-fed to apparent satiation twice daily at 05:00 and 17:00 for 8 weeks. Fish were considered satiated when they did not show a feeding behaviour towards the pellets. Daily consumption of feed and the number and weight of dead fish were recorded for each cage. During the experiment, the water temperature fluctuated from 26.5 to 31.0°C, salinity from 25 to 28 g L^{-1} and dissolved oxygen was ≥6.5 mg L^{-1}.

Sample collection and chemical analysis

At the end of the experiment, the fish were fasted for 24 h before sampling. The total number and mean body weight of the fish in each cage were measured. Ten fish from each cage were randomly collected and stored frozen (−20°C) for analysis of whole body composition. Proximate composition analysis of the feed ingredients, experimental diets and fish samples were analysed in duplicate using standard methods (AOAC 1995). Samples of diets and fish were dried to a constant weight at 105°C to determine moisture. Crude protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method (Kjeltec 2300; Tecator, Hoganas, Sweden); crude lipid by ether extraction using Soxhlet method (Soxtec 2050; Tecator); ash by combustion at 550°C and gross energy was determined by adiabatic bomb calorimeter (PARR, 1281; PARR, Moline, IL, USA).

To evaluate the effect of dietary treatments on the amino acid profile of fish muscle tissue, at the end of feeding trial three fish from each cage were collected, muscle were dissected from the back of the fish carcass and pooled. Amino acid composition of the feed ingredients, experimental diets and muscle tissue of fish was analysed in a professional laboratory using an automatic amino acid analyser (Biochrom 30+; Biochrom, Cambridge, UK) equipped with a column (Biochrom custom Na cation exchange resin).

Table 2 Amino acid composition of experimental diets (% dry matter)*

Amino acids	Diets						Whole body protein (45%)†
	D1	D2	D3	D4	D5	D6	
EAAs							
Threonine	0.71	1.03	1.42	1.75	2.13	2.46	1.90
Histidine	0.90	0.84	0.87	0.85	0.83	0.85	0.88
Isoleucine	1.80	1.77	1.79	1.75	1.82	1.78	1.76
Leucine	3.79	3.73	3.76	3.74	3.81	3.76	3.54
Lysine	3.52	3.50	3.53	3.49	3.50	3.56	3.48
Methionine	1.36	1.34	1.33	1.35	1.32	1.32	1.33
Phenylalanine	1.82	1.77	1.79	1.80	1.75	1.79	1.76
Arginine	3.50	3.55	3.57	3.51	3.56	3.53	3.54
Valine	1.99	1.95	2.01	2.00	1.98	1.99	1.97
Non-essential amino acids							
Aspartic acid	4.01	4.09	4.11	4.05	4.03	4.08	4.07
Serine	1.93	1.96	1.99	1.95	1.97	2.01	1.83
Glycine	2.52	2.50	2.54	2.49	2.55	2.50	2.47
Alanine	2.88	2.96	2.93	2.97	2.91	2.90	2.94
Cystine	0.30	0.33	0.34	0.33	0.31	0.35	0.20
Tyrosine	1.25	1.30	1.29	1.27	1.33	1.35	1.31
Glutamic acid	9.03	8.75	8.37	7.99	7.62	7.24	6.17

*Tryptophan was not determined in this study.
†Amino acid content of *Larmichthys crocea* whole body (Mai *et al.* 2006).

Calculation and statistical analysis

The parameters were calculated as follows:

$$\text{Survival}(\%) = 100 \times N_t/N_i$$

$$\text{Specific growth rate}(\text{SGR}, \%\text{day}^{-1}) = 100 \times (\text{Ln}W_t - \text{Ln}W_i)/t$$

$$\text{Protein efficiency ratio (PER)} = (W_t - W_i)/\text{protein intake in dry basis in g}$$

$$\text{Feed efficiency (FE)} = (W_t - W_i)/D_f$$

$$\text{Nitrogen retention (NR)} = (\text{N gained per fish} \times 100)/(\text{N given per fish})$$

where W_t is the mean final body weight (g), W_i is the mean initial body weight (g), t is the experimental duration in d and D_f is dry diet intake (g). N_t and N_i represent initial and final numbers of fish in each cage respectively.

All data were analysed by one-way analysis of variance (ANOVA) using the software of Statistics (SPSS 11.5 for windows, Chicago, IL, USA). When the ANOVA identified differences among groups ($P < 0.05$), multiple comparisons among means were made with Tukey's multiple range test. All data are presented as mean ± SEM. The optimum dietary threonine requirement for juvenile *L. crocea* was estimated using second-degree polynomial regression analysis ($Y = aX^2 + bX + c$) (Zeitoun, Ullrey, Magee, Gill & Bergen 1976), which is the better fitting model for growth and feed utilization data. The higher coefficient of determination (R^2) and the break point obtained represented the optimum threonine requirement of the fish.

Results

Survival and growth

Survival, feed efficiency and growth performance of *L. crocea* are shown in Table 3. Fish easily accepted the experimental diets and maintained normal behaviour during the trial period. There was no significant differences ($P > 0.05$) in survival (ranging from 99% to 100%) in fish fed all the test diets, and no deficient signs were observed among all treatments. Specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and nitrogen retention (NR) had a similar change pattern with the rising threonine level. Growth performances (SGR, FE, PER and NR) increased significantly with increasing level of diet threonine up to 1.75%, and thereafter declined as the threonine level increased ($P < 0.05$). The highest SGR

Table 3 Effect of dietary threonine levels on growth performance of juvenile *Larmichthys crocea* fed diets for 8 weeks

Diet no	Threonine level (% dry diet)	Final weight (g)	SGR (% day^{-1})	FE	PER	NR	Survival (%)
D1	0.71	13.19 ± 0.23^{a}	1.41 ± 0.03^{a}	0.42 ± 0.02^{a}	1.05 ± 0.06^{a}	13.57 ± 0.61^{a}	99.4 ± 0.57
D2	1.03	15.65 ± 0.24^{b}	1.71 ± 0.02^{b}	0.60 ± 0.01^{b}	1.46 ± 0.02^{b}	19.05 ± 0.68^{b}	99.4 ± 0.57
D3	1.42	19.02 ± 0.23^{cd}	2.06 ± 0.02^{cd}	0.71 ± 0.02^{cd}	1.72 ± 0.05^{c}	21.12 ± 0.58^{b}	100 ± 0.00
D4	1.75	20.93 ± 0.33^{e}	2.23 ± 0.02^{e}	0.78 ± 0.02^{d}	1.93 ± 0.04^{d}	26.69 ± 0.36^{c}	100 ± 0.00
D5	2.13	19.93 ± 0.39^{de}	1.96 ± 0.03^{de}	0.75 ± 0.01^{cd}	1.84 ± 0.03^{cd}	24.83 ± 0.19^{c}	99.4 ± 0.57
D6	2.46	18.00 ± 0.41^{c}	1.92 ± 0.04^{c}	0.70 ± 0.01^{c}	1.71 ± 0.03^{c}	24.50 ± 0.42^{c}	100 ± 0.00
ANOVA							
F value		84.628	98.926	69.039	74.727	91.681	0.600
P value		0.000	0.000	0.000	0.000	0.000	0.701

Values are means ± SEM of triplicate groups. Values with different superscripts in the same column are significantly different determined by Tukey's test ($P < 0.05$).
SGR (% day^{-1}) = specific growth rate; FE, feed efficiency; PER, protein efficiency ratio; NR = nitrogen retention, initial weight of fish was 6.00 ± 0.10 g, and crude protein of whole body was 16.67% (on a wet matter basis).

(2.23%), FE (0.78), PER (1.93) and NR (26.69) were observed in the fish fed Diet 4 containing 1.75% threonine (Table 3).

Body composition

Table 4 presents the body composition of juvenile *L. crocea* fed the test diets containing variational levels of threonine for 8 weeks. No significant differences were observed in body protein (14.2–15.1%), lipid (4.6–5.5%), ash (3.7–4.2%) and moisture (76.1–77.9%) among dietary treatments ($P > 0.05$).

Amino acid composition in fish muscle

As shown in Table 5, except for threonine, the levels of rest EAAs in fish muscle were very stable and independent of the dietary treatments. Fish fed the basal diet (0.71% threonine) had significantly lower threonine (2.94% of body protein) in muscle tissue ($P < 0.05$). It increased with increasing dietary threonine up to 1.42%, and then levelled off. However, the fish muscle threonine which supplemented with threonine was not significantly affected by the dietary threonine level ($P > 0.05$).

Dietary threonine requirement of juvenile *L. crocea*

Second-order polynomial regression analysis on the base of SGR, FE and NR indicated that the optimum requirements of dietary threonine were 1.86%, 1.90% and 2.06% of dry diet, corresponding to 4.13%, 4.22% and 4.58% of dietary protein (Figs 1–3) respectively. The relationship was described by the following equations: $Y = -0.6165X^2 + 2.2957X + 0.0543$, $R^2 = 0.9629$; $Y = -0.2471X^2 + 0.9376X - 0.1147$, $R^2 = 0.9645$; $Y = -6.5616X^2 + 27.017X - 2.3055$, $R^2 = 0.9171$.

Table 4 Final whole body composition of *Larmichthys crocea* fed experimental diets with graded levels of threonine for 8 weeks

Diets (threonine %)	Crude protein (%)	Crude lipid (%)	Ash (%)	Moisture (%)
D1 (0.71)	14.7 ± 0.37	5.1 ± 0.29	4.0 ± 0.16	77.1 ± 0.95
D2 (1.03)	14.5 ± 0.38	5.1 ± 0.42	3.8 ± 0.10	77.3 ± 1.19
D3 (1.42)	14.2 ± 0.15	4.6 ± 0.16	4.2 ± 0.17	77.9 ± 0.52
D4 (1.75)	14.6 ± 0.17	5.5 ± 0.47	3.7 ± 0.14	76.1 ± 0.43
D5 (2.13)	14.4 ± 0.12	4.8 ± 0.09	4.1 ± 0.06	76.9 ± 0.59
D6 (2.46)	15.1 ± 0.33	5.5 ± 0.11	4.0 ± 0.06	76.6 ± 0.54
ANOVA				
F value	1.288	1.491	2.448	0.632
P value	0.332	0.264	0.095	0.680

Values are means ± SEM of triplicate groups. Values with different superscripts in the same column are significantly different determined by Tukey's test ($P < 0.05$).

Table 5 The essential amino acid profile in the muscle of juvenile *Larmichthys crocea* fed diets with graded levels of threonine for 8 weeks (% dry matter)

	Diets (threonine %)						ANOVA	
Amino acids	**D1 (0.71)**	**D2 (1.03)**	**D3 (1.42)**	**D4 (1.75)**	**D5 (2.13)**	**D6 (2.46)**	***F* value**	***P* value**
Arginine	4.45 ± 0.04	4.47 ± 0.20	4.46 ± 0.02	4.44 ± 0.22	4.41 ± 0.14	4.52 ± 0.07	0.076	0.995
Histidine	1.51 ± 0.04	1.50 ± 0.06	1.47 ± 0.07	1.47 ± 0.05	1.50 ± 0.04	1.44 ± 0.01	0.312	0.897
Isoleucine	3.44 ± 0.05	3.43 ± 0.13	3.48 ± 0.08	3.37 ± 0.15	3.45 ± 0.12	3.44 ± 0.09	0.123	0.984
Leucine	5.57 ± 0.04	5.56 ± 0.02	5.66 ± 0.10	5.66 ± 0.13	5.45 ± 0.13	5.45 ± 0.05	1.094	0.412
Lysine	6.55 ± 0.18	6.62 ± 0.20	6.64 ± 0.12	6.59 ± 0.16	6.55 ± 0.07	6.63 ± 0.18	0.055	0.998
Methionine	2.35 ± 0.01	2.30 ± 0.05	2.31 ± 0.03	2.22 ± 0.06	2.26 ± 0.03	2.35 ± 0.02	1.852	0.177
Phenylalanine	2.75 ± 0.01	2.73 ± 0.04	2.76 ± 0.03	2.72 ± 0.06	2.71 ± 0.06	2.73 ± 0.08	0.116	0.987
Threonine	2.94 ± 0.01^{a}	3.09 ± 0.03^{ab}	3.15 ± 0.07^{b}	3.16 ± 0.04^{b}	3.14 ± 0.02^{b}	3.12 ± 0.03^{b}	4.874	0.012
Valine	3.61 ± 0.16	3.61 ± 0.16	3.59 ± 0.02	3.60 ± 0.18	3.51 ± 0.09	3.51 ± 0.10	0.136	0.981

Values are means ± SEM of triplicate groups. Values with different superscripts in the same column are significantly different determined by Tukey's test ($P < 0.05$).

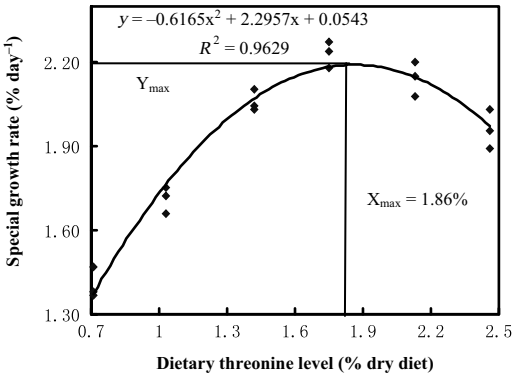

Figure 1 Second-degree polynomial regression analysis of specific growth rate (SGR; % day^{-1}) against varying levels of dietary threonine. Each data represent the mean of three triplicate groups of fish ($n = 3\times3$).

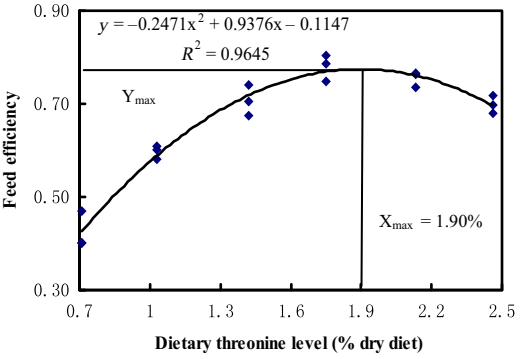

Figure 2 Second-degree polynomial regression analysis of feed efficiency (FE) against varying levels of dietary threonine. Each data represent the mean of three triplicate groups of fish ($n = 3\times3$).

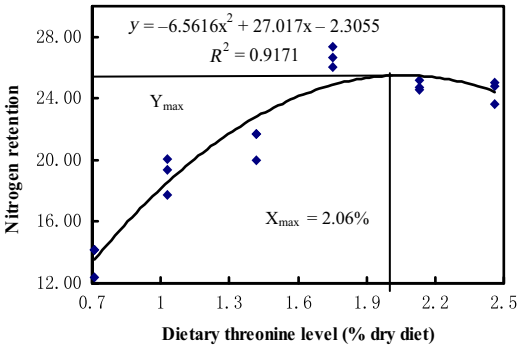

Figure 3 Second-degree polynomial regression analysis of nitrogen retention (NR) against varying levels of dietary threonine. Each data represent the mean of three triplicate groups of fish ($n = 3\times3$).

Discussion

In the current study, the effect of graded levels of threonine on growth performance, feed utilization and body composition were investigated on *L. crocea*. The results showed that fish fed threonine-deficient diet (Diet 1) displayed reduced growth and feed efficiency, but supplementing threonine in the diets significantly improved growth performance and feed utilization of *L. crocea*. It suggests that threonine is essential for growth of *L. crocea*, and the fish is able to utilize the crystalline form of threonine. In this study, the survival rate was not significantly influenced by the dietary threonine levels. Similar results were reported for red drum, *S. ocellatus* (Boren & Gatlin 1995) and European sea bass, *D. labrax* (Tibaldi & Tulli 1999). However, Borlongan (1991) reported

that the survival of milkfish, *C. chanos* fed a threonine-deficient diet (1.13% of dry diet) was significantly lower than others supplemented with threonine.

The present study indicated that the optimum dietary threonine requirement of juvenile *L. crocea* was estimated ranging from 1.86% to 2.06% of dry diet (4.13% to 4.58% of dietary protein), based on second-order polynomial regression relationship of SGR, FE and NR to dietary threonine levels. These estimated values were within the range of threonine requirements (1.8% to 5.1% of dietary protein) reported in other fish, but little higher than the requirement reported for species of Perciformes, such as European sea bass, *D. labrax* 2.3–2.6% (Tibaldi & Tulli 1999), Hybrid striped bass, *M. chrysops* × *M. saxatilis* 3.0% (Keembiyehetty & Gatlin 1997), Nile tilapia, *O. niloticus* 3.18% (Liebert & Benkendorff 2007), Red drum, *S. ocellatus* 2.3% (Boren & Gatlin 1995), Striped bass, *M. saxatilis* 2.5% (Small & Soares 1999). The wide variability among different aquaculture species, and even for the same species, are probably due to differences in following factors: (1) the use of various ingredients for basal diets such as purified or practical ingredients and the reference amino acid pattern (Borlongan 1991; Ravi & Devaraj 1991); (2) fish age, size and intensity, laboratory conditions, containing feeding regime, feed allowance, water temperature and salinity (Delong *et al.* 1962; Rodehutscord, Jacobs, Pack & Pfeffer 1995; Keembiyehetty & Gatlin 1997; Helland & Grisdale-Helland 2011); (3) different response criteria and statistical tests to estimate the requirement value (Rodehutscord, Becker, Pack & Pfeffer 1997; Liebert & Benkendorff 2007; Grisdale-Helland, Lemme & Helland 2013); (4) nutrient content, digestibility and amino acid profile, amino acid sources (Ahmed *et al.* 2004). Nevertheless, a comparison between Atlantic salmon and rainbow trout fry of the requirements for threonine showed little difference although then are different species and have such different growth rates (Bodin *et al.* 2008).

Certain dietary EAA deficiencies have been claimed to result in inferior growth and feed efficiency in most studies (NRC 2011). In the present study, there was a marked decline in growth performance and feed utilization in juvenile *L. crocea* fed the low-threonine diet. The lowest SGR, FE, PER and NR were observed in fish fed the minimum threonine diet (0.71% of dry diet). Similar growth depression was also observed in the studies on several other cultured fish species (Akiyama *et al.* 1985; Rodehutscord *et al.* 1995; Grisdale-Helland *et al.* 2013). Ahmed *et al.* (2004) reported that except for poor growth and a low feed efficiency, no pathological symptoms were observed in fingerling Indian major carp fed a threonine-deficient diet. However, Gao *et al.* (2014) reported that threonine deficiency appear some adverse effects on body protein synthesis, intestine development and integrity for grass carp, *C. idella*. With further increases in dietary threonine levels from 1.75% to 2.46%, SGR, FE and PER significantly decreased while NR did not significantly decrease. The reduction in growth of *L. crocea* fed high levels of threonine could be attributed to amino acid toxicity and amino acid catabolism (Alam *et al.* 2003). It has been reported that the disproportionate intake amino acids affects the absorption and utilization of other amino acids or decrease the diets' palatability (Harper, Benevenga & Wohlhueter 1970; Borlongan & Coloso 1993; Murthy & Varghese 1996). However, Helland and Grisdale-Helland (2011) reported that growth rate and feed efficiency were not affected by excess threonine in the salmon diets.

In the current study, the whole body composition was not significantly influenced by the dietary threonine level, which is in agreement with the study on Atlantic salmon smolts, *S. salar* (Grisdale-Helland *et al.* 2013) and Pacific white shrimp, *Litopenaeus vannamei* reared in seawater (Zhou *et al.* 2013). While in the study of Indian major carp *C. mrigala*, whole body crude protein and crude fat were significantly varied with the dietary threonine level (Ahmed *et al.* 2004). The level of threonine in fish whole body, muscle, serum or plasma have been used as an index of dietary threonine status in several experiments (Wilson *et al.* 1978; Boren & Gatlin 1995; Keembiyehetty & Gatlin 1997; Tibaldi & Tulli 1999; Alam *et al.* 2003; Bodin *et al.* 2008; Gao *et al.* 2014). In the current study, threonine concentration in muscle of *L. crocea* was significantly affected by the dietary threonine level. This could be due to the difference in free threonine in the amino acid pool in the tissues of fish. Increased threonine levels in diet have a long-term influence on the amino acid pool in fish serum or plasma (Boren & Gatlin 1995; Tibaldi & Tulli 1999) or whole body (Bodin *et al.* 2008) and seems to be useful for estimating the dietary threonine requirement (Tibaldi & Tulli 1999).

However, other amino acid was not significantly affected by the dietary threonine level.

In conclusion, results of the present investigation indicated that dietary threonine improved growth performance and feed utilization of *L. crocea*. Based on a second-degree polynomial regression analysis of the SGR, FE and NR data, the optimal dietary threonine requirement for juvenile *L. crocea* was estimated to be 1.86%, 1.90% and 2.06% of dry diet (4.13%, 4.22% and 4.58% of dietary protein) respectively.

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