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Dietary arginine requirement of juvenile cobia (*Rachycentron canadum*)

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Abstract

A 9-week feeding trial was conducted to estimate the dietary requirement of arginine in juvenile cobia in indoor flow-through and aerated aquaria. Six isonitrogenous and isoenergetic practical diets were formulated to contain graded levels of arginine ranging from 1.76% to 3.75% (dry weight) at about 0.4% increments replaced by equal proportions of glycine. Survival was not significantly different among dietary treatments. Specific growth rate (SGR) and feed efficiency ratio (FER) increased with increasing dietary arginine up to the 2.96% diet ($P < 0.05$), and thereafter declined. The whole body crude protein content was significantly affected by dietary arginine ($P < 0.05$), while moisture, crude lipid and ash showed no significant differences among dietary treatments. The essential amino acid contents of muscle were not significantly affected by dietary arginine. The serum nitric oxide synthase activities in fish fed diets with arginine from 2.18% to 3.75% were significantly higher than activities in fish fed the diet with 1.76% arginine ($P < 0.05$). On the basis of SGR and FER, the optimal dietary arginine requirements of juvenile cobia were estimated to be 2.85% of the diet (6.20% of dietary protein) and 2.82% of the diet (6.13% of dietary protein), respectively, using second-order polynomial regression analysis.

Keywords: cobia, arginine, requirement, feeding and nutrition

Introduction

Fish meal is the conventional protein source in aqua feeds for carnivorous species. However, fish meal production did not increase as much as aquaculture production, which has continued to increase over the past 20 years (Hardy 2010). To reduce the reliance on fish meal, plant feedstuffs have been used as components of commercial fish feeds (Stone 2003; Gatlin *et al.* 2007). Since most plant protein sources do not meet the essential amino acid (EAA) requirement of fish, crystalline amino acids are being increasingly used commercially to meet the EAA requirements of fish, and becoming key components of cost-effective fish feed formulations (Fournier, Gouillou-Coustans, Métailler, Vachot, Moriceau, Le Delliou, Huelvan, Desbruyeres & Kaushik 2003; NRC 2011).

Arginine is an EAA for optimal growth of fish (Wilson 1989) and limited in some plant protein sources or casein-based diets (Luo, Liu, Mai & Tian 2004). Furthermore, arginine is involved in many metabolic pathways such as protein synthesis, urea production, metabolism of glutamic acid and proline, and synthesis of creatine and polyamines (Kaushik, Fauconneau, Terrier & Gras 1988). The quantitative arginine requirement of fish species ranges from 3.3% to 6.8% of dietary protein (Luo *et al.* 2004).

The cobia, *Rachycentron canadum* (L.), is regarded as a great potential species for offshore cage culture in tropical and subtropical waters. In recent studies, dietary protein, methionine and lysine requirements have been estimated for this fish (Chou, Su & Chen 2001; Zhou, Wu, Tan, Chi & Yang 2006; Zhou, Wu, Chi & Yang 2007). Zhao, Cao, Wu, Tan, Zhou, Liang and Yang (2007) quantified the arginine requirement of cobia (2.38% diet) using casein and gelatin as intact protein sources. Commercial ingredients (white fish meal and corn gluten meal) were selected as intact protein sources in the present experiment to re-evaluate the arginine requirement of cobia and

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to provide more data for commercial feed production.

Materials and methods

Diet preparation

Six isonitrogenous and isoenergetic diets, using white fish meal and corn gluten meal as protein sources and fish oil as a lipid source, were formulated to contain graded levels of arginine (1.76%, 2.18%, 2.57%, 2.96%, 3.33% and 3.75% of dry weight respectively) (Table 1). Dietary arginine was replaced by equal proportions of glycine. A mixture of crystalline, l-amino acids was supplemented to simulate the whole body amino acid pattern of cobia except for arginine (Table 2).

Ingredients were ground into powder through 320 μm mesh. All the ingredients were thoroughly mixed with fish oil and water to produce a stiff dough. The dough was then forced through a pelletizer (F-26 (II), South China University of Technology, China) and dried in a ventilated oven at 45°C. After drying, the diets were broken up and sieved into proper pellet size. The sizes of pellets were 1.5 × 2.0 mm and 2.5 × 3.0 mm. All diets were sealed in bags and stored at −15°C until used.

Experimental procedure

Experimental fish were obtained from a commercial farm in Sanya, Hainan, China. Prior to the feeding trial the fish were fed the control diet (diet 1) for 1 week to acclimate to the experimental diet

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

	Diet (arginine level %)					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredient	0.0	0.4	0.8	1.2	1.6	2.0
White fish meal*	23.00	23.00	23.00	23.00	23.00	23.00
Corn glutenmeal*	23.00	23.00	23.00	23.00	23.00	23.00
Amino acid premix†	9.27	9.27	9.27	9.27	9.27	9.27
Menhaden fish oil	9.50	9.50	9.50	9.50	9.50	9.50
Wheat meal	23.00	23.00	23.00	23.00	23.00	23.00
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
Mould inhibitor	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Soy Lecithin	2.50	2.50	2.50	2.50	2.50	2.50
Glycine	2.00	1.60	1.20	0.80	0.40	0.00
L-arginine¶	0.00	0.40	0.80	1.20	1.60	2.00
Microcrystalline cellulose	3.58	3.58	3.58	3.58	3.58	3.58
Proximate analysis (n = 3)						
Arginine (%)	1.76	2.18	2.57	2.96	3.33	3.75
Crude protein (%)	46.5	46.0	46.0	46.3	46.4	46.2
Crude fat (%)	13.9	13.7	13.6	14.0	13.8	13.6
Ash (%)	8.4	8.4	8.5	8.2	8.5	8.5
Moisture (%)	9.9	9.0	9.4	9.8	9.0	9.2

*White fish meal, obtained from Evergreen Group (Guangzhou, China), crude protein 69.3% dry matter, crude lipid 6.1% dry matter; corn gluten meal, obtained from Evergreen Group (Guangzhou, China), crude protein 64.5% dry matter, crude lipid 2.4% dry matter.

†Amino acid premix (g 100 g^{-1} diet): L-histidine, 0.44; L-isoleucine, 0.63; L-lysine, 2.14; L-methionine, 0.46; L-Phenylalanine, 0.31; L-threonine, 0.99; L-valine, 0.54; L-aspartic acid, 1.47; glycine, 0.3; L-cystine 1.43; L-tyrosine, 0.58. Amino acids obtained from Huayang Chemical (Hebei, China), the form of L-lysine is L-lysine·HCl.

‡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 $(H_2PO_3)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; zoelite, 15.45 g.

§Vitamin premix (mg or g kg^{-1} diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinal acetate, 32 mg; cholecalciferol, 5 mg; -tocopherol, 120 mg; ascorbic acid, 2000 mg; Choline chloride, 2000 mg; ethoxyquin 150 mg; wheat middling, 14.52 g.

¶L-argine obtained from Huayang Chemical Co. (Hebei, China).

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Table 2 Amino acid composition of ingredients (g 100 g^{-1} dry matter)

Amino acid	Amount in				Total	46% Whole body protein
	23 g FM	23 g CGM	23 g WM	AAP		
EAA						
Arginine	0.97	0.48	0.15	Variable	Variable	2.72
Histidine	0.31	0.29	0.07	0.44	1.10	1.10
Isoleucine	0.61	0.54	0.12	0.63	1.91	1.91
Leucine	1.04	2.40	0.18	0.00	3.62	3.47
Lysine	1.04	0.25	0.07	2.14	3.50	3.50
Methionine	0.39	0.35	0.04	0.46	1.23	1.23
Phenylalanine	0.54	0.90	0.14	0.31	1.88	1.88
Threonine	0.59	0.48	0.08	0.99	2.14	2.14
Valine	0.69	0.63	0.12	0.54	1.99	1.99
NEAA						
Aspartic acid	1.36	0.89	0.21	1.47	3.92	3.92
Serine	0.77	0.99	0.13	0.00	1.89	1.17
Glycine	1.19	0.47	0.11	0.30	2.07	2.07
Alanine	1.07	1.60	0.12	0.00	2.79	2.35
Cystine	0.08	0.14	0.07	1.43	1.71	1.71
Tyrosine	0.33	0.71	0.09	0.58	1.71	1.71
Gulmatic acid	2.51	4.24	0.44	0.00	7.20	5.83
Proline	0.98	1.09	0.23	0.00	2.29	2.29

Since tryptophan could not be detected after acid hydrolysis, the tryptophan level from intact protein has been calculated, which was around 0.28% of diet in the range of requirements 0.1–0.3% of diet recommended by NRC (2011).
FM, fish meal; CGM, corn gluten meal; WM, wheat meal; AAP, crystalline amino acid premix; EAA, essential amino acid; NEAA, non-essential amino acid.

and conditions. Before the start of the experiment, the fish were fasted for 24 h and weighed. Juvenile cobia (3.38 ± 0.04 g, mean initial weight) were randomly sorted into eighteen 500 L cylindrical fibreglass tanks with 30 fish in each tank. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed twice daily at 8:00 and 17:00 until apparent satiation on the basis of visual observation of fish feeding behaviour. During the 9 weeks feeding trial, feed consumption and the number and weight of dead fish were recorded every day. The water temperature fluctuated from 29 to 32°C, salinity from 26 to 30‰, and dissolved oxygen was 7 mg L^{-1}.

Sample collection and analysis

Sample collection

At the end of the experiment, the fish were fasted for 24 h before harvest. Total numbers and mean body weight of fish in each tank were determined. Five fish per tank were euthanized by MS-222 (250 mg L^{-1}), and then blood samples were collected immediately from the caudal vein using syringes. Following centrifugation (3500 *g*, 10 min, 4°C), the serum was separated and stored at −80°C until use. Muscle tissues were sampled from three fish per tank for amino acid analysis. Twenty fish at the beginning and five fish at the end per tank were sampled and stored at −20°C for the analysis of whole body composition.

Laboratory analysis

Dry matter, ash, crude protein, and lipid were determined according to the established methods of AOAC (2003): dry matter after drying in an oven at 105°C until constant weight; crude protein (N × 6.25) by Kjeldahl method after acid digestion; lipid by ether extraction using Soxhlet; ash content by incineration in a muffle furnace at 600°C for 12 h. For amino acid analysis, muscle samples, experimental diets and ingredients were freeze-dried and hydrolysed for 24 h in 6 N HCl at 110°C, then analyses carried out on automatic amino acid analyser (Biochrom 30 amino acid analyser; Biochrom Ltd., Cambridge, UK). Tryptophan could not be detected after acid hydrolysis. Total-NOS activity was determined using a total-NOS kit (NanJing Jiancheng Bioengineering Institute, China).

Calculation and statistical analysis

The following variables were calculated:

Survival (%) = 100 × (final amount of fish)/(initial amount of fish)

Specific growth rate (SGR, % day^{-1}) = 100 × (Ln W_f—Ln W_i)/t

Feed efficiency ratio (FER) = wet weight gain/dry diet intake

Where W_f and W_i were final and initial fish weights; t is the experimental duration in days.

Data were transformed, if necessary, after evaluating assumptions of normality, equality of variances and outliers, and subjected to one-way analysis of variance (ANOVA) using the software of the SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant differences in the means between dietary treatments were evaluated by Tukey's multiple range tests. Probabilities of $P < 0.05$ were considered significant. The second-order polynomial regression model, described by Zeitoun, Ullrey, Magee, Gill and Bergen (1976), was used to estimate the optimum dietary arginine requirement for cobia on the basis of SGR and FER after comparing the estimation coefficient (R^2) between broken-line regression model and second-order polynomial regression model.

Results

Growth performance

Survival rates of juvenile cobia for all treatments were over 90% and there were no significant differences among dietary treatments ($P > 0.05$) (Table 3). Fish fed the arginine-deficient diet (diet 1, 1.76% arginine) showed the lowest specific growth rate (SGR, 3.49% day^{-1}) and feed efficiency ratio (FER, 0.59). The SGR and FER of fish were significantly increased with dietary arginine supplementation ($P < 0.05$). The highest SGR (3.91% day^{-1}) and FER (0.77) were observed in the fish fed diet 4 (2.96% arginine), and thereafter declined ($P < 0.05$) (Table 3).

Based on SGR and FER, the optimum arginine requirement were estimated to be 2.85% of diet (6.20% of dietary protein) (Fig. 1) and 2.82% of diet (6.13% of dietary protein) (Fig. 2), respectively, using second-order polynomial regression analysis.

Body composition and muscle essential amino acid profile

The moisture, lipid and ash content of whole body in juvenile cobia were not significantly different among dietary treatments ($P > 0.05$). Fish fed the diet with 1.76% arginine showed the lowest whole body protein content, whereas the highest values were observed in fish fed the diet with 2.96% arginine ($P < 0.05$) (Table 4). Dietary arginine level did not affect essential amino acid profile in the muscle of cobia (Table 5).

Serum NOS activity

The lowest total-NOS activity was observed in fish fed diet 1 (1.76% arginine) ($P < 0.05$) and increased

Table 3 Effects of dietary arginine on survival, final weight, SGR and FER of juvenile Cobia (*Rachycentron canadum*) fed experimental diets for 9 weeks

Diet no.	Arginine (% dry diet)	Survival (%)	Final weight (g)	SGR (% day^{-1})	FER
1	1.76	96.7	33.8^{e}	3.49^{c}	0.59^{d}
2	2.18	96.7	37.1^{cd}	3.63^{b}	0.62^{cd}
3	2.57	95.6	41.5^{b}	3.83^{a}	0.72^{b}
4	2.96	98.9	44.3^{a}	3.91^{a}	0.77^{a}
5	3.33	94.4	38.8^{c}	3.70^{b}	0.65^{c}
6	3.75	92.2	36.0^{de}	3.60^{b}	0.61^{d}
ANOVA					
Pooled SEM		0.75	0.86	0.04	0.02
F-value		1.923	52.791	47.529	65.509
P-value		0.164	<0.001	<0.001	<0.001

Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by the Tukey's test ($P > 0.05$).
SEM, standard error of mean.

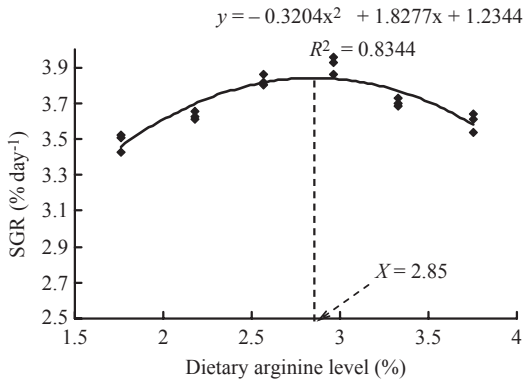

Figure 1 Relationship between specific growth rate (SGR) and dietary arginine level of juvenile cobia fed experimental diets for 9 weeks.

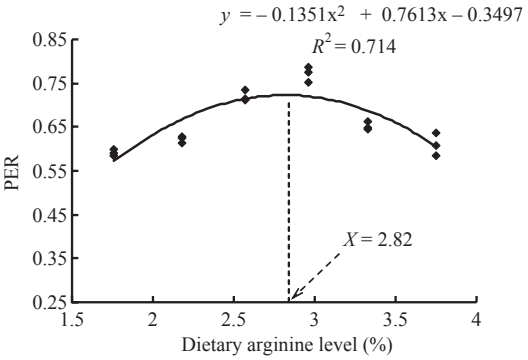

Figure 2 Relationship between feed efficiency ratio (FER) and dietary arginine level of juvenile cobia fed experimental diets for 9 weeks.

with the dietary arginine supplementation, but there were no significant differences among fish fed diet 2-6 (arginine ranging from 2.18% to 3.15% of diet) ($P > 0.05$) (Fig. 3).

Discussion

In this study, fish fed the control diet (diet 1, 1.76% arginine of diet) showed the lowest growth performance. Growth and feed utilization improved with the supplementation of dietary crystalline arginine. Results indicated arginine is an essential amino acid for cobia and juvenile cobia are able to utilize the crystalline arginine.

Dietary arginine requirements of juvenile cobia were determined to be 2.85% of diet (6.20% of dietary protein) and 2.82% of diet (6.13% of dietary protein) based on SGR and FER, respectively, using second-order polynomial regression analysis, which are similar to the values reported for Chinook salmon (6.0% of dietary protein) and Coho salmon (5.8% of dietary protein) (Klein & Halver 1970), but lower than the value reported for black sea bream (7.7–8.1% of dietary protein, Zhou, Xiong, Xiao, Shao, Bergo, Hua & Chai 2010). However, the results are higher than those reported for Asian sea bass (3.8% of dietary protein, Murillo-Gurrea, Coloso & Borlongan 2001), channel catfish (3.8% of dietary protein, Robinson, Wilson & Poe 1981), Mrigal carp (4.6% of dietary protein, Ahmed & Khan 2004), Nile tilapia (3.4% of dietary protein, Santiago & Lovell 1988) and rainbow trout (4.2% of dietary protein, Cho, Kaushik & Woodward 1992). In a previous study, the

Table 4 Effect of dietary arginine on body composition in juvenile cobia fed experimental diets for 9 weeks

Diet no.	Arginine (% dry diet)	Moisture (%)	Crude protein (% w.w.)	Crude lipid (% w.w.)	Ash (% w.w.)
1	1.76	75.67	14.13^{b}	5.57	3.56
2	2.18	74.50	14.91^{ab}	6.11	3.43
3	2.57	73.67	15.83^{a}	6.25	3.57
4	2.96	73.65	16.15^{a}	6.54	3.57
5	3.33	73.91	14.97^{ab}	6.13	3.67
6	3.75	75.27	15.01^{ab}	6.03	3.59
ANOVA					
Pooled SEM		0.28	0.19	0.10	0.04
F-value		2.01	6.608	2.192	0.741
P-value		0.149	0.004	0.123	0.608

Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by the Tukey's test ($P > 0.05$).

w.w., wet weight; SEM, standard error of mean

Table 5 Effect of dietary arginine level on the essential amino acid profile in the muscle of cobia (g 16 g N^{-1})

	Diets (arginine%)						ANOVA		
Amino acids	Diet 1 (1.76)	Diet 2 (2.18)	Diet 3 (2.57)	Diet 4 (2.96)	Diet 5 (3.33)	Diet 6 (3.75)	Pooled SEM	*F*-value	*P*-value
Threonine	4.29	4.36	4.46	4.38	4.43	4.42	0.02	2.928	0.059
Valine	4.70	4.86	5.01	4.82	4.81	4.89	0.03	2.341	0.106
Methionine	2.75	2.96	2.92	3.02	2.96	2.98	0.03	2.295	0.111
Isoleucine	3.88	4.19	4.07	4.39	4.39	4.24	0.07	1.481	0.267
Leucine	7.89	8.14	8.18	7.93	8.02	8.19	0.06	0.736	0.611
Phenylalanine	3.15	3.35	3.23	3.41	3.41	3.24	0.04	1.694	0.21
Histidine	2.27	2.18	2.26	2.18	2.19	2.25	0.02	0.684	0.644
Lysine	8.53	8.81	8.81	8.81	8.92	8.87	0.05	1.692	0.211
Arginine	6.08	6.10	6.20	6.18	6.19	6.24	0.02	2.371	0.102

Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by the Tukey's test ($P > 0.05$).
SEM, standard error of mean.

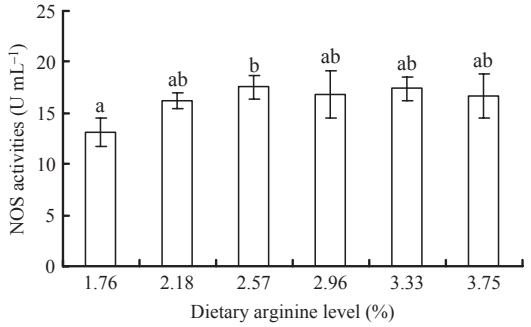

Figure 3 Relationship between total-NOS activity in serum and dietary arginine level of juvenile cobia fed experimental diets for 9 weeks.

arginine requirement for cobia (initial weight = 14.7 g) was estimated to be 5.17% of dietary protein which is lower than the results in this study (Zhao *et al.* 2007). The variances between two studies are possibly reflected by: fish of different sizes, initial weight 3.38 g compared with 14.7 g; different ingredients as intact protein sources, commercial ingredients compared with casein and gelatin; experimental conditions, indoor flow-through aquaria compared with outdoor net cage (Kim, Kayes & Amundson 1992). Although there is no information about glutamate being used for the endogenous synthesis of arginine in cobia, at least in channel catfish, glutamate appears to spare up to 33% of the arginine requirement (Buentello & Gatlin 2000). In this study, dietary arginine was replaced by equal proportions of glycine to avoid the potential effect by glutamate. However, the relationship between dietary arginine and glutamic acid needs further investigation in cobia.

Lower SGR and FER were observed in fish fed diets with high arginine level (3.33–3.75% arginine of diet) compared with those fed diets with optimum arginine (2.57–2.96% arginine of diet) in the present study. Similar results have also been found in hybrid Clarias (Singh & Khan 2007), Indian major carp (Abidi & Khan 2009) and rainbow trout (Fournier *et al.* 2003). Effects of dietary excess arginine on growth performance varied among fish species, and the hypothesis of the antagonism between lysine and arginine in fish seemed to be one reason that some species fed dietary excess arginine had reduced growth (Wan, Mai & Ai 2006). Arginine and lysine share the same brush border membrane carrier, and increasing concentrations of arginine resulted in reduced uptake of lysine in the intestine of Atlantic salmon (Berge, Bakke-McKellep & Lied 1999). Inversely some reports indicated that there was no dietary lysine-arginine antagonism in some fish species, such as hybrid striped bass (Griffin, Wilson & Brown 1994) and rainbow trout (Kim *et al.* 1992) according to growth and feed efficiency. In a previous study, lower SGR and survival were also observed in juvenile fish fed the highest arginine level diet (Zhao *et al.* 2007). Based on growth, the appropriate dietary arginine level should range from 2.57% of diet (5.58% of dietary protein) to 2.96% of diet (6.43% of dietary protein) for juvenile cobia. Whole body protein contents have a similar trend with growth performance in fish fed experimental diets, which is similar to previous

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reports in Indian major carp (Abidi & Khan 2009) and fingerling hybrid *Clarias* (Singh & Khan 2007). It indicates that appropriate supplementation of L-arginine in diet lacking of arginine for cobia could enhance body protein deposit; however, excess arginine has not been used to increase protein synthesis.

Essential amino acid contents of whole body significantly increased with dietary arginine supplementation in juvenile grouper and black sea bream (Luo, Liu, Mai, Tian, Tan & Yang 2007; Zhou *et al.* 2010). Mai, Wan, Ai, Xu, Liufu, Zhang, Zhang and Li (2006a), Mai, Zhang, Ai, Duan, Zhang, Li, Wan and Liufu (2006b) used amino acid content per 16 g N as a unit to investigate the different muscle amino acid pattern in fish fed different dietary EAA levels and results showed that lysine or methionine level in fish muscle was significantly influenced by dietary lysine or methionine level, respectively, whereas there were no significant differences in other EAAs among dietary treatments. Some reports showed that dietary amino acid did affect free amino acid content in some tissues of fish, especially in plasma (Berge, Lied & Sveier 1997; Schwarz, Kirchgessner & Deuringer 1998; Yamamoto, Unuma & Akiyama 2000; Alam, Teshima, Koshio & Ishikawa 2002; Berge, Sveier & Lied 2002), and took various times to return start level post feeding (Schuhmacher, Wax & Gropp 1997). In this study, arginine level (g 16 g N^{-1}, free and bound) in muscle of cobia showed increased trend with dietary arginine level, but there were no significant differences among dietary treatments. Juvenile cobia were fasted 24 h before sampling, and the results indicated that dietary arginine had a less than 24 h term influence on the amino acid pool in cobia muscle. Moreover, Yamamoto *et al.* (2000) found correlations in patterns between dietary and tissue EAA levels are high in plasma and very low in muscle and brain. Also, the concentration of free amino acids in plasma may be affected by the solubility of the protein source, amount of feed ingested and the sample timing post prandial (Berge *et al.* 2002).

Nitric oxide plays an important role in physiological processes controlling blood vessel tone, neurotransmission, platelet aggregation and adhesion, cell proliferation and macrophage activity (Eddy & Tibbs 2003). Arginine is a precursor for nitric oxide synthesis in fish (NRC 2011). In humans, there is a body of evidence suggesting that supplemental arginine up-regulates immune function and reduces the incidence of post-operative infection (Evoy, Lieberman, Fahey & Daly 1998). In this study, the lowest total-NOS activity in serum of cobia was observed in the dietary arginine deficient group (1.76% arginine of diet), and was improved with supplementation of dietary arginine. Similar results were observed in darkbarbel catfish (Feng, Ai, Xu, Mai & Zhang 2011). Buentello and Gatlin (1999) observed that cultured macrophages NO production in response to bacterial LPS was increased when arginine, citrulline and arginine plus glutamine were used as substrate and NO production is an arginine-dependent metabolic pathway in catfish. Dietary arginine appears to play an important role in NO production and have a close relationship with immune function in fish.

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