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RESEARCH PAPER

Dietary Arginine Requirement for Blunt Snout Bream (*Megalobrama amblycephala*) with Two Fish Sizes Associated with Growth Performance and Plasma Parameters

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Abstract

This trial was conducted to investigate the dietary arginine requirement in two sizes of blunt snout bream (*Megalobrama amblycephala*). Six isonitrogenous and isoenergetic (34% crude protein) diets containing graded levels of arginine from 8.3 to 33.6 g kg^{-1}. Triplicates of 30 fishes (body weight 52.50±0.18g) of size I or 20 fishes (body weight 101.85±1.85g) of size II were fed with one of six experimental diets. Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and weight gain (WG) significantly increased with the increase of arginine levels from 8.3 to 23.5 g kg^{-1} (size I) or from 8.3 to 18.1 g kg^{-1} (size II), and thereafter, kept decrease. The alanine aminotransferase (ALT) was significantly decreased with increase in dietary arginine levels from 8.3 to 23.5 g kg^{-1} in size I or 8.3 to 18.1 g kg^{-1} of the size II ($P<0.05$). Urea content first significantly increased ($P<0.05$), and showed the same trend in two sizes. Meanwhile, plasma arginine concentration of two sizes significantly increased with increasing dietary arginine from 8.3 to 23.5 g kg^{-1} ($P<0.05$), and significantly lower lysine content in plasma was observed in fish fed diets with arginine levels from 8.3 to 23.5 g kg^{-1} ($P<0.05$). Broken-line regression model analysis relation on the basis of SGR, the optimal dietary arginine requirement could be 20.3 g kg^{-1} in size I, and 17.9 g kg^{-1} in size II of dry matter, respectively. The results may explain the adverse effects of a deficient or an excess dietary arginine level on growth and health of fish in future. Furthermore, the present study also suggests that an optimum dietary arginine could play an important role in improving growth, and maintaining health for different size of fish, which would be useful in developing amino acid balanced commercial feeds for blunt snout bream.

Keywords: Arginine requirement; Blunt snout bream; Specific growth rate; Plasma arginine concentration.

Introduction

Blunt snout bream (*Megalobrama amblycephala*), an herbivorous fish, is one of the economically important endemic freshwater fish species with a long history in China. It is widely distributed in the middle and lower reaches of Yangtze River (Li *et al.,* 1993). The production is rapidly increasing approximately 0.72 million tons in 2014 (Ministry of Agriculture of the People's Republic of China, 2015), which is attributed to its rapid growth performance, excellent flesh quality and high larval survival rate. Besides, this species of fish has a bright future in aquaculture worldwide because of its compatibility with native species and adaptability to local environment.

In aquaculture, fish cannot synthesize all amino acids, and they must acquire essential amino acids (EAA) through formulated feed (Ren *et al.*, 2013). Arginine is one of the necessary amino acids for all fish species studied (NCR, 2011), which has played important physiological and nutritional roles (Alejandro and Delbert, 2000). It is impacted many metabolic pathways such as protein synthesis metabolism of glutamic acid (Lin *et al.*, 2015), proline, synthesis of creatine and polyamines (Kaushik *et al.,* 1988). In addition, arginine has been regarded as an EAA in diets of some fish species (Cowey, 1994), and arginine is treated as an efficient stimulant of growth hormone and insulin so that it indispensable in anabolic processes (Wan *et al.,* 2006). Meanwhile, arginine has been reported as an immunonutrient in high animal like humans, swine and rodents (Evoy *et al.,* 1998), and proved to influence immune function in fish as well (Buentello *et al.,* 2007). At present, arginine requirement studies in many kinds of commercial fish such as red drum (*Sciaenops ocellatus*) (Cheng *et al.,* 2011), black sea bream (*Sparus microcephalus*) (Zhou *et al.,* 2010), grouper (*Epinephelus coioides*) (Luo *et al.,* 2007), mrigal (*Cirrhinus mrigala*) (Ahmed and Khan, 2004) have been reported.

Recently, dietary arginine requirement of juvenile blunt snout bream (initial body weight of 2.60±0.1g) was quantified by Ren *et al*. (2013), but no information is available concerning dietary arginine requirement in other different growth stages of blunt snout bream up to now. Therefore, the present study was conducted to investigate the effects of dietary arginine level on growth performance and arginine metabolism in blunt snout bream, and quantify dietary arginine requirement in different growth stages of blunt snout bream.

Materials and Methods

Diet Preparation

Six isonitrogenous and isoenergetic (34 % crude protein) semi-purified experimental diets were formulated to contain graded levels of arginine (8.3, 13.0, 18.1, 23.5, 28.2 and 33.6 of dry weight, respectively) using soybean oil as a lipid source and casein, fish meal as protein sources, which were replaced by equal proportions of glycine (Table 1). A mixture of crystalline L-amino acids was supplemented to simulate the whole body amino acid pattern of blunt snout bream except for arginine (Table 2). All the ingredients were ground into powder through 60 mesh sieve, and then thoroughly mixed with soybean oil and water to produce a stiff dough, which was forced through a pelletizer (F-26 (II), South China University of Technology, China) and then dried in a ventilated oven at 30 °C. After drying, the diets were broken up and sieved into 1.5 × 2.0 mm pellet size and stored at -10 °C until used.

Experimental Fish and Feeding Trial

Experimental blunt snout bream in different sizes were obtained from Freshwater Fisheries Research Center (Jiangsu, China). There were two feeding trials. In Trial 1, all fishes were cultured in floating net cages (2.0 m × 2.0 m × 2.0 m, water depth: 1.5 m) in the pond (70 m × 30 m × 3 m, water depth: 2.7 m) and fed commercial diet three times a day for 2 weeks, which at prior to the experiment. At the starting of the feeding trial, all fishes were fasted for 24 h. Fishes with similar size (mean initial weight 52.50 ± 0.18 g) were selected and batch weighted. Then all fishes were randomly distributed to 18 net cages (2m × 1 m × 1 m) at a density of 30 fish per cage. Each experimental diet was randomly distributed to triplicate cages. Fishes were hand-fed three times daily at 8:00, 12:00 and 16:00 until apparent satiation on the basis of visual observation. During the 9 week feeding trial, the number and weight of dead fish and feed consumption were recorded every day. The water temperature fluctuated from 21 to 24 °C and dissolved oxygen was approximately 6 mg/l throughout the feeding trial. In Trial 2, the experimental conditions and procedure were the same with Trial 1 except for the fish initial body weight and quantity (101.85 ± 1.85 g, 20 fish per cage).

Sample Collection and Analysis

At the end of feeding trial, approximately 24 h after the last feeding, all fish were individually weighed and counted from each cage to calculate the survival, weight gain, feed efficiency ratio and feeding rate. Nine fish of each group (three fish per cage, 3 cages / each group) were anesthetized by MS-222 (150 mg/l), and then blood samples were collected immediately from the caudal vein with disposable medical syringes. Following centrifugation (3500 ×g, 10 min, 4℃), supernatants were removed and stored at -80 °C for subsequent plasma composition measurement. Nine fish from each group were killed, and then samples of liver and viscera were collected and weighed. All the samples were stored at -80℃ for further analysis.

Growth Performances

IBW and FBW are initial body weight and final body weight, and the following variables were calculated:

Specific growth rate, (SGR, % day^{-1}) = 100 × (Ln FBW−Ln IBW) / days.

Feed conversion ratio, (FCR) = Wet weight gain / Dry feed intake.

Protein efficiency ratio, (PER) = wet weight gain / protein intake.

Weight gain, (WG, %) = 100 × (FBW−IBW) / IBW.

Hepatosomatic index, (HSI, %) = 100 × (liver wet weight, g) / (body wet weight, g).

Condition factor, (CF, %) =100 × FBW / (body $length^3$).

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

Biochemical and Immune Parameters in Plasma

The plasma biochemical parameters such as aspartate aminotransferase (AST), alanine transaminase (ALT), albumin (ALB), total protein (TP) and Urea were measured by an automatic biochemical analyzer Mindary BS-400 (Shenzhen, China) using assay kits purchased from Shenzhen Mindary Bio-medical Electronics Co., Ltd., following previously described method (Liu *et al*., 2012; Wang *et al.*, 2014).

Table 1. Formulation and proximate composition of the experimental diets

Ingredients	Arginine level (g kg^{-1}, dry matter)					
	8.3	13.0	18.1	23.5	28.2	33.6
White fish meal[a]	50.0	50.0	50.0	50.0	50.0	50.0
Casein[a]	150.0	150.0	150.0	150.0	150.0	150.0
Gelatin[a]	37.5	37.5	37.5	37.5	37.5	37.5
Soybean oil	60.0	60.0	60.0	60.0	60.0	60.0
Soybean lecithin	10.0	10.0	10.0	10.0	10.0	10.0
Amino acid premix[b]	115.6	115.6	115.6	115.6	115.6	115.6
Vitamin premix[c]	20.0	20.0	20.0	20.0	20.0	20.0
Mineral premix[d]	50.0	50.0	50.0	50.0	50.0	50.0
Corn starch	350.0	350.0	350.0	350.0	350.0	350.0
Cellulose	76.9	76.9	76.9	76.9	76.9	76.9
Carboxymethyl cellulose	50.0	50.0	50.0	50.0	50.0	50.0
Ethoxyquin	5.0	5.0	5.0	5.0	5.0	5.0
Glycine	25.0	20.0	15.0	10.0	5.0	0.0
L-Arginine-HCl	0.0	5.0	10.0	15.0	20.0	25.0
Proximate analysis (g kg^{-1} of dry diet)						
Dry matter (DM)	892.1	892.2	892.2	892.4	892.3	892.5
Gross energy (kJ/g DM)	188.0	188.0	189.0	188.0	189.0	188.0
Crude protein	338.0	340.0	339.0	336.0	338.0	343.0
Crude lipid	83.1	84.0	85.4	82.6	83.5	84.2

[a] Casein, obtained from Hua'an Biological Products Lit. (Gansu, China), crude protein 90.2%; gelatin, obtained from Zhanyun Chemical Lit. (Shanghai, China), crude protein 91.3%; white fish meal, obtained from Copeinca (Lima, Peru), crude protein 67.4%, and crude lipid 9.3%.
[b] Amino acid premix (g/100 g diet): L-histidine, 0.31; L-isoleucine, 0.68; leucine, 0.87; L-lysine, 1.09; L-methionine, 0.43; L-phenylalanine, 0.66; L-threonine, 0.71; L-valine, 0.56; L-aspartic acid, 1.46; serine, 0.55; glycine, 1.37; alanine; 1.25; L-cystine 0.14; L-tyrosine,0.27; tryptophan, 0.12; glumatic acid, 1.11; proline 0.12. Amino acids obtained fromFeeer Co., LTD (Shanghai, China).
[c] Vitamin premix (IU or mg/kg of diet): vitamin A, 25,000 IU; vitamin D3, 20,000 IU;vitamin E, 200 mg; vitamin K3, 20 mg; thiamin, 40 mg; riboflavin, 50 mg; calciumpantothenate,100 mg; pyridoxine HCl, 40 mg; cyanocobalamin, 0.2 mg; biotin, 6 mg; folic acid, 20 mg; niacin, 200 mg; inositol, 1000 mg; vitamin C, 2000 mg; choline, 2000 mg, and cellulose was used as a carrier.
[d] Mineral premix (g/kg of diet): calciumbiphosphate, 20 g; sodiumchloride, 2.6; potassiumchloride, 5 g;magnesiumsulphate, 2 g; ferrous sulphate, 0.9 g; zinc sulphate, 0.06 g; cupric sulphate, 0.02; manganese sulphate, 0.03 g; sodium selenate, 0.02 g; cobalt chloride, 0.05 g; potassium iodide, 0.004; and zeolite was used as a carrier.

Table 2. Analysis of amino acid composition in the experimental diets

Amino acid	Arginine level (g kg^{-1}, dry matter)					
	8.3	13.0	18.1	23.5	28.2	33.6
EAA[a]						
Histidine	7.61	7.64	7.59	7.62	7.57	7.60
Isoleucine	14.87	14.89	14.93	15.11	14.90	14.90
Leucine	24.47	24.18	24.21	24.33	24.41	24.13
Lysine	24.21	24.42	24.25	23.92	25.01	24.38
Methionine	8.98	9.11	9.07	9.24	8.99	9.01
Phenylalanine	14.73	14.65	14.69	14.78	14.71	14.67
Threonine	14.11	14.08	14.21	14.10	14.11	14.06
Valine	15.73	15.76	15.69	15.70	15.70	15.68
NEAA[b]						
Aspartic acid	28.44	28.47	28.35	28.51	28.43	28.40
Serine	14.51	14.57	14.46	14.48	14.51	14.50
Glycine	24.97	24.99	25.03	25.01	24.95	25.12
Alanine	21.44	21.39	21.48	21.49	21.46	21.38
Cystine	1.92	1.87	1.89	2.04	2.01	1.90
Tyrosine	10.69	10.65	10.71	10.68	10.73	10.71
Gulmatic acid	46.43	46.39	46.44	46.40	46.53	46.35
Proline	18.11	18.21	18.07	18.10	18.11	18.22

[a] EAA, essential amino acid.
[b] NEAA, non-essential amino acid.

Amino Acid Concentrations Analyses

About the total amino acid contents analysis, the diet and ingredients were freeze-dried overnight, then hydrolyzed for 24 h in 6 N HCl at 110 °C. For free amino acid content analysis, the plasma were deproteinized by trichloroacetic acid (5%). After pretreatment, all the samples were analyzed with an Agilent-1100 amino acid determination system (Agilent Technologies Co., Ltd., Santa Clara, USA).

Tryptophan could not be detected after acid hydrolysis (Ren *et al.*, 2015).

Statistical Analysis

Data were subjected to one way analysis of variance (ANOVA) using the software SPSS 13.0 for Windows. Significant differences between different dietary treatments were evaluated by Tukey's multiple range test. Mean differences were considered significant at a P value equal or less than 0.05.

Results

Growth Performance and Feed Utilization

During the 9-weeks feeding trial, no pathological signs or anomalies occurred during the feeding experiment, and the experimental diets were well accepted in all treatments. Results of feed utilization and growth performance in two sizes of blunt snout bream fed different arginine levels were listed in Table 3. With increasing arginine level of the diet, SGR, FCR, PER and WG of blunt snout bream increased significantly for fish of two sizes.

The condition factor (CF) was significantly ($P< 0.05$) increased with increase of dietary arginine level and the plateau occurred at 23.5 g kg^{-1}. No significant ($P>0.05$) relationships between dietary arginine level and hepatosomatic index (HIS) in two sizes of fish. FCR, PER, WG and CF were higher in small fish than those in big fish. Furthermore, based on the broken-line regression model analysis relation on SGR, the optimum arginine requirement could be 20.3 g kg^{-1} (Figure 1) in size I fish, and greater than 17.9 g kg^{-1} in size II fish (Figure 2).

Blood Biochemical Measurements

Results of plasma biochemical parameters in two sizes of blunt snout bream fed different arginine levels were listed in Table 4. The ALT significantly ($P<0.05$) decreased with increasing dietary arginine level from 8.3 to 23.5 g kg^{-1} in size I or 8.3 to 18.1 g kg^{-1} of the size II. The AST and TP activities were not significantly ($P>0.05$) affected by dietary arginine levels in small and big fish. No significant ($P>0.05$) relationships between dietary arginine level and albumin (ALB) in size II. But in small fish, fish fed 23.5 g kg^{-1} arginine diet had a higher ALB than those fed other diets ($P<0.05$). Moreover, the Urea concentration increased significantly ($P<0.05$) in both of two sizes along with the increased of dietary arginine level. The fish fed with 28.2 g kg^{-1}, 33.6 g kg^{-1} arginine diet was higher in the Urea concentration than those fed other diets ($P<0.05$)

Plasma Free Amino Acid Profile

The plasma free amino acid profiles of the fish fed different diets show in Table 5. Plasma arginine concentration of size I and II fish increased with increasing dietary arginine from 8.3 to 33.6 g kg^{-1} ($P<0.05$), and the decreasing trend of lysine content in plasma was observed in fish fed diets with arginine level from 8.3 to 33.6 g kg^{-1} ($P<0.05$), respectively.

Table 3. Effects of dietary arginine level on growth performance morphological indices in different growth stages of blunt snout bream (*Megalobrama amblycephala*)

Item	Arginine level (g kg^{-1}, dry diet)					
	8.3	13.0	18.1	23.5	28.2	33.6
Size I (initial body weight of 52.49g)						
IBW	52.4±0.13	52.5±0.03	52.6±0.10	52.4±0.17	52.4±0.17	52.4±0.15
FBW	105.2±2.82^{a}	117.6±5.33^{b}	130.7±3.63^{c}	127.1±3.15^{bc}	124.1±4.94^{bc}	123.8±2.31^{bc}
SGR	1.05±0.03^{a}	1.27±0.03^{b}	1.37±0.06^{bc}	1.43±0.08^{c}	1.28±0.03^{b}	1.26±0.02^{b}
FCR	0.53±0.03^{a}	0.57±0.04^{ab}	0.58±0.02^{ab}	0.64±0.04^{b}	0.62±0.03^{b}	0.61±0.01^{b}
PER	1.31±0.09^{a}	1.47±0.13^{ab}	1.62±0.07^{b}	1.68±0.11^{b}	1.67±0.09^{b}	1.66±0.03^{b}
WG	100.52±5.03^{a}	123.85±10.14^{b}	148.70±6.29^{c}	149.50±5.35^{c}	136.77±10.16^{bc}	136.34±4.28^{bc}
HSI	1.38±0.03	1.35±0.04	1.43±0.06	1.33±0.12	1.41±0.04	1.40±0.09
CF	2.91±0.02^{a}	3.14±0.07^{ab}	3.20±0.04^{b}	3.25±0.04^{c}	3.19±0.08^{bc}	3.16±0.04^{bc}
SR	91.7±1.67	95.0±5.00	93.3±4.41	95.0±5.00	98.3±1.67	95.0±2.89
Size II (initial body weight of 101.85g)						
IBW	101.9±0.38	101.4±0.49	100.4±0.20	100.5±0.19	100.4±0.20	100.4±0.20
FBW	279.89±4.37^{a}	314.54±6.92^{b}	343.03±10.74^{bc}	332.72±15.41^{c}	315.58±5.77^{bc}	310.71±3.06^{b}
SGR	1.14±0.04^{a}	1.33±0.08^{b}	1.53±0.01^{c}	1.48±0.04^{c}	1.44±0.08^{c}	1.43±0.04^{c}
FCR	0.43±0.01^{a}	0.47±0.01^{ab}	0.51±0.04^{bc}	0.57±0.03^{c}	0.54±0.02^{bc}	0.56±0.01^{c}
PER	1.01±0.04^{a}	1.28±0.02^{b}	1.59±0.11^{c}	1.43±0.08^{bc}	1.57±0.06^{bc}	1.52±0.05^{bc}
WG	80.52±2.91^{a}	103.26±3.51^{b}	123.99±7.15^{c}	115.49±5.68^{bc}	105.28±3.79^{bc}	102.11±1.72^{b}
HSI	1.75±0.17	1.78±0.07	1.73±0.01	1.79±0.09	1.80±0.06	1.76±0.02
CF	2.90±0.08^{a}	3.10±0.13^{bc}	3.06±0.15^{ab}	3.19±0.05^{c}	3.07±0.08^{ab}	3.13±0.21^{c}
SR	100±0.00	100±0.00	100±0.00	97.8±3.84	100±0.00	100±0.00

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

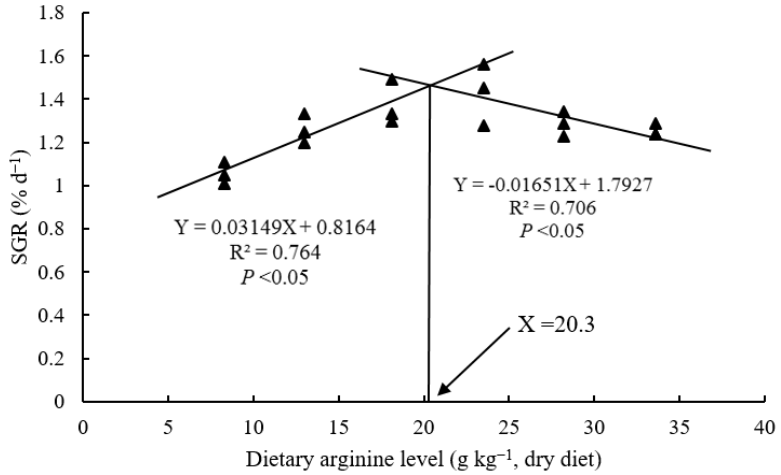

Figure 1. Broken-line analysis on specific growth rate of size I to dietary arginine level. Note: Each point represents the means of three replicate groups.

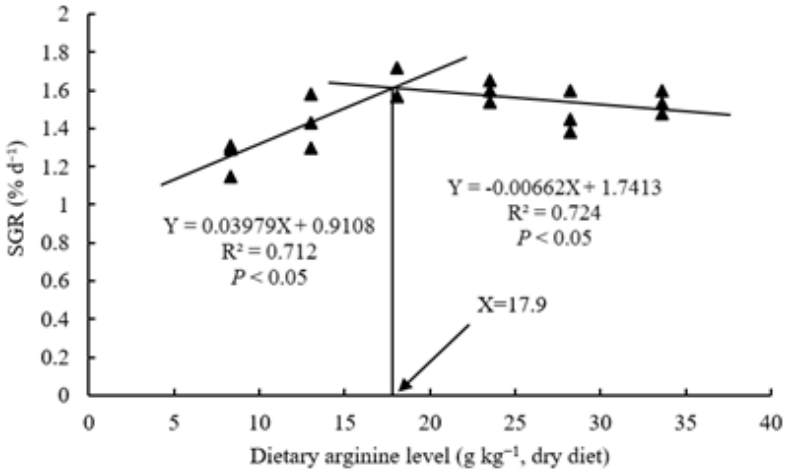

Figure 2. Broken-line analysis on specific growth rate of size II to dietary arginine level. Note: Each point represents the means of three replicate groups.

The fish of size I and II stage fed with 18.1 g kg^{-1}, 23.5 g kg^{-1}, 28.2 g kg^{-1}, 33.6 g kg^{-1} arginine diet was higher in the concentration of plasma arginine than those fed other diets of 8.3 g kg^{-1}, 13.0 g kg^{-1} arginine diet (P<0.05). Furthermore, the fish of size I fed with 13.0 g kg^{-1}, 18.1 g kg^{-1}, 23.5 g kg^{-1}, 28.2 g kg^{-1}, 33.6 g kg^{-1} arginine diet was lower in the concentration of plasma lysine than that of 8.3 g kg^{-1} arginine diet (P< 0.05). The fish of size II fed with 23.5 g kg^{-1}, 33.6 g kg^{-1} arginine diet was lower in the concentration of plasma lysine than that of 8.3 g kg^{-1} arginine diet (P<0.05). Besides, arginine concentration of size I was lower than fish II. However dietary arginine levels had no significant effect on plasma histidine, isoleucine, leucine, phenylalanine, threonine, valine and methionine concentratio n at different growth stages (P>0.05).

Discussion

Arginine shortage may lead to reduced growth rate, feed intake, feed efficiency and protein retention in most fish species (Wilson, 2002; Fournier *et al.*, 2003), and also the increased mortality and incidence of lordosis were also discovered, such as in common carp, *C. carpio* fed arginine deficiency diet (Tacon, 1992). In this study, no fish disease or apparent symptoms, but poor feed utilization and growth performance were found in fish of two sizes fed arginine unsuitable diets. Arginine supplementation significantly improved their FBW, SGR, FCR, PER and WG. This is an indication that, arginine was indispensable for growth of blunt snout bream, and well utilized the crystalline arginine, which supplemented in the experimental diets by this species. Besides, the survival rate (SR) in all groups were not significantly affected by dietary arginine levels in this experiment, and this result in accordance with juvenile blunt snout bream (initial body weight of 2.60±0.1g) (Ren et al., 2013), possibly 8.3 g kg^{-1} dietary arginine level could be basically satisfy the demand of blunt snout bream.

The promotion of amino acid on feed utilization and growth has been reported in numerous fishes, the

Table 4. Effects of dietary arginine level on blood biochemical indices in different growth stages of blunt snout bream (*Megalobrama amblycephala*)

Item	Arginine level (g kg^{-1}, dry diet)					
	8.3	13.0	18.1	23.5	28.2	33.6
Size I (initial body weight of 52.49g)						
ALT [1] (U / L)	1.81±0.06^{b}	1.75±0.05^{b}	1.43±0.08^{a}	1.30±0.05^{a}	1.60±0.10^{b}	2.21±0.09^{b}
AST [2] (U / L)	52.86±3.67	50.02±4.81	48.60±4.37	46.18±4.11	51.05±4.23	55.51±5.21
TP [3] (U / L)	35.32±1.14	36.63±0.68	37.23±0.75	34.16±1.85	37.62±0.71	36.77±1.27
ALB [4] (U / L)	14.5±0.57^{a}	15.5±0.38^{ab}	14.32±0.75^{a}	16.50±0.31^{b}	15.83±0.44^{ab}	15.31±0.43^{ab}
Urea (μmol / L)	0.68±0.07^{ab}	0.63±0.04^{a}	0.87±0.08^{b}	0.80±0.04^{ab}	1.13±0.09^{c}	1.15±0.10^{c}
Size II (initial body weight of 101.85g)						
ALT (U / L)	1.85±0.12^{c}	1.18±0.20^{ab}	0.96±0.10^{a}	1.72±0.11^{bc}	1.43±0.09^{bc}	2.25±0.13^{c}
AST (U / L)	55.25±4.65	51.60±4.64	42.53±4.21	47.28±4.60	48.01±4.40	50.70±4.20
TP (U / L)	34.83±0.99	34.62±0.72	33.68±1.69	34.48±0.99	32.93±0.81	35.88±1.60
ALB (U / L)	14.50±0.39	14.38±0.28	15.22±1.26	14.45±0.68	13.20±0.18	13.70±0.67
Urea (μmol / L)	0.48±0.03^{a}	0.54±0.04^{a}	0.73±0.07^{ab}	0.69±0.06^{a}	0.94±0.01^{bc}	1.04±0.10^{c}

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

[1] ALT, alanine aminotransferase.

[2] AST, aspartate aminotransferase.

[3] TP, total protein.

[4] ALB, albumin.

Table 5. Effects of dietary arginine level on the concentration of plasma essential amino acids in different growth stages of blunt snout bream (*Megalobrama amblycephala*)

Item	Arginine level (g kg^{-1}, dry diet)					
	8.3	13.0	18.1	23.5	28.2	33.6
Size I (initial body weight of 52.49g)						
Arg	18.86±1.29^{a}	18.19±1.27^{a}	29.18±2.47^{b}	38.95±2.54^{c}	38.88±1.89^{c}	39.75±1.62^{c}
His	40.99±4.09	39.64±1.99	40.38±1.45	41.93±2.94	35.92±2.63	39.92±3.17
Ile	10.39±0.21	13.12±1.30	10.16±1.01	11.47±0.55	12.60±1.12	10.66±0.41
Leu	19.75±0.69	27.29±0.51	19.15±1.48	29.94±0.87	24.89±2.07	15.52±0.70
Lys	82.79±3.15^{c}	59.02±1.48^{b}	45.14±4.05^{a}	44.03±1.14^{a}	46.14±1.04^{a}	39.45±1.57^{a}
Phe	13.57±0.62	14.73±1.43	13.73±1.21	15.14±0.87	14.99±1.01	12.82±0.35
Thr	35.71±13.02	34.80±3.29	30.60±2.20	32.03±1.04	35.57±1.67	33.02±2.20
Val	20.57±1.10	21.04±1.76	19.52±1.46	17.16±1.02	19.82±1.20	16.28±1.60
Met	7.72±0.17	7.41±0.19	7.62±0.26	7.73±0.15	7.09±0.63	6.82±0.48
Size II (initial body weight of 101.85g)						
Arg	26.83±2.44^{a}	29.10±1.39^{ab}	34.03±1.73^{b}	42.24±1.57^{c}	41.33±1.99^{c}	42.28±1.16^{c}
His	40.28±1.39	43.77±3.50	45.58±5.10	44.76±3.41	44.81±2.83	44.22±2.54
Ile	7.35±0.33	7.44±0.24	7.88±0.22	7.78±0.93	7.70±0.01	7.12±0.75
Leu	16.15±1.49	20.09±1.66	27.16±0.31	18.62±1.83	14.08±0.06	12.34±1.22
Lys	58.33±2.69^{c}	46.96±0.63^{bc}	37.86±2.41^{bc}	32.97±1.15^{b}	39.24±2.47^{bc}	26.16±1.50^{a}
Phe	19.66±1.42	20.45±1.99	16.98±1.49	20.47±1.80	17.42±1.06	17.49±0.85
Thr	63.82±6.91	64.65±8.97	61.15±3.73	66.22±3.51	67.49±4.01	65.06±4.12
Val	21.12±1.75	22.60±2.23	22.85±1.51	20.62±2.01	19.13±1.01	20.35±1.82
Met	10.43±0.85	10.24±0.28	10.41±0.20	9.83±0.64	10.31±0.33	9.32±0.36

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

different body size at different nutrition turnover rate, growth performance, requirements and utilization of amino acid for fish are different. To our knowledge, previous studies has reported the amino acid requirement in different size of fish, such as gibel carp (*Carassis auratus gibeliovar*. CAS III) (Tu et al., 2015), which was reported that dietary arginine requirement for gibel carp of these two sizes were 16.4 (small fish: initial weight 51.6 ± 0.3 g) and 12.9 g kg $^{-1}$ (big fish: initial weight 147 .8 ± 0. 5 g) of dry matter. In this study, maximum SGR were observed at dietary arginine level of 23.5 g kg^{-1} (size I) or 18.1 g kg^{-1} (size II), it was similar to the growth that reported in jian carp, which showed normal growth with the purified diet (Chen *et al*., 2015). Based on the broken-line regression model analysis of SGR data showed that the optimum arginine requirement in size I was 20.3 g kg^{-1} or size II fish was 17.9 g kg^{-1} under our experimental condition, respectively. These findings are in accordance with those reported on channel catfish, *Ictalurus punctatus* (Alejandro and Delbert, 2000) and silver perch, and higher than grass carp,

Ctenopharyngodon idella, which reported that dietary arginine requirement for grass carp was determined to be 13.45 g kg^{-1} diet (Wang *et al.*, 2015). Also while lower than the values reported for Atlantic Salmon, *Salmo salar* (Gerd *et al.*, 1997). The difference within or among fish species is possibly affected by fish Size, fish species, dietary levels or experimental conditions (Kim *et al.*, 1992; Luo *et al.*, 2004). Meanwhile, the optimal requirement of arginine for small fish (size I) was higher than that for larger fish (size II). This results were supported the findings in red sea bream *Pagrus major* (Rahimnejad and Lee, 2014), which showed decreased requirement of dietary arginine levels with increase in body sizes. In this study, all performance values tended to decline that hereafter the optimum level, and similar with Indian major carp, *Labeo rohita* (Abidi and Khan, 2009), which showed that excess dietary arginine level might decrease growth performance and feed utilization. It is indicated that the excess dietary arginine may generate extra energy expenditure toward deamination and excretion of the same caused by unbalanced dietary amino acids profile, which may result in stress and toxic effects in the fish that have adverse effects on growth performance and feed utilization (Walton *et al.*, 1986).

Protein synthesis is a key component of organism growth (Xu *et al.*, 2016), and evidence has shown that arginine play a vital role in regulating protein synthesis (Ren *et al.*, 2014). Previous studies reported that arginine is the nitrogen carrier for tissue proteins and also essential for the synthesis of biologically crucial molecules, such as creatine, proline, nitric oxide, ornithine and polyamines (Coutinho *et al*., 2016). In this study, PER was observed in arginine deficient diets, but it was significantly enhanced when optimal arginine supplemented in diet, which might due to disturbance of absorption and utilization of other amino acids, and lower palatability in arginine deficient diets (Habte-Tsion *et al.*, 2015). It indicated that the optimal supplementation of dietary arginine might improve the protein synthesis. Besides, the higher PER was observed in small fish compared with larger fish, and this should be a key point to explain why small fish showed better than larger fish on utilization of dietary arginine.

ALT and AST are usually used as general indicators of the functioning of the hepatopancreas (Chaplin *et al.*, 1967) and the liver (Yamamoto, 1981). In this study, the ALT activity in size I fish fed 23.5 g kg^{-1} dietary arginine level was decreased compared to other levels. In size II fish, the same tendency was observed in those fed 18.1 g kg^{-1} dietary arginine level. This result inconsistent with the results of Lin *et al.* (2015) on golden pompano *Trachinotus ovatus*. This illustrated that arginine level might affect the whole amino acid deamination and the metabolism (Tu *et al.*, 2015), and this might be another reason for reduced growth and feed efficiency in fish (size I and II) fed excess dietary arginine. Besides, plasma Urea had been treated as a supportive parameter to determine dietary arginine intake assessed by growth data such as in European sea bass (Tibaldi *et al.*, 1995), Japanese flounder (Alam *et al.*, 2002) and Atlantic salmon (Berge *et al.*, 1997). Some studies showed that plasma urea concentrations could be as sensitive as growth in determining the arginine intake of the fish, which might be estimated by growth data (Cho *et al.*, 1992). In this study, Urea values showed a significant increasing tendency, and similar to result observed in rainbow trout (Cho *et al.*, 1992). Urea is synthesized in the liver and excreted by the kidneys (Nieves and Langkamp-Henken, 2002), and is an end product of purine catabolism (Zhou *et al.*, 2010). Thus, our result could be ascribed to the arginine ability to enhance Urea synthesize and excretion, an indication that plasma Urea concentrations in blunt snout bream was sensitive in determining the arginine requirement.

Furthermore, excessive dietary arginine level was negatively affected growth and feed utilization, this may be due to the lysine and arginine antagonism resulting in the imbalance amino acid profiles as well (Ren *et al.*, 2013). This antagonism is well known in poultry, rats and fish (Luo *et al.*, 2004). In the present study, the lysine content was significantly lower in the plasma of fish fed diet with highest dietary arginine levels from 13.0 to 33.6 g kg^{-1} compared with those fed diets with arginine 8.3 g kg^{-1} in two sizes, so that the dietary high arginine level would probably result in the deficiency of lysine, which limited the growth of fish and affect the accurate requirement of arginine. Similar findings were observed in rainbow trout (Berge *et al.*, 1999) and Atlantic salmon (Kaushik *et al.*, 1988), which indicated antagonism between arginine and lysine at the various species of fish. However, using different fish species have yielded no convincing evidence of competition between lysine and arginine (NRC, 2011). There were different results in Japanese flounder (Tibaldi *et al.*, 1994), which no negative effect of feeding excess lysine has been detected on high plasma arginine levels. Besides, it needed to note that the arginine content in small fish (size I) was lower than larger fish (size II), which showed that the utilization of arginine for small fish was higher than larger fish, and similar phenomenon was appeared in Japanese flounder *Paralichthys olivaceus* (Han *et al.*, 2014).This result might be due to the fact that small fish need to utilize more arginine to growth than larger fish.

In conclusion, dietary arginine levels were significantly influenced the growth performance and blood biochemical indices in different stages of blunt snout bream, which showed that the arginine requirement is playing a significant role in growth and metabolism of blunt snout bream. Furthermore, results of the present investigation indicated that the arginine requirement of blunt snout bream of two body sizes were 20.3 g kg^{-1} (initial body weight of

52.50±0.18g) and 17.9 g kg^{-1} (initial body weight of 101.85±1.85g) of dry matter, respectively.

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