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Dietary arginine requirement of juvenile blunt snout bream, *Megalobrama amblycephala*

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ABSTRACT

A 9-week feeding trial was conducted to quantify the dietary arginine requirement of juvenile blunt snout bream. Six isonitrogenous and isoenergetic semi-purified diets (34.0% crude protein) were formulated to contain graded arginine levels (0.83% to 3.36% of dry weight) at 0.5% increments replaced by equal proportions of glycine. At the end of the feeding trial, the results showed that survival rate was not significantly affected by dietary arginine level. Final weight, specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER) and protein productive value (PPV) increased with increasing dietary arginine level from 0.83 to 1.81% ($P < 0.05$), thereafter showed a declining trend but the differences were not significant. Whole body compositions were independent of dietary arginine levels ($P > 0.05$). Plasma arginine concentration increased with the increase of dietary arginine from 0.83% to 1.81%, and thereafter was relatively constant, while lower lysine content in plasma was observed in fish fed the diet with 3.36% arginine level compared to those fed diets with 0.83–2.35% arginine ($P < 0.05$). Significantly higher plasma urea content was observed in fish fed diet with 3.36% arginine compared with those fed 0.83% arginine diet (0.83%). Significantly lower plasma total nitric oxide synthase (T-NOS) activities were observed in fish fed 0.83% arginine diet than those fed diets with 2.35–3.36% arginine. Plasma superoxide dismutase (SOD) activities and ammonia contents were not significantly affected by dietary arginine levels. Based on SGR, FER and PER, the optimal dietary arginine requirement of juvenile blunt snout bream was estimated to be 2.46% of the diet (7.23% of dietary protein), 2.28% (6.71% of dietary protein) and 2.26% of the diet (6.65% of dietary protein), respectively.

1. Introduction

Fish cannot synthesize all amino acids, and it is important to satisfy the essential amino acid requirements of fish by formulating balanced nutrients in feed (NRC, 2011). Arginine has been shown to be an essential amino acid for optimal growth of fish, which is the most limiting amino acid in some plant protein sources such as corn meal, sesame meal and zein (Singh and Khan, 2007). 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 (Luo et al., 2004). In addition, as a precursor for nitric oxide synthesis, arginine was identified to play a major role in the immunity response in some fish species (Buentello and Gatlin, 1999). The dietary arginine requirement has been estimated for several fish species, and a large range (from 3.0% of dietary protein for turbot, *Pseua maxima* to 8.1% of dietary protein for black sea bream, *Sparus macrocephalus*) has been observed between species (NRC, 2011), and this wide range makes the extrapolation of dietary needs from one fish species to another unfeasible (Luo et al., 2007).

Blunt snout bream, *Megalobrama amblycephala*, a freshwater fish, has a long history of cultivation in China because of its excellent flesh quality, rapid growth performance and high larval survival rate (Zhou et al., 2008). Its production has a fast increase and approximately 0.63 million tons in 2010 (Ministry of Agriculture of the People's Republic of China, 2010). Nonetheless, the information of nutritional requirements in blunt snout bream is still quite limited and traditional formulated feed production relies on formulas for grass carp (*Ctenopharyngodon idella*). Recently, dietary protein and lipid were quantified in blunt snout bream fingerlings based on growth performance (Li et al., 2010). To our knowledge, no information is available concerning the dietary essential amino acid requirement in blunt snout bream up to now.

Therefore the present study was conducted to investigate the effects of dietary arginine level on growth performance, feed utilization, whole body composition and immunity response in blunt snout bream,

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and to quantify dietary arginine requirement in juvenile blunt snout bream.

2. Materials and methods

2.1. Diet preparation

Six isonitrogenous and isoenergetic diets (34% crude protein), using fish meal, casein and gelatin as protein sources and soybean oil as a lipid source, were formulated to contain graded levels of arginine (0.83%, 1.30%, 1.81%, 2.35%, 2.82% and 3.36% of dry weight, respectively) 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 and thoroughly mixed with soybean oil and water, then forced through a pelletizer (4–2 style, Xinchang Machinery LTD, China) and dried in a ventilated oven at 30 °C. After drying, all diets were sealed in bags and stored at −15 °C until used.

2.2. Experimental procedure

Experimental fish were obtained from a commercial farm (Jiangsu, China). Prior to the feeding trial, the fish were fed with diet 1 for 2 weeks to acclimate to the experimental diet and conditions. After fasting for 24 h, juvenile blunt snout bream (initial weight 2.6 ± 0.1 g) were randomly sorted into eighteen floating cages (1 m × 1 m × 1 m) with 30 fish in each cage. Each diet was randomly assigned to triplicate cages. Fish 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^{-1} throughout the feeding trial.

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

Ingredients	Diet number					
	1	2	3	4	5	6
White fish meal[a]	5.00	5.00	5.00	5.00	5.00	5.00
Casein[a]	15.00	15.00	15.00	15.00	15.00	15.00
Gelatin[a]	3.75	3.75	3.75	3.75	3.75	3.75
Soybean oil	6.00	6.00	6.00	6.00	6.00	6.00
Soybean lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Amino acid premix[b]	11.56	11.56	11.56	11.56	11.56	11.56
Vitamin premix[c]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[d]	5.00	5.00	5.00	5.00	5.00	5.00
Corn starch	35.00	35.00	35.00	35.00	35.00	35.00
Cellulose	7.69	7.69	7.69	7.69	7.69	7.69
Carboxymethyl cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Ethoxyquin	0.50	0.50	0.50	0.50	0.50	0.50
Glycine	2.50	2.00	1.50	1.00	0.50	0.00
L-Arginine	0.00	0.50	1.00	1.50	2.00	2.50
Proximate analysis (% of dry diet)						
Arginine	0.83	1.30	1.81	2.35	2.82	3.36
Crude protein	33.8	34.0	33.9	33.6	33.8	34.3
Crude lipid	8.31	8.40	8.54	8.26	8.35	8.42
Gross energy (KJ g^{-1})	18.8	18.8	18.9	18.8	18.9	18.8

[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 from Feeer 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; calcium pantothenate, 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): calcium biphosphate, 20 g; sodium chloride, 2.6; potassium chloride, 5 g; magnesium sulphate, 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
Amino acid composition of ingredients (g·100 g^{-1} dry matter).

Amino acid	Amount in				Total	34% Whole body protein
	15 g C	3.75 g G	5 g FW	AAP		
EAA						
Arginine	0.43	0.24	0.18	0.00	0.85	2.01
Histidine	0.33	0.01	0.11	0.31	0.76	0.76
Isoleucine	0.63	0.05	0.14	0.68	1.49	1.49
Leucine	1.21	0.09	0.23	0.87	2.40	2.40
Lysine	1.01	0.11	0.23	1.09	2.43	2.43
Methionine	0.37	0.02	0.09	0.43	0.90	0.90
Phenylalanine	0.62	0.06	0.14	0.66	1.47	1.47
Threonine	0.55	0.05	0.11	0.71	1.41	1.41
Valine	0.78	0.08	0.16	0.56	1.57	1.57
NEAA						
Aspartic acid	0.96	0.16	0.27	1.46	2.84	2.84
Serine	0.70	0.09	0.12	0.55	1.45	1.45
Glycine	0.24	0.69	0.19	1.37	2.50	2.50
Alanine	0.43	0.27	0.20	1.25	2.14	2.14
Cystine	0.03	0.00	0.02	0.14	0.19	0.19
Tyrosine	0.68	0.02	0.10	0.27	1.07	1.07
Gulmatic acid	2.79	0.32	0.42	1.11	4.64	4.64
Proline	1.19	0.39	0.10	0.12	1.81	1.81

C, casein; G, gelatin; FM, fish meal; AAP, crystalline amino acid premix; EAA, essential amino acid; NEAA, non-essential amino acid. Tryptophan could not be detected after acid hydrolysis.

2.3. Sample collection and analysis

2.3.1. Sample collection

At the end of the feeding trial, fish were fasted for 24 h before sampling. Total numbers and mean body weight of fish in each cage were determined. Five fish per cage were euthanized by MS-222 (100 mg·L^{-1}), and then blood samples were collected immediately from the caudal vein using heparinized syringes. Following centrifugation (3500 ×*g*, 10 min, 4 °C), the plasma was separated. All the samples were stored at −80 °C until analysis. Ten fish at the beginning and five fish at the end of the experiment per cage were sampled and stored at −20 °C for the analysis of whole body composition.

2.3.2. Laboratory analysis

Dry matter, 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. Gross energy of the experimental diets was analyzed by an adiabatic bomb calorimeter (PARR1281, USA).

Amino acid concentrations were determined by a professional laboratory (at the Institute of Feed Science, Jiangnan University, China). For total amino acid content analysis, the diet and ingredients were freeze-dried overnight, and 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.

The plasma urea content was determined by the diacetyl monoxime method using a medical detection kit (Nanjing Jiancheng Bioengineering Institute, China). The determination of plasma ammonia content was performed using a diagnostic kit (Nanjing Jiancheng Bioengineering Institute, China) by Berthelot reaction. Total nitric oxide synthase