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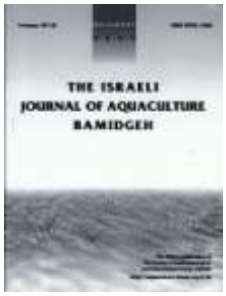

Effects of Dietary Protein Level on Growth Performance, Muscle Composition, Blood Composition, and Digestive Enzyme Activity of Wuchang Bream (*Megalobrama amblycephala*) Fry

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Key words: Wuchang bream (*Megalobrama amblycephala*), dietary protein, growth, muscle composition, blood composition, digestive enzymes activity

Abstract

The purpose of this study was to determine the dietary protein requirement and effects of dietary protein level on growth performance, muscle composition, blood composition, and digestive enzyme activity in Wuchang bream fry. Five isoenergetic and isolipidic semi-purified diets were formulated to contain 28%, 30%, 32%, 34%, or 36% (dry matter) dietary protein. Diets were fed to triplicate groups of 25 fishes (16.08±0.03 g) to near satiation three times a day in a closed recirculation system for 10 weeks. Weight gain, specific growth rate, and feed conversion ratio significantly improved as the dietary protein content increased up to 34%. The protein efficiency ratio, hepatosomatic index, and viscerosomatic index significantly dropped as the dietary protein rose while the Fulton condition factor was positively correlated to the dietary protein level. Increased dietary protein resulted in increased muscle protein content and decreased lipid content. Red blood cell, hemoglobin, and hematocrit counts increased significantly with the increase in dietary protein. Serum triiodothyronine and thyroxine significantly rose as the dietary protein rose but serum aspartate aminotransferase significantly dropped. Intestinal protease and amylase activity rose significantly with the increase in dietary protein while lipase tended to drop. On the basis of broken-line regression analysis of weight gain and FCR, the dietary protein requirement of Wuchang bream fry is 32-33%.

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Introduction

Feed is a principal factor in aquaculture for increasing growth and production of cultured fish. Protein is the most expensive ingredient in formulated diets and thus should be carefully formulated to meet the needs of the cultured species. Protein is one of the primary cost components of formulated diets and the effect of the dietary protein level on response variables of fish is an important nutritional consideration. Protein efficiency decreases with increasing dietary protein (Kanazawa et al., 1980) because, in most cases, fish cannot synthesize excess dietary protein but utilize it for energy (Santinha et al., 1996). Further, when dietary protein levels exceed the requirement, ammonia is excreted and water quality is affected (Kim and Lee, 2005). Therefore, it is important to optimize protein utilization for body protein synthesis rather than for energy.

Megalobrama amblycephala, also known as blunt snout, Wuchang bream, and Chinese bream fish, is a typical herbivorous freshwater fish native to China that has been introduced to Africa, North America, Japan, Europe, and other Asian countries. *Megalobrama amblycephala* is a good candidate for freshwater intensive culture because of its fast growth rate, use of natural food, high larvae survival rate, tender flesh, and high disease resistance. Aquaculture of this fish in China has expanded rapidly during the last decade because of the increasing consumer demand. Production in China reached approximately 652,215 tons in 2010, sixth among whole Chinese freshwater fish production (Ministry of Agriculture of the People's Republic of China, 2011).

The effects on growth performance and other parameters in Wuchang bream of protein and lipid (Li et al., 2010), carbohydrate/lipid ratio (Li et al., 2012), and carbohydrate (Zhou et al., 2013) have been studied. The objective of the present study was to quantify the dietary protein requirement and investigate the effects of the dietary protein level on growth performance, muscle composition, blood composition, and digestive enzymes activities of Wuchang bream fry.

Materials and Methods

Fish. The experiment was carried out at the Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Freshwater Fisheries Research Center (FFRC), Chinese Academy of Fishery Sciences, Wuxi, P.R. China. The experiment was conducted in a closed water recirculation system with a water flow rate of approximately 3 l/min and with continuous aeration. Wuchang bream fry were obtained from FFRC and acclimatized to the experimental facilities and conditions for two weeks. During acclimatization, fish were fed a commercial feed (no. 191, Tongwei Feed Group Co., Ltd., Wuxi, China) containing 30% crude protein and 5% crude lipid to near satiation. After acclimatization, fish (16.08±0.03 g) were selected and randomly assigned to 15 tanks at a density of 25 fish each. Three tanks were arranged randomly and assigned to each test diet.

Diets. Five isoenergetic (15.72 kJ/g dry matter) and isolipidic (6.17% dry matter) semi-purified diets were formulated to contain 28%, 30%, 32%, 34%, and 36% dietary protein (Table 1). Casein, gelatin, and fishmeal (Coprinca, Brazil) were the main protein sources. The powdered ingredients were thoroughly mixed, then oils and water were added. The dough was pelletized in the lab pelletizer (die diameter 2 mm) and dried in an oven at 65°C for 12 h. After drying, the diets were packed into airtight plastic bags and stored at 4°C until use.

Husbandry. The fish were provided a continuous flow of sand-filtered water (3 l/min) with continuous aeration to maintain the dissolved oxygen level above saturation. Water temperature was monitored with a data logger. The experimental diets were fed to the fry by hand to near satiation three times a day (8:00-8:30, 12:00-12:30, 16:00-16:30) for 10 weeks. Water temperature (24-26°C), dissolved oxygen (≥6.0 mg/l), total ammonia-nitrogen (≤0.05 mg/l), and pH (7.0-7.5) were monitored weekly. The photoperiod was 12 h light/12 h dark.

Sampling. At the start of the feeding trial, 12 fish were sampled after 24 h starvation and kept frozen at -20°C for subsequent initial proximate chemical composition analysis of the muscle. At the end of the feeding trial, fish were starved for 24 h to evacuate the alimentary tract contents prior to harvest, and three fish from each tank were sampled,

Table 1. Ingredients and proximate compositions of experimental diets for Wuchang bream fry (*Megalobrama amblycephala*).

	Dietary protein level (%)				
	28	*30*	*32*	*34*	*36*
Ingredient (%)					
α-starch[1]	30.75	28.50	25.20	22.85	20.00
Casein[2]	18.86	19.50	20.80	22.00	23.50
Fishmeal[3]	9.95	10.70	11.50	12.26	13.00
Dextrin[4]	10.00	10.00	10.00	10.00	10.00
Carboxylmethyl cellulose[5]	10.00	10.00	10.00	10.00	10.00
Microcrystalline cellulose[6]	5.84	6.20	6.90	7.23	7.84
Soybean oil	5.50	5.45	5.40	5.35	5.30
Gelatin[5]	4.25	4.85	5.50	5.71	5.86
Calcium dihydrogen phosphate	2.65	2.60	2.50	2.40	2.30
Vitamin/mineral additives[7]	1.00	1.00	1.00	1.00	1.00
Soy lecithin	1.00	1.00	1.00	1.00	1.00
Chlorinated choline	0.15	0.15	0.15	0.15	0.15
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Proximate composition (dry matter basis)					
Moisture (%)	7.89	7.83	6.81	7.58	7.47
Crude protein (%)	28.06	30.03	32.16	34.36	36.06
Crude lipid (%)	6.19	6.19	6.17	6.16	6.14
Ash (%)	8.49	8.57	8.77	8.85	9.44
Carbohydrate (%)	31.37	29.21	26.01	23.77	21.02
Gross energy (kJ/g)[8]	15.70	15.79	15.56	15.84	15.73
Protein/energy (mg/kJ)	17.88	19.02	20.66	21.70	22.92

[1] Jin Ling Tower Starch Co., Ltd., P.R. China
[2] Lin Xia Huaan Biological Products Co., Ltd., P.R. China
[3] Coprinca, Brazil
[4] Xi Wang Chemical Co., Ltd., P.R. China
[5] Shanghai Zhan Yun Chemical Co., Ltd., P.R. China
[6] Linghu Xinwang Chemical Co., Ltd., P.R. China.
[7] per kg premix: vitamin A 900,000 IU, vitamin D 250,000 IU, vitamin E 4500 mg, vitamin K3 220 mg, vitamin B_1 320 mg, vitamin B_2 1090 mg, vitamin B_6 5000 mg, vitamin B_{12} 116 mg, biotin 50 mg, pantothenate 1000 mg, folic acid 165 mg, choline 60,000 mg, inositol 15,000 mg, niacin acid 2500 mg, $CuSO_4 \bullet 5H_2O$ 2.5 g, $FeSO_4 \bullet 7H_2O$ 28 g, $ZnSO_4 \bullet 7H_2O$ 22 g, $MnSO_4 \bullet 4H_2O$ 9 g, Na_2SeO_3 0.045 g, KI 0.026 g, $CoCl_2 \bullet 6H_2O$ 0.1 g
[8] calculated as 23.64 kJ/g protein, 39.54 kJ/g lipid, 17.15 kJ/g carbohydrate

individually weighed, and body length was measured. After anesthetization with MS-222 (tricaine methane-sulfonate, Sigma, USA) at a concentration of 200 mg/l, two blood samples were obtained from the caudal veins. The first was obtained using heparinized syringes (with anticoagulant) to measure blood parameters; the second, without anticoagulant, was left to clot at 4°C for 1-2 h and centrifuged at 3,000 × *g* at 4°C for 10 min to prepare serum. The supernatant was removed and stored at -80°C for subsequent serum biochemical measurement. At the same time, the sampled fish were dissected, samples of liver and viscera were collected and weighed, and the hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated. Gut samples were stored at -80°C for subsequent digestive enzyme assay. Dorsal muscles were scratched off the fish, pooled, chopped, and stored frozen (-20°C) until analysis of proximate chemical composition.

Proximate composition analysis. Moisture, crude protein, crude lipid, and ash contents of the diets and fish muscle were determined by standard methods (AOAC, 1997). Moisture was determined by oven drying until constant weight (105°C), crude protein (nitrogen × 6.25) by the Kjeldahl method using an Auto Kjeldahl System (FOSS KT260, Switzerland), crude lipid by ether-extraction using Soxtec System HT6 (FOSS, Tecator, Sweden), and ash by combustion at 560°C for 5 h.

Hematological measurements. Red blood cell, white blood cell, hemoglobin, hematocrit, and platelets were counted using an Auto Hematology Analyzer (BC-5300Vet, Mindray, P.R. China) with a test kit from Shenzhen Mindray Medical International Co. Ltd., P.R. China.

Biochemical measurements. Serum glucose, total cholesterol, triacyglycerol, total protein content, aspartate aminotransferase, and alanine aminotransferase activities were determined by the colorimetric method (Mindray Bio Medical Co., Ltd., P.R. China) using a Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R. China). Serum triiodothyronine and thyroxine were measured by the chemiluminescence immune competition method using an Automated Chemiluminescence Immunoassay System (MAGLUMI 1000, Snibe, P.R. China) with a test kit from Shenzhen New Industries Biomedical Engineering Co., Ltd., P.R. China.

Digestible enzyme activity assay. The fish gut was divided into three sections: stomach, anterior intestines, and posterior intestines. The anterior and posterior sections of the intestine of three fish/tank were weighed and homogenized in 0.01M Tris buffer,

pH 7.4, at a ratio of 1:9 (tissue:buffer) with the Teflon pestle of a motor-driven tissue-cell disruptor under an ice bath. The extract was later centrifuged at 4,000 × *g* at 4°C for 20 min and the supernatant was used as the enzyme source.

Protein concentration of the tissue supernatant was determined using the Coomassie brilliant blue method (Jiancheng Bioengineering Institute, Nanjing P.R. China) as a standard to enable calculation of enzyme-specific activities. Protease activity in the intestine was assayed following the Forint phenol-reagent method in 0.01M Tris-HCl (pH 7.4) buffer using 2% casein as a substrate. Reactions were carried out at 30°C for 10 min, stopped with 0.1M trichloroacetic acid, and centrifuged at 3,000 × *g* (4°C) for 5 min. Then, 0.5 ml supernatant was added to 2.5 ml 0.4M $NaHCO_3$ and 0.5 ml 50% Folin's phenol reagent and the optical density was read at 680 nm against tyrosine as the standard. Specific activity of protease is expressed in micromole of hydrolyzed tyrosine/min/mg protein (U/mg tissue protein). Activity of lipase and amylase in the intestine was assayed by the colorimetric method using commercial kits (Jiancheng Bioengineering Institute, Nanjing, P.R. China), and the optical density of the supernatant was read in a spectrophotometer at 660 nm. Specific activity of amylase was expressed in l mol of reducing sugars/min/mg protein (U/mg tissue protein). Specific activity of lipase was defined as the amount of substrate hydrolyzed in µmol/min/mg protein (U/mg tissue protein). A substrate-free control and an enzyme-free control were run with the experimental samples.

Statistical analysis. Data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) program for Windows (version 19, Chicago, IL, USA). Data were subjected to one-way analysis of variances (ANOVA) to compare the effects of dietary protein level between treatments. Differences between means were determined by Duncan's multiple range tests and $p<0.05$ was considered statistically significant. Data are presented as means±standard error of three replicates. The broken-line regression model (Robbins et al., 1979) was used to estimate the dietary protein requirement of Wuchang bream fry.

Results

Final body weight, weight gain rate, and specific growth rate increased as the dietary protein level increased to 34% but there was no further increase as the level rose to 36% (Table 2). The feed conversion ratio improved as the dietary protein level increased and was lowest in fish fed the 34% diet. Muscle protein content was highest and lipid was lowest in fish fed the 34% diet. Red blood cells, hemoglobin, and serum total protein rose as the dietary protein level increased. Protease and amylase activity increased with the dietary protein while lipase activity decreased. Broken-line regression analyses indicated that the ideal protein levels for rate of weight gain, specific growth rate, and feed conversion ratio in Wuchang bream fry are 32.25%, 32.81%, and 32.85%, respectively (Fig. 1).

Table 2. Growth performance, feed utilization, muscle composition, serum biochemical parameters, and digestive enzyme activity of Wuchang bream fry (*Megalobrama amblycephala*) fed diets with different dietary protein levels for 10 weeks (means±SE, n = 3).

	Dietary protein level (%)				
	28	*30*	*32*	*34*	*36*
Initial body wt (g)	16.11±0.03	16.12±0.04	16.05±0.03	16.03±0.03	16.11±0.03
Final body wt (g)	36.23±1.32^{a}	36.77±0.11^{a}	37.74±1.52^{ab}	40.69±0.64^{b}	37.78±0.85^{ab}
Wt gain rate (%)[1]	124.91±8.01^{a}	128.13±1.20^{a}	135.09±9.79^{ab}	153.93±4.22^{b}	134.63±5.96^{ab}
Specific growth rate (%/day)[2]	1.16±0.05^{a}	1.18±0.01^{a}	1.22±0.06^{ab}	1.33±0.02^{b}	1.22±0.04^{ab}
Feed conversion rate[3]	2.17±0.21^{a}	2.16±0.01^{a}	1.88±0.02^{ab}	1.77±0.08^{b}	2.20±0.06^{a}
Protein efficiency ratio[4]	1.67±0.15^{a}	1.54±0.01^{a}	1.65±0.02^{a}	1.66±0.07^{a}	1.26±0.04^{b}
Hepatosomatic index (%)[5]	1.32±0.09^{a}	1.20±0.02^{ab}	1.20±0.03^{ab}	1.18±0.03^{ab}	1.12±0.05^{b}
Viscerosomatic index (%)[6]	9.64±0.25^{a}	8.73±0.30^{b}	8.39±0.21^{b}	8.20±0.10^{b}	8.48±0.29^{b}
Fulton condition factor (%)[7]	1.72±0.07^{a}	1.88±0.02^{ab}	2.03±0.08^{b}	1.91±0.03^{b}	1.93±0.06^{b}
Survival rate (%)	98.67±1.33	94.67±1.33	97.33±1.33	97.33±1.33	96.00±1.79

Table 2 (cont.).

Muscle composition (%)					
Moisture	79.02±0.37	75.47±0.88	76.18±0.41	76.88±0.07	76.26±0.48
Crude protein	15.45±0.15	14.47±0.10^{a}	16.38±0.21^{b}	17.41±0.23^{c}	16.37±0.30^{b}
Crude lipid	1.98±0.14	2.83±0.25^{a}	2.25±0.16^{ab}	2.03±0.03^{b}	2.05±0.29^{b}
Ash	1.57±0.02	1.58±0.04	1.40±0.02	1.66±0.18	1.45±0.01
Hematological parameters					
White blood cell count (× 10^{9}/l)	24.94±1.53	18.53±1.91	22.85±4.03	20.26±2.26	21.78±0.75
Red blood cell count (× 10^{12}/l)	0.18±0.03^{a}	0.18±0.01^{a}	0.22±0.02^{ab}	0.20±0.01^{ab}	0.24±0.01^{b}
Hemoglobin count (g/l)	7.33±1.20^{a}	7.44±0.99^{a}	8.56±0.99^{ab}	8.50±0.58^{ab}	10.33±0.33^{b}
Hematocrit concentration (%)	1.78±0.24^{a}	1.86±0.24^{ab}	2.26±0.25^{ab}	1.88±0.12^{ab}	2.44±0.01^{b}
Platelet count (× 10^{9}/l)	10.56±1.28	9.67±1.68	8.72±0.15	10.33±0.51	8.44±0.29
Serum biochemical parameters					
Glucose (mmol/l)	32.52±0.53	30.23±2.24	32.65±1.15	30.18±2.63	33.75±1.75
Total cholesterol (mmol/l)	5.13±0.19	5.13±0.55	4.11±0.34	4.53±0.18	4.25±0.28
Triacyglycerol (mmol/l)	0.77±0.01	0.67±0.04	0.67±0.03	0.71±0.04	0.69±0.04
Total protein (g/l)	23.04±0.18^{a}	23.69±0.58^{ab}	24.33±0.61^{ab}	24.78±0.61^{b}	23.86±0.39^{ab}
Aspartate aminotransferase (U/l)	126.52±20.86^{a}	93.31±1.62^{ab}	76.16±4.85^{bc}	73.76±5.39^{bc}	57.03±9.75^{c}
Alanine aminotransferase (U/l)	2.26±0.46	3.39±1.11	2.53±0.77	3.43±1.32	1.66±0.28
Triiodothyronine (ng/ml)	0.43±0.15^{a}	1.00±0.21^{ab}	1.06±0.25^{ab}	0.99±0.10^{ab}	1.14±0.34^{b}
Thyroxine (ng/ml)	7.31±0.78^{a}	7.99±0.84^{ab}	10.70±1.41^{b}	7.57±0.41^{a}	6.84±0.52^{a}
Digestive enzyme activities (U/mg tissue protein)					
Protease	0.413±0.078^{a}	0.620±0.076^{ab}	0.680±0.015^{bc}	0.877±0.046^{cd}	0.973±0.100^{d}
Lipase	0.050±0.006^{a}	0.040±0.006^{ab}	0.047±0.003^{ab}	0.037±0.003^{b}	0.040±0.000^{ab}
Amylase	0.567±0.079^{a}	0.663±0.041^{ab}	0.700±0.044^{ab}	0.623±0.070^{ab}	0.810±0.055^{b}

Means in a row with different superscripts significantly differ (p<0.05).
[1] 100(final body wt - initial body wt)/initial body wt
[2] 100(ln final body wt - ln initial body wt)/feeding days
[3] feed intake/wt gain
[4] wt gain/protein intake
[5] 100(wt of liver/total body wt)
[6] 100(wt of viscera/total body wt)
[7] 100(fish wt/fish length ^{3})

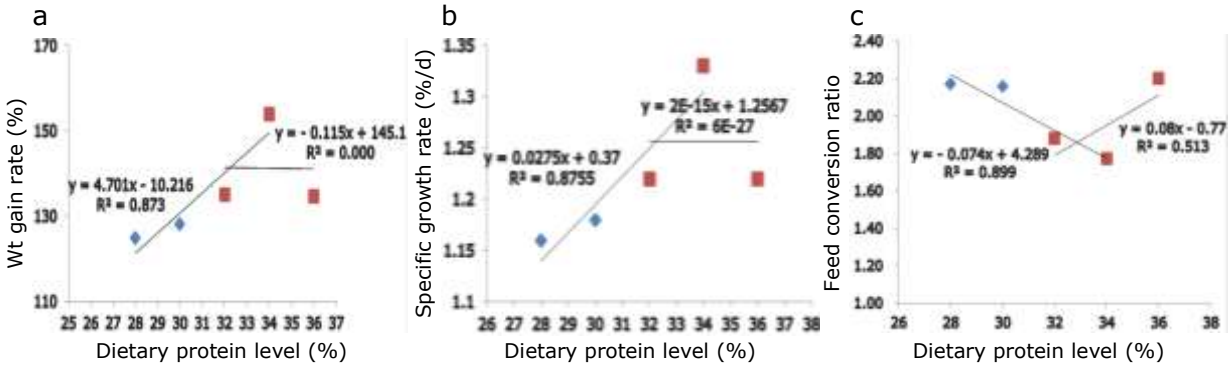

Fig. 1. Broken-line model of dietary protein level to (a) weight gain rate, (b) specific growth rate, and (c) feed conversion ratio for Wuchang bream fry (*Megalobrama amblycephala)* fed diets with different protein levels for 10 weeks. Each point represents the means of triplicate groups, with 25 fish per group. On the bases of the broken-line models for weight gain, specific growth rate, and feed conversion ratio, the dietary protein requirements for Wuchang bream fry are 32.25%, 32.81%, and 32.85%, respectively.

Discussion

Weight gain rate and specific growth rate increased as the dietary protein level rose from 28% to 34% then either leveled off or declined when the protein level increased to 36%, indicating that dietary protein beyond 34% has no significant benefit for growth improvement of Wuchang bream fry. Excess dietary protein can result in additional energy costs by deamination and consequent reduction to energy for growth (Vergara et

al., 1996). FCR significantly decreased as the dietary protein level increased up to 34%, but thereafter increased, which can be attributed to the effect of the enhanced weight gain (Deng et al., 2011a). Similar trends were observed in Nile tilapia by Abdel-Tawwab et al. (2010) and sea bream by Lupatsch et al. (2003).

Based on the broken-line analyses of weight gain rate, SGR, and FCR against the dietary protein level, the optimum dietary protein requirement for Wuchang bream fry is 32.25-33.81% (P/E = 20.52 and 20.99 mg/KJ, respectively), within the reported 25.6-41.4% for blunt snout bream fingerlings (Shi et al., 1988). However, our result is a bit higher than the requirement for the growth and effective protein utilization of blunt snout bream fingerlings: 31% protein, with 7% lipid and 18.57 MJ/kg diet (Li et al., 2010). The variation in protein requirements could be due to differences in feeding methods, feeding frequency, age, stocking density, water temperature, or rearing system (closed recirculation system vs cage system). However, the greatest difference between the two studies seems to be the level of available energy and type of diet (semi-purified vs practical diet). In our study, the dietary protein requirement was lower when analyzed by the broken-line model than when analyzed by the statistical method. The statistical method used to analyze data, such as the dose-response model, may affect the estimated requirements, and requirements estimated by the broken-line model are often lower than those estimated by a nonlinear model (Baker, 1986).

Fish fed the 28% diet had significantly higher PER than those fed the 36% diet, suggesting that fishes cannot utilize excess dietary protein for protein synthesis but can utilize it as an energy source (Santinha et al., 1996; Kim and Lee, 2009; Abdel-Tawwab, 2012). HSI and VSI significantly decreased as the protein level increased in our study, similar to results in Asian catfish fry (Singh et al., 2009). Wuchang bream fry fed the 28% diet had the highest HSI and VSI and poorest growth performance, possibly due to the low dietary protein content and the ratio between protein and carbohydrate (Moreira et al., 2008; Deng et al., 2011b). High HSI and VSI are often related to poor growth and fish health due to increased levels of dietary carbohydrate (Hamre et al., 2003; Moreira et al., 2008). The Fulton condition factor positively correlated with the dietary protein level, indicating that a higher dietary protein level increases the nutrient content in the fish body (Ali et al., 2005).

The dietary protein level significantly influenced muscle protein and lipid contents but moisture and ash contents were not significantly influenced, similar to observations in the whole body of sunshine bass juvenile (Gallagher, 1999). The increase of muscle protein content in fish fed the 32% diet is similar to results in the slender walking catfish (Kiriratnikom and Kiriratnikom, 2012). Fish fed the high dietary protein levels tended to have significantly higher muscle protein content, but lower lipid contents, as in juvenile *Zacco barbata* (Shyong et al., 1998). The reason for changes in protein and lipid contents in the fish body could be linked to changes in the synthesis and/or deposition rate of protein and lipid in the muscle (Abdel-Tawwab et al., 2006). The lipid content of the fish fed the 28% diet was significantly higher than in fish fed the higher dietary protein levels, suggesting that the lipid levels in this study were low and the same in all diets and did not induce muscle lipid deposition. More likely, the higher dietary carbohydrate level in the 28% diet induced lipid deposition in the muscle.

The dietary protein level significantly affected red blood cell count, hemoglobin, and hematocrit, comparable to changes in RBC count and hemoglobin reported by Abdel-Tawwab (2012). The increase in RBC count may have occurred because of its release from the storage pool in the spleen (Pulsford et al., 1994) as spleenic activity is affected by the dietary protein level (Abdel-Tawwab, 2012).

Total protein content of the serum tended to increase with the increase in dietary protein level, likely due to the enhancement of digested protein (Lundstedt et al., 2002), and similar to Nile tilapia (Abdel-Tawwab et al., 2010). Generally, high aspartate aminotransferase (AST) and alanine aminotransferase (ALT) indicate a weakening or damage of normal liver function. AST and ALT are extensively used in studies that evaluate finfish response to toxins, stress, disease, and malnutrition. The highest serum AST was observed in fish fed the 28% diet. These fish were possibly affected by

malnutrition as suggested by their poor growth performance. Results were comparable in juvenile tiger puffer (Kim and Lee, 2009). Serum triiodothyronine (T_3) concentration increased as the dietary protein increased. The lowest concentration was observed in fish fed the 28% diet, indicating that a dietary protein level below 30% may lead to reduced growth in Wuchang bream fry, as in blunt snout bream (Li et al., 2011). The highest thyroxine (T_4) concentration was observed in fish fed the 32% diet. The T_4 concentration may have enhanced the weight gain of the fish up to the optimum protein level.

The dietary protein level significantly affected the activity of protease, lipase, and amylase in the intestine of Wuchang bream fry. Unspecific protease activity increased as the dietary protein level increased with the highest activity in fry fed the 36% diet. This is reasonable, considering the fish's, history, position in the course of evolution, size, and diet. In jundiás, protease had great heterogeneity between diets (Lazzari et al., 2010). Lipase activity significantly decreased as the dietary protein level increased and the highest lipase activity was observed in fish fed the 28% diet. The availability of lipid from dietary carbohydrate seems to be higher in fish fed diets with lower protein levels, similar to results in tambaqui (De Almeida et al., 2006). Amylase activity correlated with dietary protein, reasonable since lower dietary protein levels and the consequent reductions in some amino acids are responsible for reduced amylase expression (De Almeida et al., 2006).

In conclusion, on the basis of broken-line regression analyses of weight gain rate, specific growth rate, and feed conversion ratio against dietary protein level, the present study indicates that Wuchang bream fry require 32-33% protein (20 mg protein/21 kJ energy). Within this range, growth was maximum, feed conversion ratio was lowest, muscle protein content was highest, and physiological performance was highest. However, the increase of dietary protein level above this range resulted in poorer growth, feed utilization, and physiological performance in Wuchang bream fry.

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