Effect of Dietary Carbohydrate Level on Growth Performance, Blood Chemistry, Hepatic Enzyme Activity, and Growth Hormone Gene Expression in Wuchang Bream (*Megalobrama amblycephala*)

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Key words: *Megalobrama amblycephala*, cassava starch, growth, plasma parameters, hepatic enzymes activities

Abstract

An 8-week feeding trial investigated the effect of dietary carbohydrate level on growth performance, blood chemistry, hepatic enzyme activity, and growth hormone gene expression of juvenile Wuchang bream (*Megalobrama amblycephala*). Six isonitrogenous (34% crude protein) and isolipidic (8% crude lipid) semi-purified diets containing graded levels of carbohydrate (0, 19%, 25%, 31%, 38%, 47%) were fed to triplicate groups of juveniles. The significantly highest weight gain and specific growth rate were obtained in fish fed the 31% diet. Whole body moisture dropped while lipid and hepatic glycogen rose as the carbohydrate level increased but there were no significant differences in whole body protein or ash content. The hepatosomatic index, plasma glucose, and liver glycogen levels positively correlated with the dietary carbohydrate level. Glucokinase activity significantly rose and glucose-6-phosphatase activity significantly dropped as the carbohydrate level rose, but there were no significant differences in phosphoenolpyruvate carboxykinase activity. Growth hormone gene expression was positively related to growth performance. Based on the measured parameters, the appropriate dietary carbohydrate supplementation of juvenile Wuchang bream was 31-34% of the diet.

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Introduction
Carbohydrates are excellent sources of energy and carbon, and one of the major elements from which living organisms are composed. Carbohydrates are relatively lower in cost than proteins and lipids, and are thus used in fish diets to improve their physical quality, reduce catabolism of proteins and lipids for energy, and provide metabolites for biological syntheses (Wilson, 1994). In some species, growth improves when fish are fed a diet with an appropriate carbohydrate level compared to a diet devoid of carbohydrates (Hemre et al., 2002). Excessive dietary carbohydrates, however, can lead to inhibited growth performance and disorder of certain physiological functions (Hemre et al., 2002). In general, herbivorous fish are more capable of utilizing dietary carbohydrate than carnivorous and omnivorous fish (Wilson, 1994; Hemre et al., 2002).

Wuchang bream (*Megalobrama amblycephala*) is a Chinese freshwater herbivorous species with great potential for aquaculture. This fish was originally found in Newshan and Yuli Lakes. Its main distribution is in the mid reach of the Yangtze River, China (Zhu, 1995). The merits of this species include tender flesh, fast growth, feeding on natural foods, high disease resistance, economic profitability, and cultural value (Ke, 1986; Zhou et al., 2008). Wuchang bream is widely cultured in China. Production reached 625,789 tons in 2009, an increase of 31.5% over the previous decade. The species has been introduced to North America (from northern Canada to southern Mexico), Africa, Europe, and other Asian countries. It adapts well to local environments, is compatible with native species, and thus has a bright future for culture worldwide.

The dietary requirements of Wuchang bream for crude protein and lipid were reported by Zhu (1995), Zhou et al. (1997), and Li et al. (2010). The purpose of the present study was to quantify the appropriate level of dietary carbohydrate (cassava starch) in diets for Wuchang bream.

Materials and Methods
Fish. Wuchang bream juveniles were obtained from the fish farm at the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, China, and reared in fifteen 300-l cylindrical fiberglass tanks. Fish were acclimated for 15 days during which they were fed a commercial diet (carp feed no. 1038, Tongwei Feed Group Co., Ltd., Yancheng, China).

Diet preparation. Following methods of Liu et al. (20120), six isonitrogenous (34% crude protein) and isolipidic (8% crude lipid) semi-purified diets were formulated to contain 0, 19%, 25%, 31%, 38%, or 47% carbohydrate in the form of cassava starch (Table 1). Dry ingredients were thoroughly mixed in a mixer, then water was added and mixed. Pellets of 2 mm were wet-extruded, dried in a forced air oven at 40°C to a moisture content of 10%, and stored at -20°C until use.

Experimental procedure. At the start of the experiment, the fish were fasted for 24 h and weighed. Juvenile bream (15.73±0.03 g) were randomly sorted into eighteen 300-l cylindrical fiberglass tanks at 20 fish per tank. The tanks were supplied a continuous flow of sand-filtered fresh water and continuous aeration. Fish were subjected to a natural photoperiod regime and all tanks had similar light conditions. The diets were randomly assigned to triplicate tanks and fed to the fish three times per day (06:30, 11:30, 16:30). To prevent the waste of pellets, fish were slowly hand-fed to satiation, based on visual observation of their feeding behavior. The feeding trial lasted eight weeks during which feed consumption and the number and weight of dead fish were recorded daily. The water temperature fluctuated 24-26°C and dissolved oxygen was approximately 6.0 mg/l. At the end of the feeding trial, the fish were fasted for 24 h, then counted and weighed.

Sample collection. At the end of the feeding trial, three fish from each tank were fasted for 24 h, anesthetized in diluted MS-222 (tricaine methanesulfonate, Sigma, USA) at 200 mg/l, and blood samples were collected from the caudal vein using heparinized syringes. The blood was centrifuged at 3000 g for 10 min at 4°C, and the plasma was separated and stored at -80°C. After blood collection, livers were quickly sampled and weighed. The liver samples were frozen in liquid nitrogen and stored at -80°C until assay.
Effect of dietary carbohydrate level on Wuchang bream (Megalobrama amblycephala).

For body composition analysis, an initial sample of 20 fish at the beginning of the 8-week experiment and final samples of three fish per tank were collected and stored at -20°C. Dry matter, crude protein, crude lipid, and ash contents of the diets and fish bodies were chemically analyzed following AOAC (1995) procedures: dry matter by drying in an oven at 105°C to a constant weight; crude protein (nitrogen × 6.25) using the Kjeldahl method after acid digestion (FOSS KT260, Switzerland); crude lipid by ether extraction in a Soxtec System HT (Soxtec System HT6, Tecator, Sweden); and ash by incineration in a muffle furnace at 550°C for 4 h. The hepatic glycogen content was determined as described in Plummer (1987).

Plasma glucose, triglyceride, and cholesterol. Plasma glucose, triglyceride, and cholesterol contents were measured using the glucose oxidase method, the enzymatic (glycerol phosphate oxidase) and colorimetric (PAP) method, and the enzymatic (cholesterol oxidase) and colorimetric method, respectively, with kits purchased from Junshi Biotechnology Co., Ltd. (Shanghai, China).

Enzyme activity. A frozen sample of liver was homogenized (dilution 1/10) in ice-cold buffer consisting of 80 mM Tris (pH 7.5), 5 mM EDTA, 1 mM KH₂PO₄, 2 mM NaHCO₃, and 1.4 mM dithiothreitol. The homogenate was centrifuged at 4000 rpm for 10 min at 4°C, and the supernatant was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was divided into three parts for measurement of glucokinase (GK), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase) activity. Enzyme activity is expressed per mg of total protein (specific activity). The total protein content in crude extracts was determined at 30°C using bovine serum albumin as the standard, based on the method of Bradford (1976).

Table 1. Formulation and nutrient composition of diets for juvenile Wuchang bream (Megalobrama amblycephala).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet (% carbohydrate level)</th>
<th>0</th>
<th>19</th>
<th>25</th>
<th>31</th>
<th>38</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47</td>
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<td>47</td>
<td>47</td>
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<tr>
<td>Microcrystalline cellulose</td>
<td>45</td>
<td>27</td>
<td>21</td>
<td>15</td>
<td>9</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Cassava starch</td>
<td>0</td>
<td>18</td>
<td>24</td>
<td>30</td>
<td>36</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Fish oil</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Carboxyl-methyl cellulose</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>2</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Zeolite power</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Proximate analysis. For body composition analysis, an initial sample of 20 fish at the beginning of the 8-week experiment and final samples of three fish per tank were collected and stored at -20°C. Dry matter, crude protein, crude lipid, and ash contents of the diets and fish bodies where chemically analyzed following AOAC (1995) procedures: dry matter by drying in an oven at 105°C to a constant weight; crude protein (nitrogen × 6.25) using the Kjeldahl method after acid digestion (FOSS KT260, Switzerland); crude lipid by ether extraction in a Soxtec System HT (Soxtec System HT6, Tecator, Sweden); and ash by incineration in a muffle furnace at 550°C for 4 h. The hepatic glycogen content was determined as described in Plummer (1987).

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1 Norwegian fishmeal, Shanghai Imports and Exports Co. Ltd., China; crude protein 67.70%, crude fat 7.90%, carbohydrate 0.45%, ash 16.70%.
2 Zhejiang Joinway Pharmaceutical Co., Ltd., China
3 Wuxi Yongfeng starch Engineering Co., Ltd., China
4 Wuxi Xunda Ocean Biological Co., Ltd., China
5 Shanghai Jiande Industrial Co., Ltd., China
6 per kg premix: CuSO₄·5H₂O 2.5 g, FeSO₄·7H₂O 28 g, ZnSO₄·7H₂O 22 g, MnSO₄·4H₂O 9 g, Na₂SeO₃ 0.045 g, KI 0.026 g, CoCl₂·6H₂O 0.1 g
7 per kg premix: vitamin A 900,000 IU, vitamin D 250,000 IU, vitamin E 4500 mg, vitamin K3 220 mg, vitamin B1 320 mg, vitamin B2 1090 mg, vitamin B5 2000 mg, vitamin B6 500 mg, vitamin B12 116 mg, vitamin C 5000 mg, pantothenate 1000 mg, folic acid 165 mg, folic acid 165 mg, choline 60,000 mg
8 Nanjing Huamu Animal Research Institute, China
9 calculated according to protein 23.64 kJ/g, fat 39.54 kJ/g, carbohydrate 17.15 kJ/g
GK activity. GK (EC 2.7.1.2) activity was measured using the G6PDH coupling method (Tranulis et al., 1996; Panserat et al., 2000a). The reaction system contained 65 mU/ml G6PDH, 2 mM NADH, 7 mM ATP, 80 mM Tris, 5 mM EDTA, 8 mM MgSO₄, 1 mM KH₂PO₄, 2 mM NaHCO₃, 0.2 mM dithiothreitol, and 100 mM glucose (pH 8.2). The glucose concentration was 0.64-1277 mM in the kinetic study; the assay for measuring GK activity using frozen samples necessitates correction by measuring glucose dehydrogenase activity (EC 1.1.1.47) as previously described (Tranulis et al., 1996). One unit of GK activity was defined as the amount of NADPH generated/g protein/min at 30°C.

PEPCK activity. PEPCK (EC 4.1.1.32) activity was measured according to the method described by Kirchner et al. (2003). The reaction system contained 2 mM IDP, 5 mM MnCl₂, 1.1 U/ml MDH, 2.5 mM PEP, 0.12 mM NADH, and 0.1 mol/l Tris-HCl (pH 7.4). One unit of PEPCK activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μM of glucose-6-phosphate/min at 30°C.

G6Pase activity. G6Pase (EC 3.1.3.9) activity was measured following the method described by Panserat et al. (2000a,b). The reaction system contained 26.5 mM G6P, 1.8 mM EDTA, 2 mM NAD⁺, 0.5-0.7 U/ml mutarotase, 5-7 U/ml glucose dehydrogenase, and 100 mM imidazole-HCl. One unit of G6Pase activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μM of glucose-6-phosphate/min at 30°C.

Real-time PCR measurement of hepatic growth hormone (GH) gene. We used the M. amblycephala cDNA sequences in GenBank to design primers for GH, accession no. AF463498.2, and β-actin, accession no. AY170122.2 (Ming et al., 2010). The primers consisted of 5'-GCCGAGCCATCTCAAAACGCC-3' and 5'-AACGCAAGCCAGAAGGAAA-3' for GH and 5'-TCGTCACCTTCACCGTTCA-3' and 5'-CGGTACCTTCCAGTTCCAGT-3' for β-actin and were synthesized by Shanghai Generay Biotech Co., Ltd. (Shanghai, China). The primers amplified a single PCR product of the size determined by the melting temperature, agarose gel electrophoresis, and melting curve analysis, i.e., 100-150 bp.

RNA was extracted from the liver tissue using RNAiso Plus (Dalian Takara Co. Ltd., China). The RNA samples were treated with RQ1 RNase-Free DNase (Dalian Takara) to avoid genomic DNA amplification. cDNA was generated from 500 ng DNase-treated RNA using ExScript™ RT-PCR Kit (Dalian Takara). The reverse transcription PCR reaction solution consisted of 500 ng RNA, 2 μl 5 x buffer, 0.5 μl dNTP mixture (10 mM each), 0.25 μl RNase inhibitor (40 U/μl), 0.5 μl dT-AP primer (50 mM), and 0.25 ml ExScript™ RTase (200 U/μl), with DEPC-treated H₂O to a final volume of 10 μl. The reaction conditions were 37°C for 15 min, 85°C for 5 s, and 4°C thereafter.

We used real-time PCR to determine mRNA levels using an SYBR Green I fluorescence kit (Ming et al., 2010). Real-time PCR was performed in a Mini Opticon Real-Time Detector (Bio-Rad, USA). The fluorescent quantitative PCR reaction solution consisted of 12.5 μl SYBR premix Ex Taq™ (2×), 0.5 μl PCR forward primer (10 μM), 0.5 μl PCR reverse primer (10 μM), 2.0 μl RT reaction mix (cDNA solution), and 9.5 μl H₂O. The reaction conditions were 95°C for 2 min, followed by 44 cycles consisting of 9°C for 10 s, 62°C for 20 s, and 72°C for 15 s. The fluorescent flux was recorded and the reaction continued at 72°C for 3 min. We measured the dissociation rate between 65°C and 92°C. Each increase of 0.2°C was maintained for 1 s and the fluorescent flux was recorded. We calculated the relative quantification of the target gene transcript (GH) with a chosen reference gene transcript (β-actin) using the 2^{-ΔΔCT} method. This mathematical algorithm, which does not require a calibration curve, computes an expression ratio based on real-time PCR efficiency and the crossing point deviation of the sample versus a control. We measured the PCR efficiency by constructing a standard curve using a serial dilution of cDNA: ∆ΔCT = (Cₜ, Target - Cₜ, β-actin at time x) - (Cₜ, Target - Cₜ, β-actin at time 0).

Statistical analysis. Results are presented as means±SE. All data were subjected to one-way analysis of variance (ANOVA) to test the significance of the effects of the experimental diets. When differences were significant (p<0.05), the means among the dietary treatments were compared using Duncan’s multiple range test. All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA).
Results

No fish died during the growth trial. Weight gain and specific growth rate (SGR) were significantly highest in the diet containing 31% dietary carbohydrate (Table 2). The broken-line regression model between weight gain and dietary starch level was Y = 233.09 - 2.12(33.66 - X), 33.66 - X = 0 when X > 33.66, R² = 0.947 (Fig. 1). The optimum dietary starch level was 34% for maximum weight gain. The feed conversion ratio was significantly lowest in fish fed the 31% diet while the feeding rate was significantly highest with this diet.

The hepatosomatic (HSI) and viscerosomatic (VSI) indices increased as the dietary carbohydrate increased while the protein efficiency ratio (PER) rose up to the 25% dietary level, then remained nearly the same thereafter. Hepatic glycogen concentration increased significantly with the dietary carbohydrate level. Whole body protein and ash contents were not significantly affected by the dietary carbohydrate level. Plasma glucose increased with the dietary carbohydrate level, while plasma cholesterol was significantly lower in fish fed the 31% diet. Hepatic GH gene expression increased as the dietary carbohydrate level increased to 38%, and thereafter declined. PEPC activity did not differ among treatments but GK activity increased and G6Pase activity decreased significantly as the dietary carbohydrate rose.

Table 2. Effects of diets with different carbohydrate levels on Wuchang bream, Megalobrama amblycephala (means±SE; n = 3).

<table>
<thead>
<tr>
<th></th>
<th>Diet (% carbohydrate level)</th>
<th>0</th>
<th>19</th>
<th>25</th>
<th>31</th>
<th>38</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth and feed performance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Specific growth rate¹</td>
<td></td>
<td>1.74±0.03a</td>
<td>1.96±0.01a</td>
<td>1.98±0.01d</td>
<td>2.18±0.00a</td>
<td>2.07±0.03ab</td>
<td>2.04±0.03bc</td>
</tr>
<tr>
<td>Feed conversion ratio²</td>
<td></td>
<td>3.60±0.15a</td>
<td>2.93±0.06b</td>
<td>2.67±0.09bc</td>
<td>1.60±0.04a</td>
<td>2.11±0.10ef</td>
<td>2.31±0.20de</td>
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<tr>
<td>Feeding rate³</td>
<td></td>
<td>2.57±0.08ab</td>
<td>2.60±0.04a</td>
<td>2.77±0.01a</td>
<td>2.81±0.07a</td>
<td>2.61±0.04a</td>
<td>2.59±0.02bc</td>
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<td>Protein efficiency ratio⁴</td>
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<td>1.50±0.09ab</td>
<td>2.09±0.12ab</td>
<td>2.27±0.04a</td>
<td>2.65±0.45a</td>
<td>2.67±0.08a</td>
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<td>Condition factor⁵</td>
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<td>2.00±0.07a</td>
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<td>2.15±0.03ab</td>
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<td>2.08±0.05ab</td>
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<td>Hepatosomatic index⁶</td>
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<td>10.12±0.23ab</td>
<td>10.74±0.72ab</td>
<td>11.24±0.66ab</td>
<td>11.95±0.51ab</td>
<td>11.59±0.61ab</td>
<td>12.39±0.81ab</td>
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<td>Viscerosomatic index⁷</td>
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<td>1.08±0.02c</td>
<td>1.53±0.04b</td>
<td>1.99±0.23a</td>
<td>1.97±0.08ab</td>
<td>1.80±0.22ab</td>
<td>2.10±0.06bc</td>
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<td>Hepatic glycogen content</td>
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<td>13.84±1.11c</td>
<td>17.18±0.69b</td>
<td>17.41±0.97bc</td>
<td>17.90±0.93ab</td>
<td>18.76±0.68ab</td>
<td>20.71±0.80ab</td>
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<td><strong>Body composition (%)</strong></td>
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<tr>
<td>Moisture (%)</td>
<td></td>
<td>75.73±1.2a</td>
<td>75.52±1.15a</td>
<td>74.38±1.14ab</td>
<td>73.46±0.38ab</td>
<td>73.46±0.32ab</td>
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<tr>
<td>Crude protein (% wet wt)</td>
<td></td>
<td>14.68±0.69</td>
<td>15.50±0.97</td>
<td>14.91±0.85</td>
<td>15.14±0.68</td>
<td>15.04±0.32</td>
<td>15.95±0.59</td>
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<tr>
<td>Crude lipid (% wet wt)</td>
<td></td>
<td>4.15±0.15b</td>
<td>5.04±0.66ab</td>
<td>5.44±0.73ab</td>
<td>5.49±0.68ab</td>
<td>5.87±0.16ab</td>
<td>5.97±0.47b</td>
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<tr>
<td>Ash (% wet wt)</td>
<td></td>
<td>5.11±0.29</td>
<td>4.61±0.29b</td>
<td>5.10±0.62</td>
<td>4.40±0.36</td>
<td>4.86±0.23</td>
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<td><strong>Blood chemistry (mmol/l)</strong></td>
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<tr>
<td>Glucose</td>
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<td>4.19±0.57a</td>
<td>5.08±0.18ab</td>
<td>6.18±0.98ab</td>
<td>6.17±0.87ab</td>
<td>6.53±0.58b</td>
<td>6.99±0.30a</td>
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<td>Cholesterol</td>
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<td>4.41±0.16a</td>
<td>3.56±0.07b</td>
<td>3.59±0.07b</td>
<td>4.41±0.23a</td>
<td>4.18±0.25ab</td>
<td>4.47±0.37b</td>
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<td>Triglyceride</td>
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<td>2.37±0.14</td>
<td>2.89±0.24</td>
<td>3.06±0.25</td>
<td>3.01±0.29</td>
<td>2.99±0.19</td>
<td>3.00±0.60</td>
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<tr>
<td><strong>Enzyme activity (per mg total protein/min at 30°C)</strong></td>
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<tr>
<td>Glucokinase</td>
<td></td>
<td>3.24±0.20abc</td>
<td>4.88±1.07cd</td>
<td>6.11±0.63c</td>
<td>8.79±0.88b</td>
<td>13.41±0.77a</td>
<td>14.08±0.68a</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxykinase</td>
<td></td>
<td>13.07±0.68</td>
<td>12.21±0.47</td>
<td>12.73±0.71</td>
<td>12.66±0.31</td>
<td>11.25±0.53</td>
<td>11.92±0.83</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td></td>
<td>24.43±0.68a</td>
<td>23.23±0.45a</td>
<td>20.99±1.05a</td>
<td>18.68±0.47c</td>
<td>16.04±0.34d</td>
<td>14.62±0.60d</td>
</tr>
<tr>
<td>Hepatic growth hormone of mRNA (n = 9)</td>
<td></td>
<td>1.01±0.06b</td>
<td>3.25±0.21c</td>
<td>3.64±0.53c</td>
<td>3.99±0.33bc</td>
<td>6.56±0.51a</td>
<td>4.83±0.21b</td>
</tr>
</tbody>
</table>

Means in a row with different superscripts significantly differ (p<0.05).
¹ 100(LnWt - LnW0)/T, where W0 and Wt are initial and final body wt and T is the culture period in days
² total diet fed/total wet wt gain
³ 100(dry feed intake)/T(initial wet body mass + final wet body mass)/2
⁴ 100(final wet wt gain/protein intake)
⁵ 100(body wt/total body length)³
⁶ 100(liver wt/final wet body wt)
⁷ 100(liver wt/body wt)
Increased dietary carbohydrate intake led to a significant increase in growth of Wuchang bream juveniles, and the best growth and feed utilization were observed in fish fed the diet containing 31% carbohydrate. The broken-line model based on regression analysis of the weight gain versus dietary carbohydrate level indicated that the optimum dietary carbohydrate level for maximum weight gain was 34%, similar to that reported for herbivorous common carp (30-40%; Satoh, 1991) but higher than for carnivorous Asian seabass (Boonyaratpalin, 1991) and Atlantic salmon (Helland et al., 1991). In natural habitats, Wuchang bream feed mainly on Vallisneria natans, Hydrilla verticillata, and zooplankton.

Carbohydrates supply energy at low cost, but sufficient quantities of protein to meet anabolic requirements should be supplied in the diet to gain the protein-sparing effect of the carbohydrate (Stone et al., 2003). In the present study, fish fed diets with higher carbohydrate showed higher PER. To some extent, the carbohydrate in the diet supplied energy to gain the protein-sparing effect.

In this study, whole body lipid content was significantly affected by the dietary carbohydrate level, as in European sea bass (Moreira et al., 2008). A positive correlation between plasma glucose concentration and dietary carbohydrate level was found in cobia, Rachycentron canadum L. (Ren et al., 2011). In the present study, the result was similar: after 24 h of fasting, the plasma glucose level increased with the increasing dietary carbohydrate, indicating that Wuchang bream have a relatively lower capacity to metabolize glucose.

Altering the carbohydrate content of the fish diet induces pronounced changes in carbohydrate metabolism, especially in the activity of key enzymes of carbohydrate metabolism in the liver (Leung and Woo, 2011). In this study, glucokinase activity increased with the increase in dietary carbohydrate in agreement with results in other fish species (Panserat et al., 2000b; Metón et al., 2004). The glucose-6-phosphatase activity decreased significantly with the increased dietary carbohydrate level, similar to reports for other fish species (Panserat et al., 2002). Higher activity of the glycolytic and gluconeogenesis enzymes suggests that Wuchang bream likely have the metabolic ability to adapt to relatively high dietary carbohydrate levels.

Growth hormone is synthesized in the pituitary gland and secreted into the bloodstream under the regulation of neuronal, hormonal, and nutritional factors (Duan, 1998). In fish, growth hormone can participate in protein, fat, and carbohydrate metabolism (Donaldson et al., 1979). Similarly, GH mRNA was detected in the pituitary gland, brain, gill, heart, kidney, muscle, and liver of common carp (Kumar et al., 2011) and rainbow trout and coho salmon (Tymchuk et al., 2009). For all common carp diet groups, there was an overall negative correlation between expression of the GH gene and growth performance (Kumar et al., 2011). In contrast, in the current study, growth performance was enhanced in parallel with GH gene expression. The greatest weight gain was obtained in bream fed the 31% diet, and the highest hepatic GH gene expression was obtained in fish fed the 38% diet.

In conclusion, this study provides insight into the carbohydrate requirements of juvenile Wuchang bream. Results indicate that the appropriate carbohydrate (cassava starch) level for juvenile Wuchang bream is 31-34%. Dietary starch enhanced the glycolytic pathway in the liver of Wuchang bream.
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