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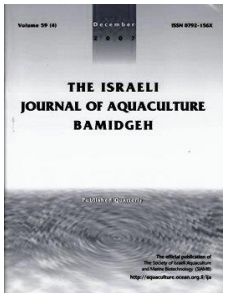

Food Deprivation of Blunt Snout Bream, *Megalobrama Amblycephala* Fingerlings and the Subsequent Effect of Feeding with Different Dietary Starch Levels on Glucose Metabolism

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Key words: blunt snout bream; starvation and feeding; metabolism; enzymes activities

Abstract

A study was conducted to assess the specific glucose metabolic pathways of the blunt snout bream (*Megalobrama amblycephala*) after a short starvation period followed by feeding with different dietary starch levels. Blunt snout bream (54±1.7 g) were fasted for 20 days, and then fed with three isonitrogenous and isolipidic diets containing 0%, 30% and 45% starch, respectively. Fish were sampled on the 1st, 7th, and 20th day of starvation (day 1S, 7S and 20S) and at the 3rd hour (H3) after the first feeding, and on the 1st, 3rd, 10th and 20th day of feeding (day 1R, 3R, 10R and 20R). The results showed that seven days starvation induced a sharp decrease in hepatosomatic index, tissue glycogen contents, plasma triglycerides, and hepatic lipid levels. Plasma protein content significantly decreased on day 20S. Fish weight and glucose content significantly decreased with prolonged starvation. Hepatic glucokinase (GK) and pyruvate kinase (PK) activities were not significantly affected by short-term starvation. Glucose-6-phosphate dehydrogenase (G6PDH) activity showed a significant decrease on day 20S. Hepatic fructose-1, 6-bisphosphatase (FBPase) activity showed a significant increase on day 7S. The level of metabolic activity prior to starvation returned on day 10R, with reestablishment of muscle and liver composition, plasma metabolites, and hepatic glucose metabolic enzymes activity. However, liver glycogen content recovered faster on day 3R. High dietary carbohydrate levels stimulated GK, PK and G6PDH enzyme activity, but decreased FBPase and aspartate transaminase (AST) activity at H3. The results indicated that glucose is an important energy source for blunt snout bream, which is mobilized as a result of nutritional challenges. 45% dietary starch level induced faster recovery of glycogen in the liver and muscle, and significantly reduced the gluconeogenesis pathway (FBPase activity) and the restoration of glycogen from three-carbon compounds compared to groups fed a lower starch level at the early feeding stage.

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Introduction

Farmed fish may experience starvation as a result of several factors such as transportation, acute water temperature changes, poor aquaculture procedures, and specific medical treatments. Feed deprivation is also used in the aquaculture industry as an efficient tool to improve the production quality, and to induce compensatory growth in overstocking situations (Caruso et al., 2012). Hence, it is essential to know how fish adapt to food deprivation and their response to subsequent feeding. Generally, fish reduce their energy expenditure which dictates metabolic adjustments, and mobilizes body energy reserves to survive during the food deprivation period (Pérez-Jiménez et al., 2012). However, these adjustments are species specific. For most fish species, glycogen and lipids are utilized as an energy sources in the early stage of starvation. With prolonged starvation, protein is metabolized when reserve glycogen and lipids are practically depleted (Pérez-Jiménez et al., 2007). In contrast, some species protect liver glycogen stores, degrading protein for gluconeogenesis and using lipid and protein as energy sources (Gillis and Ballantyne, 1996). Metabolic responses of feeding may be affected by fish species, environmental conditions, and previous feeding history (Navarro and Gutiérrez, 1995).

Composition of diets significantly influences the metabolic responses during the starvation and feeding periods in fish (Pérez-Jiménez et al., 2007). Carbohydrate is the cheapest dietary energy source, and the appropriate supplementation into fish feed may reduce the cost of formulated diets, especially for herbivorous and omnivorous fish species. Long term, fish generally show poor utilization of dietary carbohydrate compared to mammals (Hemre et al., 2002). However, during short intervals between starvation and feeding periods, fish, even carnivorous species, have the ability to regulate glucose metabolism. This suggests that higher dietary carbohydrate levels could serve as an effective energy source after starvation in fish (Metón et al., 2003).

Blunt snout bream, *Megalobrama amblycephala* is a Chinese native freshwater fish with a long history of cultivation. It is renowned for its excellent flesh quality, rapid growth, and high larval survival rate. In previous studies, 31% dietary starch was estimated to be optimal for growth performance in juvenile fish (Zhou et al., 2013) but needed to be correlated to the lipid levels in the feeds. The combined effects of dietary lipid and carbohydrate in blunt snout sea bream has been studied (Li et al, 2014). However, the mechanism of the intermediary metabolic modifications following nutritional challenges remains unclear. Based on the above information, the present study was conducted to investigate the metabolic mechanisms of blunt snout bream after a short starvation-feeding period and the effects of dietary starch levels on their metabolic responses during the feeding period. The results will enable us to learn about the glucose metabolic strategy in blunt snout bream during starvation and subsequent feeding with different carbohydrate levels, in order to prevent possible damage to fish health, and optimize the culture of this species.

Materials and Methods

Experimental diet. Three isonitrogenous and isolipidic diets (32% crude protein and 8% crude lipid), supplemented with fish meal, casein, and gelatin as protein sources, and soybean oil as a lipid source, were formulated to contain graded levels of starch (0%, 30% and 45% of dry weight, respectively). These were replaced by equal proportions of cellulose (Table 1). All the ingredients were ground into powder, thoroughly mixed with soybean oil and water, forced though a pelletizer (4-2 style, Xinchang machinery LTD, China), and dried in a ventilated oven at 30°C. After drying, the diets were sealed in bags and stored at -15°C until use.

Table 1. Composition and proximate analyses of the experimental diets

	Diet		
Ingredients (%)	*45%*	*30%*	*0% starch*
Casein[1]	22.0	22.0	22.0
Gelatin[1]	5.0	5.0	5.0
White fish meal[1]	13.0	13.0	13.0
Soybean Oil	6.0	6.0	6.0
Soybean lecithin	1.0	1.0	1.0
Wheat starch[2]	45.0	30.0	0.0
Microcrystalline	1.0	16.0	46.0
Carboxyl-methyl	4.0	4.0	4.0
Vitamin premix[4]	0.5	0.5	0.5
Mineral premix[5]	1.0	1.0	1.0
Calcium biphosphate	1.5	1.5	1.5
Proximate analysis (%dry matter)			
Crude protein	32.7	32.3	32.5
Crude lipid	8.1	8.2	8.2
Starch	44.3	29.3	0.7
Ash	7.1	7.3	7.3

[1]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%; fish meal, obtained from Copeinca (Lima, Peru), crude protein 67.4%, crude lipid 9.3%.

[2]Wheat starch obtained from Guangsheng starch Lit. (Jiangsu, China).

[3]Microcrystalline cellulose, obtained from Xinwang chemical Lit. (Zhejiang, China).

[4]Vitamin premix (IU or per kg premix): vitamin A 900000 IU, vitamin D, 250000 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 60000 mg.

[5]Mineral premix (per kg premix): $CuSO_4 \cdot 5H_2O$ 2.5g, $FeSO_4 \cdot 7H_2O$ 28g, $ZnSO_4 \cdot 7H2O$ 22g, $MnSO_4 \cdot 4H_2O$ 9g, Na_2SeO_3 0.045g, KI 0.026g, $CoCl_2 \cdot 6H_2O$ 0.1g.

Experimental fish and procedure. Blunt snout bream fingerlings (average weight 54±1.7g) were obtained from a commercial farm (Jiangsu, China), and fed with a commercial feed for 2 weeks to acclimate them to the experimental conditions. They were then randomly divided into nine 300L fiberglass cylindrical tanks with 30 fish per tank thermo-regulated in a recirculated fresh water system. Thereafter, fish were fasted for 20 days after which each of the diets was randomly assigned to triplicate tanks, and hand-fed twice daily at 8:00 and 16:00 until apparent satiation as assessed by visual observation. During the experiment, the water temperature was a constant 25±0.5°C. There were no mortalities during the trial.

Sample collection. Three fish per tank were randomly sampled on the 1st, 7th, and 20th day of starvation (day 1S, 7S and 20S), and on the 1st 3rd, 10th and 20th day of feeding (day 1R, 3R, 10R and 20R). In addition, fish were also sampled at the 3rd hour (H3) after the first feeding. Fish were euthanized with MS-222 (100mg/L), and blood samples were immediately drawn from the caudal veins with heparinzed syringes. Following centrifugation (3500×g, 10 min, 4°C), the plasma was separated. Livers and muscles were excised, immediately frozen in liquid nitrogen and stored at -80°C until analysis. Whole body fish, liver and viscera weights were recorded to determine their hepatosomatic and visceral indexes.

Laboratory analysis. Dry matter, crude protein, and lipid in the diets and muscle were determined according to 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 by Soxhlet procedure. Crude protein in the liver was determined according to the method of Bradford (1976) using bovine serum albumin as a standard. Extraction of lipids from the liver was performed by the method of Folch et al. (1957). Glycogen was determined by the method described by Hassid and Abraham (1957). Analyses of glucose and triglyceride in plasma were carried out on an automatic biochemical analyzer (Mindray BS-400, Mindray Medical International Ltd., Shenzhen, China), using glucose, total cholesterol and triglycerides kits (GPO-POD Method) respectively.

Hepatic enzyme activities were determined as described by Pérez-Jiménez et al. (2007) and Metón et al. (1999). Alanine aminotransferase (ALT) and aspartate transaminase (AST) activities were measured by detection kits (Nanjing Jiancheng Bioengineering Institute, China).

Statistical analysis. Statistical analyses were carried out using the SPSS version 11.5 for Windows software package. Significant differences in the means among dietary treatments (e.g. plasma glucose of diet 0, 30 and 45% starch on day 3R) and among different samplings (e.g. plasma glucose of diet 45% starch on day 1S, 7S, 20S, 1R, 3R, 10R, 20R, and at H3) were evaluated by Duncan's multiple range tests and $p \leq 0.05$ was considered to be statistically significant. Results are expressed as mean ± S.E.M.

Results

Fish weight and feed intake. Starvation induced a significant decrease in fish weight that was regained on day 10R ($P<0.05$). There were no significant differences among the treatments. Significantly higher feed intake values were observed on day 20R ($P<0.05$) in fish fed with diets containing 30% and 45% starch level compared to those fed the non-starch diet.. Starvation induced a significant decrease in hepatosomatic index (HSI), while feeding increased the value. Significantly higher HSI was observed in fish fed the diet containing higher starch levels during the feeding period ($P<0.05$) (Table 2).

Table 2. Fish weight, feed intake and hepatosomatic index of blunt snout bream during starvation (S) and feeding (R) period[1]

	1S	*7S*	*20S*	*3R*	*10R*	*20R*
Fish weight (g)						
45% starch	54.6±0.67^{c}	48.4±0.78^{b}	39.9±0.46^{a}	44.6±1.17^{b}	53.6±1.68^{c}	64.9±1.23^{d}
30% starch	54.2±0.33^{c}	48.0±1.32^{b}	39.6±0.66^{a}	43.6±0.96^{b}	53.3±1.53^{c}	63.5±2.25^{d}
0% starch	54.2±0.12^{c}	48.1±0.56^{b}	39.3±0.67^{a}	44.8±1.87^{b}	56.3±1.09^{c}	61.9±0.92^{d}
Feed intake[2]						
45% starch	-	-	-	3.44±0.07^{b}	3.16±0.20^{b}	2.03±0.10^{a*}
30% starch	-	-	-	3.51±0.05^{b}	3.28±0.19^{b}	2.43±0.09^{a*}
0% starch	-	-	-	3.58±0.05^{b}	3.36±0.19^{b}	2.87±0.06^{a**}
Hepatosomatic index (%)						
45% starch	1.35±0.06^{b}	0.76±0.03^{a}	0.53±0.01^{a}	2.06±0.09^{c***}	2.76±0.09^{d***}	1.91±0.11^{c**}
30% starch	1.36±0.08^{b}	0.74±0.01^{a}	0.54±0.01^{a}	1.46±0.07^{b**}	2.22±0.11^{c**}	2.09±0.11^{c**}
0% starch	1.35±0.08^{d}	0.75±0.04^{b}	0.53±0.04^{a}	1.04±0.07^{c*}	1.52±0.05^{d*}	1.40±0.08^{d*}

[1]Values are means± S.E.M.; different superscript letters in the same row indicate significant differences (P<0.05) among sampling points in each group; asterisk indicates significant differences (P<0.05) among groups at each sampling point.
Feed Intake (FI) as g/fish/day was calculated: dry diet fed in g/((Wf + Wi)/2×t), t is the experimental duration in days from last weighing in each tank.
[2]Fish were not weighed in each tank at hour H3 and day 1R in order not to stress fish. Feed intake (FI, g/fish/day) = dry diet fed in g / ((Wf + Wi)/2 ×t), t is the experimental duration in days from last weighed in each tank.

Plasma metabolites. Plasma total protein levels significantly decreased on day 20S, and recovered to the pre-starvation levels in all the groups on day 10R (P<0.05). Plasma total protein levels were similar among the three groups throughout the experiment (P>0.05). On day 7S, plasma triglyceride levels were sharply reduced (P<0.05). From day 7S onwards, these values remained low. Feeding restored the plasma triglyceride to pre-starvation levels in all groups from day 10R. No significant differences were observed among the three groups (P>0.05). Plasma protein and glucose content showed a fluctuating trend during starvation and feeding periods. However, significantly higher plasma glucose levels were found in fish fed diet containing 45% starch compared with those fed with diets containing 0% and 30% starch at H3 (P<0.05). Results of plasma parameters are presented in Table 3.

Table 3. Plasma protein, triglyceride and glucose content of blunt snout bream during starvation (S) and feeding (R) period[1]

	1S	*7S*	*20S*	*H3*	*1R*	*3R*	*10R*	*20R*
Protein (g/L)								
45% starch	35.4±1.74^{c}	35.6±0.97^{a}	26.0±2.07^{a}	24.6±1.33^{a}	27.6±0.49^{ab}	26.0±1.93^{a}	32.1±1.53^{bc}	31.2±1.92^{bc}
30% starch	35.8±0.73^{c}	35.3±1.13^{c}	27.8±0.66^{b}	24.5±2.35^{ab}	23.4±1.04^{a}	23.5±0.73^{a}	31.8±1.23^{c}	32.2±1.77^{c}
0% starch	35.8±0.90^{c}	35.1±1.20^{c}	27.9±1.17^{ab}	25.0±1.02^{a}	24.9±1.03^{a}	26.6±1.15^{a}	35.1±1.52^{c}	30.9±1.30^{c}
Triglyceride (mmol/L)								
45% starch	3.20±0.05^{b}	0.77±0.02^{ab}	0.54±0.03^{a}	0.41±0.04^{a}	0.66±0.01^{a}	2.39±0.32^{cd**}	3.34±0.27^{e}	2.25±0.12^{d}
30% starch	3.35±0.09^{f}	0.78±0.02^{b}	0.52±0.02^{ab}	0.39±0.04^{a}	0.57±0.04^{ab}	1.25±0.11^{c*}	2.85±0.12^{e}	2.25±0.15^{d}
0% starch	3.32±0.09^{d}	0.73±0.02^{ab}	0.54±0.02^{a}	0.44±0.04^{a}	0.62±0.04^{a}	1.22±0.11^{bc*}	3.40±0.12^{c}	1.66±0.15^{d}
Glucose (mmol/L)								
45% starch	4.33±0.07^{bc}	2.58±0.10^{ab}	2.23±0.07^{a}	2.78±0.01^{ab**}	2.47±0.35^{ab}	2.90±0.39^{ab}	4.31±0.53^{bc}	4.86±0.35^{c}
30% starch	4.43±0.06^{cd}	2.61±0.07^{abc}	2.23±0.03^{ab}	2.16±0.10^{a*}	2.28±0.14^{a}	2.40±0.34^{ab}	4.19±0.53^{bcb}	4.85±0.35^{d}
0% starch	4.51±0.11^{c}	2.68±0.06^{abc}	2.23±0.09^{ab}	1.83±0.05^{a*}	1.90±0.18^{a}	2.94±0.54^{abc}	3.04±0.23^{abc}	3.78±0.68^{bc}

Values are means± S.E.M.; different superscript letters in the same row indicate significant differences (P<0.05) among sampling points in each group; asterisk indicates significant differences (P<0.05) among groups at each sampling point.

Liver and muscle composition. On day 7S, glycogen content in liver and muscle rapidly decreased to extremely low levels after which, the values remained low. After feeding, glycogen content in liver and muscle was gradually restored. The glycogen levels restored more rapidly in fish fed higher dietary starch levels. From day 3R, liver and muscle glycogen were significantly higher in both the 45% and 30% groups than in the 0% group (P<0.05). Muscle protein and hepatic protein contents were relatively constant throughout the experiment. Hepatic lipid decreased on day 7S, and gradually recovered after day 3R. However, protein content in the whole liver significantly decreased on day 10S and recovered only on day 10R (P<0.05). Results of liver and muscle composition are present in Table 4 and Figure 1 respectively.

Table 4. Liver composition of blunt snout bream during starvation (S) and feeding (R) period[1]

	1S	*7S*	*20S*	*H3*	*1R*	*3R*	*10R*	*20R*
Protein (%/liver)								
45% starch	3.22±0.49	3.92±0.39	3.70±0.69	3.70±0.21	3.23±0.13	3.16±0.27	3.68±0.20	3.22±0.45
30% starch	3.42±0.32	3.75±0.46	3.89±0.33	3.21±0.17	3.50±0.10	3.02±0.21	3.74±0.07	3.25±0.21
0% starch	3.09±0.37	3.77±0.21	3.89±0.33	3.81±0.17	3.24±0.16	3.08±0.16	3.20±0.07	3.28±0.06
Protein (mg/whole liver)								
45% starch	21.6±2.79^{b}	16.1±1.30^{ab}	6.53±1.20^{a}	8.65±0.23^{a}	9.97±1.78^{a}	28.5±1.58^{cd}	60.7±6.40^{e}	37.6±4.48^{d}
30% starch	22.9±3.13^{b}	15.4±2.34^{abc}	6.78±1.02^{a}	7.51±0.59^{ab}	10.7±1.47^{a}	27.3±2.51^{c}	61.7±5.87^{e}	46.5±9.22^{d}
0% starch	21.8±3.31^{bc}	15.4±1.27^{ab}	6.77±1.01^{a}	8.89±0.21^{a}	9.87±1.30^{a}	27.7±0.95^{c}	54.4±3.28^{e}	42.6±6.54^{d}
Lipid (%/liver)								
45% starch	10.6±0.23^{d}	7.28±0.81^{abc}	7.22±0.48^{ab}	6.61±0.57^{a}	7.38±1.13^{abc}	9.65±0.42^{cd}	9.55±0.42^{cd}	10.9±1.61^{d}
30% starch	10.6±0.87^{c}	7.28±0.75^{ab}	7.02±0.82^{a}	6.91±0.32^{a}	6.76±0.41^{a}	9.08±0.21^{bc}	9.55±0.86^{c}	9.80±1.04^{c}
0% starch	10.5±0.54^{b}	7.58±0.85^{ab}	7.00±0.94^{a}	7.05±0.82^{a}	7.64±1.21^{ab}	9.04±0.41^{ab}	8.96±0.81^{ab}	8.64±1.26^{ab}
Glycogen (%/liver)								
45% starch	6.82±0.19^{c}	0.12±0.01^{a}	0.21±0.02^{a}	1.47±0.41^{a}	4.18±0.37^{b}	8.63±0.14^{c**}	7.88±0.70^{c**}	6.15±1.88^{c}
30% starch	6.54±0.16^{d}	0.14±0.02^{a}	0.25±0.01^{a}	0.99±0.16^{a}	2.90±1.49^{b}	7.54±0.77^{c**}	7.45±0.80^{c**}	4.49±0.30^{b}
0% starch	6.88±0.14^{d}	0.13±0.01^{a}	0.28±0.01^{a}	1.26±0.21^{a}	3.61±1.77^{b}	6.36±0.45^{cd*}	5.07±0.18^{bc*}	4.03±0.52^{b}

Values are means ± S.E.M.; different superscript letters in the same row indicate significant differences (P<0.05) among sampling points in each group; asterisk indicates significant differences (P<0.05) among groups at each sampling point.

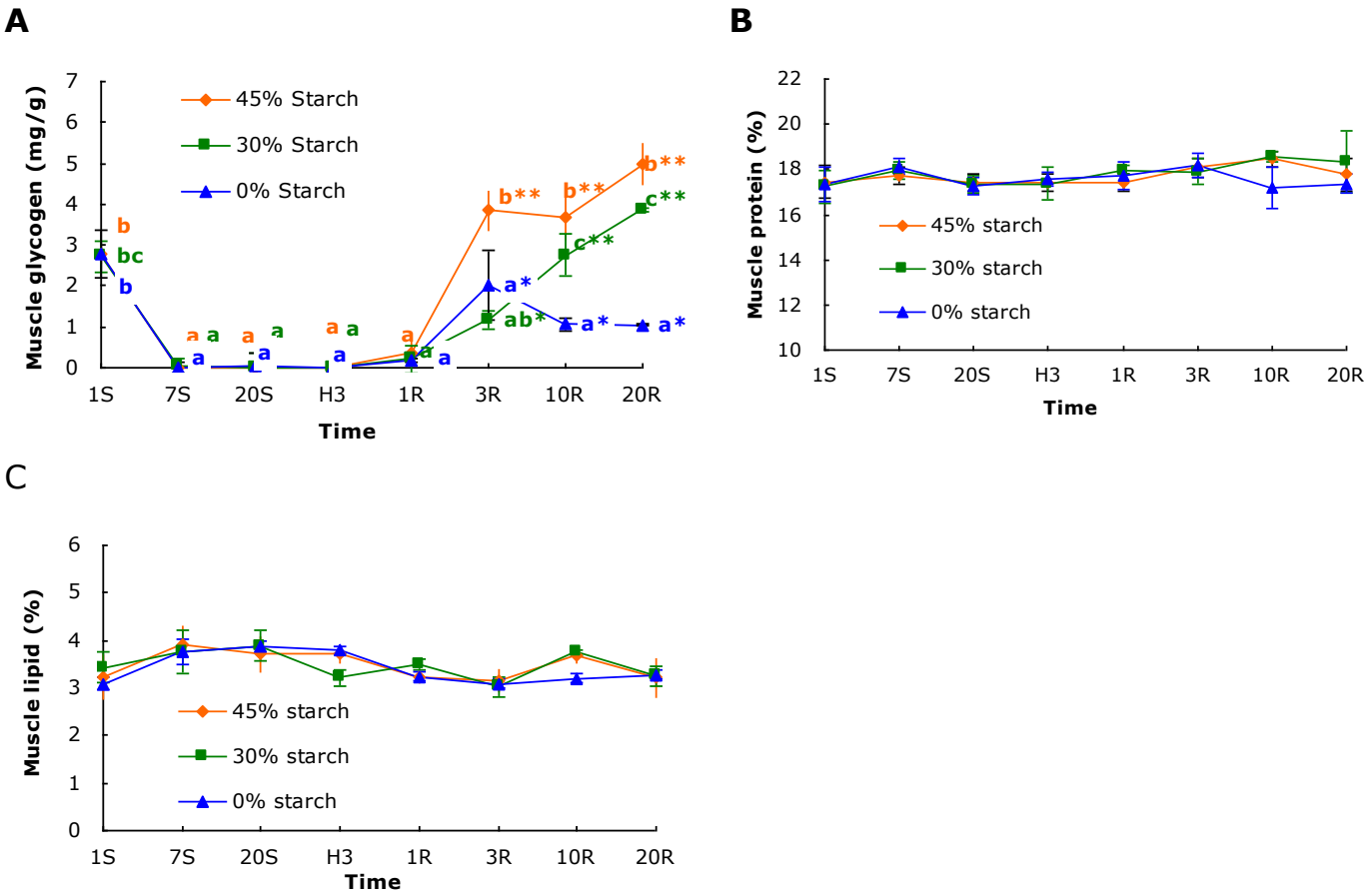

Figure 1. Muscle glycogen (A), protein (B) and lipid (C) contents of blunt snout bream during starvation (S) and feeding (R) period. Different letters indicate significant differences (P<0.05) among sampling points for each group. Asterisk indicates significant differences (P<0.05) among groups at each sampling point.

Hepatic enzyme activities. Hepatic GK, PK and G6PDH activity showed a decreasing trend on day 20S (P>0.05) and there were no significant differences among the groups throughout the experiment, except H3 where higher activities of these enzymes were observed in fish fed diets with higher starch levels (P<0.05). Hepatic FBPase activity increased with prolonged starvation. In 0% starch group, FBPase activity was significantly higher compared to that in starvation period, and values decreased on day R3. FBPase activity increased on day 1R in both 45% and 30% starch groups. However, after reaching the peak on day 1R in 45% starch group and on day 3R in 30% starch group, activity showed a decreasing trend. The FBPase activity in fish fed 0% starch diet was significantly higher than those fed with 30% and 45% starch diets at H3 (P<0.05). Higher value of PK: FBPase ratio was observed in fish fed diet with higher starch level during the feeding period. Results of hepatic glucose enzyme activities are presented in Figure 2.

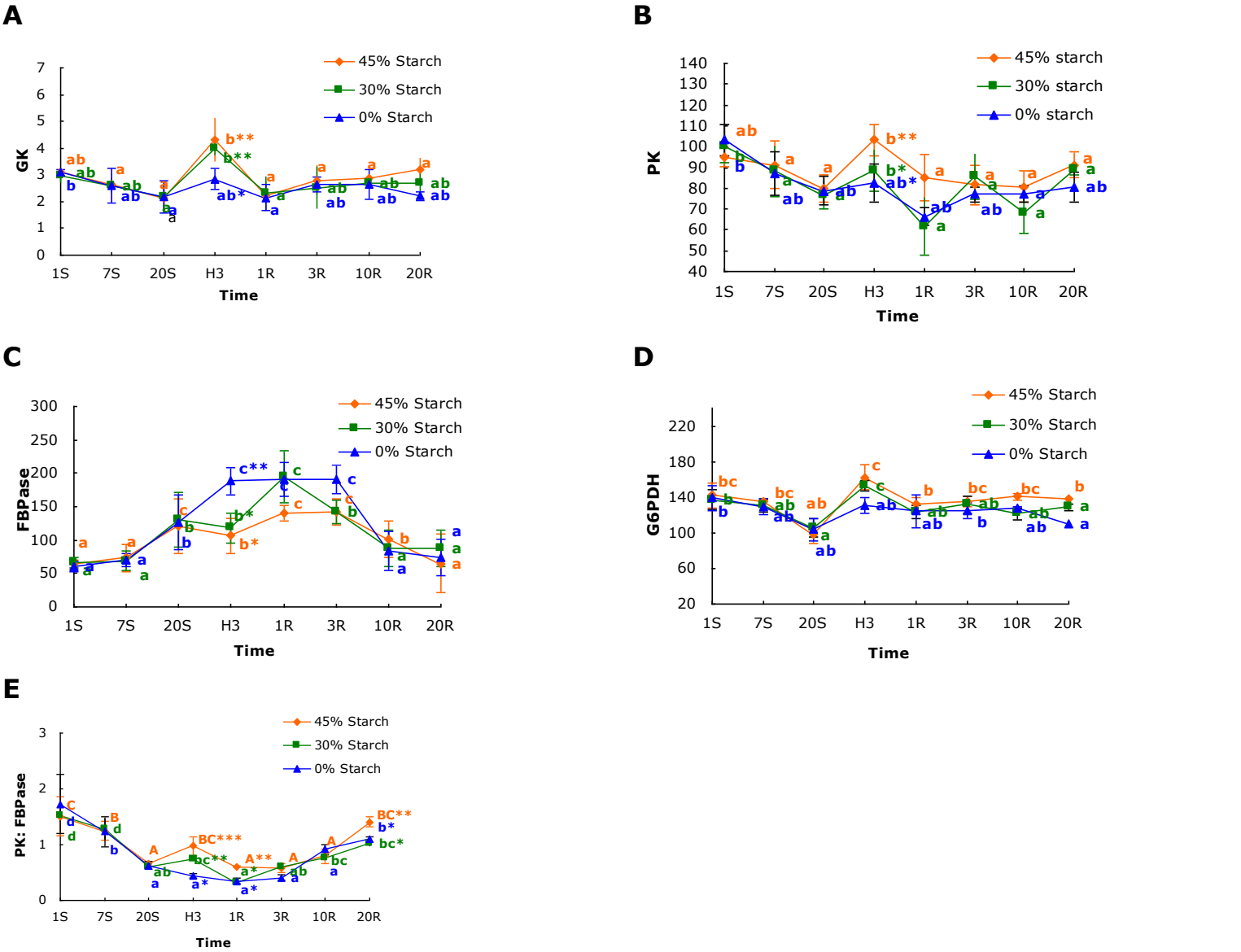

Figure 2. Hepatic GK (A), PK (B), FBPase (C), G6PDH (D) activities (mU/mg protein) and PK: FBPase ratio (E) of blunt snout bream during starvation (S) and feeding (R) period. Different letters indicate significant differences (P<0.05) among sampling points for each group. Asterisk indicates significant differences (P<0.05) among groups at each sampling point.

Hepatic ALT activity was independent of this nutritional challenge. However, day 7S showed significant increase in AST activity. High carbohydrate diets promoted high AST activity (P<0.05) at H3 and day 1R (Figure 3).

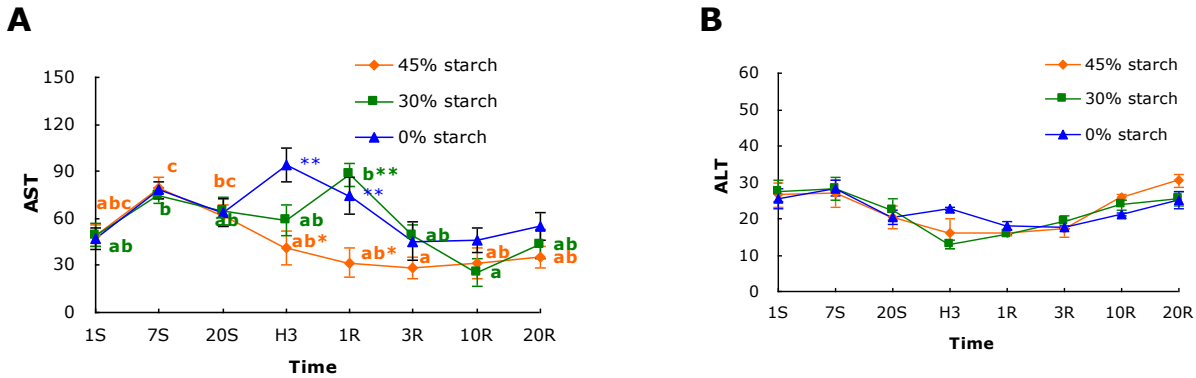

Figure 3. Hepatic AST (A) and ALT (B) activities (U/mg protein) for blunt snout bream during starvation (S) and feeding (R) period. Different letters indicate significant differences (P<0.05) among sampling points for each group. Asterisk indicates significant differences (P<0.05) among groups at each sampling point.

Discussion

Starvation period. In this study, seven days starvation induced a sharp decrease of glycogen content in the liver and muscle, which suggests that glycogen is the first substrate used as an energy source at the early stage of starvation, as in most fish species such as common carp, *Cyprinus carpio* (Shimeno et al., 1997). Similarly declining trends were observed in hepatic lipid content, but not in muscle lipid content. This suggests that lipid in the liver was used for energy in parallel with glycogen utilization. If starvation is prolonged, protein is mobilized as an energy source in some fish species (Navarro and Gutiérrez, 1995; Metón et al., 2003). In this study, significantly lower plasma protein levels were observed on day 20S compared to the values on day 7S and 1S, whereas protein levels in liver and muscle were not affected by starvation. The absolute total protein quantity of the whole liver is a meaningful physiological measurement (Navarro and Gutiérrez 1995); in fact it did decline on day 20S compared to day 1S and 7S in this study, suggesting that protein in blunt snout bream could be used as the energy source after most glycogen and lipid were consumed.

In the present study, there was a continuous reduction in plasma glucose level during the starvation period, which was in agreement with the reports on European sea bass (Pérez-Jiménez et al., 2007), sturgeon, and rainbow trout, *Oncorhynchus mykiss* (Furné et al., 2012). However, compared to plasma triglyceride, the reduction of blood glucose content was slight in this study, and the plasma glucose maintenance was directly related to the capacity to mobilize liver glycogen at day 7S. Blood glucose maintenance may be due to a metabolic adaptation involving the decrease of glycolysis and the pentose phosphate pathway enzymes activities, and increase of gluconeogenic enzyme activity (Caseras et al., 2002). In this study, gluconeogenic enzyme activity (FBPase) was significantly stimulated on day 20S, and the activity of hepatic GK, PK, and G6PDH, showed a decreasing trend. These results indicated that during the starvation period, the source of metabolized glucose could derive from glycogen and gluconeogenic pathway in blunt snout bream.

ALT and AST enzyme activity are the most important amino transferases in fish (Cowey and Walton, 1989). The effects of starvation on ALT and AST activity in fish were species dependent, as both increases and no changes after starvation have been reported (Moon and Johnston, 1981; Cowey and Walton, 1989; Kim et al., 1992). In this study, nutritional status did affect hepatic AST activity, but not ALT activity indicating that AST plays a more prominent role in protein mobilization compared to ALT in blunt snout bream.

Feeding period. There were no significant differences in fish weight among the treatments during the feeding period. Blunt snout bream seem to have the ability to regulate blood glucose levels efficiently after early feeding, which is similar to results in a previous report on this species (Li et al., 2013). The ability to regulate blood glucose possibly involves glucose metabolic enzyme changes at the early stage of refeeding. At H3, a significantly higher level of plasma glucose was observed in fish fed 45% starch diet compared to those fed 0% and 30% starch diets, which was in response to improving glycolysis (GK and PK activity) and pentose phosphate pathway (G6PDH activity), and reducing gluconeogenesis (FBPase activity) pathway. This differed to the report on gilthead sea bream, a carnivorous species. In gilthead sea bream, PK, FBPase, G6P-DH and 6PG-DH activities were recovered to the levels on day 10R (Metón et al., 2003). These differences might suggest that herbivorous fish can utilize higher dietary carbohydrate levels compared to carnivorous species.

Liver glycogen content returned to pre-starvation levels in fish fed three different diets on day 3R, and recovery speed was faster than that of protein and lipid in this study, indicating that blunt snout bream initially restores glycogen levels at the early stage of refeeding. On the other hand, 3 days feeding were not sufficient to reduce hepatic FBPase activity in fish fed 0% starch, and the values increased steadily until day 10R, similarly to gilthead seabream *Sparus aurata*, (Metón et al., 2003). However, FBPase activity was significantly reduced in fish fed a diet containing 45% starch in the present study, which indicated that dietary starch supplementation could improve glycogen restoration directly, and reduce the contribution of three-carbon compounds via gluconeogenesis (Pilkis and Granner, 1992).

In the present study, higher values of PK:FBPase ratio were observed in fish fed diets with higher starch levels throughout the feeding period, indicating an increase in glycolysis and thus metabolic adaptation to high-carbohydrate diets. During the feeding period, high dietary carbohydrate could stimulate the glycolysis pathway in order to metabolize the excess glucose to produce pyruvate in blunt snout bream. Similar results have been reported in some carnivorous fish species such as in gilthead sea bream (Metón et al., 1999). In a previous study, the administration of high carbohydrate diets also increased the liver PK activity in juvenile blunt snout bream after a long-term feeding trial (Li et al., 2013). In contrast, no induction of PK activity by dietary carbohydrates

was observed in some carnivorous fish species (Suarez et al., 2002; Dias et al., 2004; Enes et al., 2008).

At H3 and day 1R, significantly lower hepatic AST activity was observed in fish fed the 45% starch diet compared to those fed 0% starch diet. A possible explanation is that high dietary carbohydrate levels in feeds reduce utilization of amino acids as a substrate for gluconeogenesis at early feeding stages. Our results further confirmed that excess dietary protein and lipid intake by the lower dietary starch group is probably utilized to replenish the hepatic glycogen.

We can conclude that glucose is an important energy source in blunt snout bream, and is mobilized from glycogen and gluconeogenesis pathway during the starvation period. Juvenile blunt snout bream, as herbivorous fish species fed diets containing 45% starch, experienced rapid metabolic adjustments after food deprivation.

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