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Dietary protein-to-carbohydrate ratios affect metabolism and growth of juvenile surubim cachara (*Pseudoplatystoma reticulatum*)

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Abstract This study was conducted to evaluate the effects of dietary carbohydrates (CHO) and protein on growth performance, nutrient retention, blood metabolites, tissue energy reserves (hepatic glycogen and lipids, mesenteric fat index, and hepatosomatic index), and key metabolic enzyme activities (hexokinase, glucokinase, glucose-6-phosphate dehydrogenase, and aspartate aminotransferase) of surubim cachara (*Pseudoplatystoma reticulatum*). Six experimental diets containing one of two CHO levels (170 or 300 g kg^{-1}) and three crude protein (CP) levels (360, 400, or 440 g kg^{-1}) were fed to four replicate groups of *P. reticulatum* (69.2 ± 4.4 g) for 60 days. The results showed that 300 g kg^{-1} CHO resulted in a worse final weight (FW, 208.4 ± 26.9 g), weight gain (WG, 139.4 ± 23.9 g), specific growth rate (SGR, 1.8 ± 0.2% day^{-1}), apparent feed conversion (AFC, 1.5 ± 0.3), and protein efficiency ratio (PER, 163.7 ± 23.4%) than fish fed 170 g kg^{-1} CHO (FW, 241.4 ± 37.3 g; WG, 171.9 ± 36.6 g; SGR, 2.1 ± 0.2% day^{-1}; AFC, 1.2 ± 0.2; PER, 203.7 ± 36.9%). Fish fed 170 g kg^{-1} CHO showed greater retention of CP (REcp, 36.3 ± 6.9%) and energy (REge, 23.9 ± 5.6%) in their carcass than fish fed 300 g kg^{-1} CHO (REcp, 28.9 ± 3.9%, REge, 18.5 ± 3.7%). The highest blood glucose levels (97.7 ± 32.7 mg dL^{-1}) were observed in fish fed the 300CHO/400CP diet, and they had a significantly lower mesenteric fat index

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(0.85 ± 0.26%) than fish fed with the 170CHO/400CP diet (1.25 ± 0.33%). Hepatic hexokinase activity increased in fish fed 300 g kg^{-1} CHO, resulting in an activity of 5.0 μmol mg^{-1} of protein, whereas fish fed 170 g kg^{-1} CHO had an activity of 3.2 μmol mg^{-1} of protein. These results demonstrate that dietary CHO directly affect the intermediary metabolism of *P. reticulatum* and feeding 300 g kg^{-1} CHO and 440 g kg^{-1} CP should be considered excessive as it results in decreased nutrient retention in the carcass and decreased growth performance despite metabolic adjustments.

Keywords Hepatic enzymes · Growth · Dietary starch · Feed formulation

Introduction

Carnivorous fish do not frequently consume carbohydrates (CHO) due to the composition of their natural diet (Hemre et al. 2002). The digestive and metabolic systems of carnivorous fishes appear to be more adapted to use proteins and lipids as energy sources (Dabrowski and Guderley 2002). However, this generalization may not always hold true as there are striking morphological and physiological differences even within fishes that have similar feeding habits, and these differences can lead to differences in the dietary utilization of CHO (Kamalam et al. 2017).

Adequate levels of non-structural CHO, such as starch, can promote a protein-sparing effect, which ultimately results in an optimal cost/benefit ratio and a reduction in ammonia excretion (Enes et al. 2009; Pérez-Jiménez et al. 2015). Non-protein energy sources in the diet, like CHO, allow proteins to be used for fish growth (Kamalam et al. 2017). Glucose is an important metabolic stimulant for glucose-dependent tissues because available dietary CHOs can reduce gluconeogenic activity and lead to the use of amino acids for building structural proteins instead of in the oxidative pathway (Hemre et al. 2002).

Complex CHO sources, such as starch and dextrins, are more efficiently used than simple sugars in most fish species (Wilson 1994). However, in some carnivorous fishes, such as gilthead seabream (*Sparus aurata*), dietary glucose is more effective at stimulating hepatic glycolytic activity than dietary starch. This result has been attributed to higher glycaemia and an increase in glucose uptake by the liver (Enes et al. 2010).

The inclusion of high levels of CHO in diets in an attempt to reduce the protein levels has been attempted for some carnivorous species, such as European sea bass (*Dicentrarchus labrax*), brook trout (*Salvelinus fontinalis*), Senegalese sole (*Solea senegalensis*), and golden pompano (*Trachinotus ovatus*), with no impairment of productive performance and with a direct relationship to intermediary metabolism (Enes et al. 2006; Amin et al. 2014; Guerreiro et al. 2014; Zhou et al. 2015).

South American catfishes (*Pseudoplatystoma* sp.) are the most important and widely cultivated group of piscivorous fish in Brazil (Brasil 2012). Their meat is of excellent quality, and it has few spines in the fillets, a clear coloration, and a firm texture (Campos 2010). The surubim cachara (*Pseudoplatystoma reticulatum* formerly *P. fasciatum* from the Amazon and Paraná rivers) (Buitrago-Suárez and Burr 2007) is preferentially carnivorous and has a gastrointestinal tract that is typical of carnivorous fish. However, this species retains some anatomical peculiarities that indicate adaptations to an omnivorous diet, such as the longitudinal pattern of its gastrointestinal tract and the presence of several anastomoses that slow the advancement of food, providing a longer digestive period and, consequently, better nutrient utilization (Rodrigues et al. 2009).

P. reticulatum is a commercially important carnivorous species, but there is a lack of knowledge about its physiological mechanisms related to nutritional requirements, especially about CHO metabolism. The commercial feeds commonly used in *P. reticulatum* farming contain approximately 420 g crude protein (CP) kg^{-1} and 80 g crude lipid kg^{-1} (Campos 2010), but these levels should be optimized, mainly regarding the non-protein energy content. The objective of the present study was to evaluate the effects of dietary protein and CHO on the growth performance and physiological responses of juvenile *P. reticulatum*.

Materials and methods

Experimental diets

Six experimental diets were formulated to be isoenergetic (18.0 MJ gross energy (GE) kg^{-1}) and isolipidic (70 g crude lipid (CL) kg^{-1}) with three CP levels (360, 400, or 440 g kg^{-1}) and two CHO levels (170 or 300 g kg^{-1}), as shown in Table 1. Fish meal and poultry viscera meal were used as the main protein sources, fish oil as the lipid source, and pre-gelatinized starch as the CHO source.

The proximate composition of the ingredients and experimental diets were determined according to the methods described by the AOAC (2000). Dry matter (DM) content was determined by drying samples in an oven at 105 °C for 24 h, CP content was determined as the total nitrogen (N) content using the Kjeldahl method, CL content was analyzed by Soxhlet extraction with petroleum ether, and ash content was determined by incinerating the dried samples at 600 °C for 4 h in an electric furnace. GE was determined by using an oxygen bomb-calorimeter (C200 Control, Ika, Guangzhou, China). Dietary starch was analyzed according to the methodology described by Beutler (1984) using enzymatic analysis after samples had been pretreated to convert the starch into a soluble form. The ingredients were milled and mixed, and the diets were pelleted with added water (200 g kg^{-1}) and dried at 40 °C for 12 h. The diets were stored in plastic containers and kept at 8 °C during the experiment.

Fish and experimental conditions

Juveniles of *P. reticulatum* from the same spawning were acquired from a commercial producer (Piraí fish farm, Terenos, Mato Grosso do Sul, Brazil). Fish were allocated in 130-L polyethylene tanks and maintained for 4 weeks to adapt to the laboratory condition. Tanks were supplied with continuously circulating freshwater and aeration (electric aerator CR3, Ibram Ind. Brasileira de Máquinas Ltda, São Paulo, São Paulo, Brazil), and the temperature was controlled with electric heaters (Guangdong Boyu Group Co., Raoping, Guangdong, China). A constant 12 h light/12 h dark photoperiod was maintained. The fish were fed to apparent satiation twice a day (18 h 00 min and 00 h 30 min) with a commercial feed for carnivorous fish (Pirá 40 4–5 mm: 400 g CP kg^{-1}, 80 g CL kg^{-1}; Guabi Nutrição e Saúde Animal S.A., Campinas, São Paulo, Brazil).

After acclimation, 20 fish were sampled for the initial whole-body composition analysis. The remaining fish were starved (12 h), anesthetized (benzocaine, 0.1 g L^{-1}), and individually weighed. Fish (69.2 ± 4.4 g) were distributed among 24 130-L polyethylene tanks (4 tanks/treatment) at a stocking density of eight fish/tank.

During the experiment, fish were fed with the experimental diets twice a day (18 h 00 min and 00 h 30 min) until apparent satiation for 60 days. Each experimental diet was distributed to four replicates (tanks). Apparent satiation was defined as the time when the fish refused to eat the food offered. Food intake was determined by weighing the food containers daily.

Table 1 Composition of the experimental diets

7	170CHO			300CHO		
	360CP	400CP	440CP	360CP	400CP	440CP
Ingredients[1], g kg^{-1}						
Fish meal[2]	260	260	260	260	260	260
Poultry viscera meal[3]	243	305	366	243	305	366
Soy protein concentrate[4]	50	50	50	50	50	50
Pre-gelatinized starch[5]	171	171	171	285	285	285
Fish oil[6]	29	22	14	29	22	14
Vitamins and minerals[7]	18	18	18	18	18	18
Cellulose[8]	182	127	74	68	13	7
Kaolin	47	47	47	47	47	0
Analyzed composition						
Dry matter, g kg^{-1}	965	964	957	953	947	949
Crude protein, g kg^{-1}	374	417	448	375	399	445
Crude lipid, g kg^{-1}	73	76	66	68	63	80
Ash, g kg^{-1}	118	152	155	140	148	113
Carbohydrates (starch), g kg^{-1}	172	173	190	306	299	303
Gross energy, MJ kg^{-1}	18.23	17.92	18.01	17.90	17.94	19.03

[1] Enrichment per kilogram of feed: vit A (3000 IU), vit D_3 (3000 IU), vit E (200.0 mg), vit B_1 (6.0 mg), vit B_2 (8.0 mg), vit B_6 (3.0 mg), vit B_{12} (20.0 mg), vit C (350.0 mg), vit K (6.0 mg), folic ac. (1.0 mg), pantothenic ac. (20.0 mg), Biotine (0.1 mg), Niacin (100.0 mg), Choline (150.0 mg), Cu (10.0 mg), Fe (100.0 mg), I (5.0 mg), Mn (70.0 mg), Zn (150.0 mg), B.H.T. (125.0 mg)

[2] Pesquera Pacific Star, Puerto Montt, Chile. CP 651.7 g kg^{-1}

[3] Adamantina Alimenta, Adamantina, São Paulo, Brazil. CP 652.1 g kg^{-1}

[4] ADM do Brasil Ltda, Paranagua, Paraná, Brazil. CP 634.4 g kg^{-1}

[5] Ingredion Incorporated, Mogi Guaçu, São Paulo, Brazil

[6] Cristalina Fish Farm (Mauro Nakata), Fartura, São Paulo, Brazil

[7] Poli-Nutri Alimentos SA., Osasco, São Paulo, Brazil

[8] Rhoster Indústria e Comércio Ltda, Araçoiaba da Serra, São Paulo, Brazil

The organic matter deposited on the bottom of the tanks was removed via siphoning with a rubber hose. The water temperature, dissolved oxygen content (YSI 55 dissolved oxygen, YSI Incorporated, Yellow Springs, Ohio, USA), pH, and total ammonia content (YSI Professional Plus, YSI Incorporated, Yellow Springs, Ohio, USA) were monitored daily. Water quality parameters were as follows: temperature (25.7 ± 1.9 °C), dissolved oxygen (5.4 ± 0.9 mg L^{-1}), pH (7.6 ± 0.06), and total ammonia (0.06 ± 0.002 mg N-NH_4 L^{-1}).

Measurements and analytical methods

After 60 days of feeding, fish were starved for 12 h, anesthetized (benzocaine, 0.1 g L^{-1}), and weighed to determine their final weight (FW), weight gain (WG), specific growth rate (SGR), feed consumption index (FCI), apparent feed conversion (AFC), and protein efficiency ratio (PER). Three fish per tank were randomly collected and stored at − 20 °C for whole-body composition analysis.

WG (g)	final weight − initial weight;
SGR (% day^{-1})	100 × [(ln final weight − ln initial weight)/experimental period];
FCI (% BW day^{-1})	100 × (daily consumption/average body weight);

AFC food supplied/weight gain;
PER (%) 100 × (weight gain/crude protein consumed).

The DM, CP, CL, and ash contents of whole fish were analyzed according to the methods described by the AOAC (2000), as cited above. Whole-body GE was determined using an oxygen bomb-calorimeter (C200 Control, Ika, Guangzhou, China). Whole-body composition was determined to evaluate the retention efficiency of crude protein (REcp) and retention efficiency of gross energy (REge).

REcp (%) 100 × [(CP final carcass × FW) − (CP initial carcass × IW)]/CP consumed;
REge (%) 100 × [(GE final carcass × FW) − (GE initial carcass × IW)]/GE consumed.

Blood samples were taken from the caudal vasculature of another three fish per tank (12 fish/treatment) with heparinized syringes and were centrifuged (827*g*, 10 min, 4 °C) and stored at − 20 °C to measure blood metabolites. The concentrations of the following metabolites were analyzed: plasma glucose (GOD-Trinder method, Gold Analisa Diagnostica, Belo Horizonte, Brazil), serum triglycerides (Enzimatic-Trinder method, Gold Analisa Diagnostica, Belo Horizonte, Minas Gerais, Belo Horizonte, Brazil), and total serum protein (Biuret method, Reinhold 1953).

Then, fish were euthanized, and a laparotomy was performed to collect and weigh the mesenteric fat in the visceral cavity and the liver. Liver samples were stored at − 80 °C and used for the measurement of hepatic metabolic enzymes, total lipids (Bligh and Dyer 1959), and glycogen (Perry et al. 1988). The mesenteric fat index (MFI) and hepatosomatic index (HSI) were evaluated.

MFI (%) 100 × (weight of mesenteric fat/FW);
HSI (%) 100 × (weight of liver/FW).

Hepatic enzyme activity was evaluated by measuring glucokinase (GK, EC 2.7.1.2) and hexokinase (HK, EC 2.7.1.1), both of which are enzymes from the glycolytic pathway. Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), from the lipogenic pathway, and aspartate aminotransferase (AST, EC 2.6.1.1), from the amino acid metabolism, were also measured.

Briefly, to measure the activity of key enzymes, liver samples were homogenized (dilution 1:4) in ice-cold buffer (100 mM Tris-HCl, 0.1 mM EDTA, and 0.1% (*v*/*v*) Triton X-100 at pH 7.8). Homogenates were centrifuged at 30,000*g* for 30 min at 4 °C. HK and GK activities were determined as described by Vijayan et al. (1990). The reaction mixture contained 50 mM imidazole-HCl buffer (pH 7.4), 2.5 mM ATP, 5 mM $MgCl_2$, 0.4 mM NADP, 2 units mL^{-1} G6PD, and 1 mM (HK) or 100 mM (HK-IV) glucose.

G6PD activity was measured as described by Morales et al. (1990) using a reaction mixture containing 50 mM imidazole-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 2 mM NADP, and 1 mM glucose-6-phosphate. AST activities were measured using a commercial kit (Gold Analisa Diagnostica, Belo Horizonte, Minas Gerais, Brazil). All enzyme activities were measured in a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc., Madison, WI, USA). The specific activities of the enzymes are expressed in units of tissue protein, with bovine serum albumin as the standard (Cain and Skilleter 1987).

Statistical analyses

After being tested for normality (Cramer Von Mises) and homoscedasticity (Brown-Forsythe), data were analyzed by two-way ANOVA followed by Tukey's test for the comparison of

means. Treatment effects were considered significant at $P < 0.05$. Results are expressed as the means ± SD. All analyses were performed using SAS 9.0 (SAS Inst. Inc., Raleigh, NC, USA).

Results

Growth performance

The levels of protein influenced ($P < 0.05$) the SGR, FCI, and AFC, and the best results were observed in fish that consumed 440 g kg^{-1} CP (Table 2). Fish that consumed diets with 170 g kg^{-1} CHO showed better ($P < 0.05$) FW, WG, SGR, AFC, and PER than those that consumed diets with 300 g kg^{-1} CHO.

Whole-body composition and nutrient retention

Fish fed 170 g kg^{-1} CHO showed higher REcp and REge ($P < 0.05$) than those that consumed 300 g kg^{-1} CHO, but CP did not have a significant effect ($P > 0.05$) (Table 3). The levels of dietary CP and CHO did not influence ($P > 0.05$) the whole-body composition (Table 3).

Blood and tissue metabolism

No differences were observed ($P > 0.05$) in the serum triglycerides and total protein concentrations (Table 4). An interaction was observed between the levels of dietary CP and CHO in the plasma glucose. Fish that consumed the 300CHO/400CP diet showed the highest blood glucose level ($P < 0.05$).

The HSI and hepatic glycogen and lipid contents were not influenced by the treatments ($P > 0.05$) (Table 4). Fish that were fed 360 g kg^{-1} CP displayed higher hepatic lipid levels than fish fed higher CP content in both CHO levels. An interaction was observed between the levels of CP and CHOs on the MFI (Table 4), which was lower in fish fed the 300CHO/400CP diet than in the other groups ($P < 0.05$).

Hepatic metabolic enzymes

No interactions were observed between the dietary levels CP and CHO for the hepatic activities of GK, HK, and G6PD ($P > 0.05$) (Table 5). The hepatic activity of HK increased ($P < 0.05$) in fish that consumed diets with 300 g kg^{-1} CHO, and the activity of G6PD increased ($P < 0.05$) in fish that were fed diets with 440 g kg^{-1} CP. A significant interaction effect was found for hepatic AST activity (Table 5), which was increased in fish fed with the 170CHO/440CP and 300CHO/400CP diets.

Discussion

Dietary CHO directly affect the intermediary metabolism of *P. reticulatum*, but high levels result in worse growth performance. The dietary CHO requirements of fish have not been determined, but previous studies have shown that dietary CHO can interfere with growth performance when administered at very high levels, as observed in grass carp

Table 2 Growth performance in *Pseudoplatystoma reticulatum* fed diets with different levels of protein and carbohydrates

	170CHO			300CHO			ANOVA	($P < 0.05$)	
	360CP	400CP	440CP	360CP	400CP	440CP	CP	CHO	CPxCHO
FW (g fish^{-1})	223.4 ± 32.2	246.1 ± 45.9	254.7 ± 35.5	199.8 ± 8.9	189.1 ± 18.0	236.2 ± 25.2	0.082	0.015	0.400
WG (g fish^{-1})	152.8 ± 31.3	175.9 ± 46.7	187.2 ± 30.3	129.3 ± 10.9	123.4 ± 16.0	165.3 ± 19.1	0.058	0.011	0.492
SGR (% day^{-1})	1.91 ± 0.23	2.07 ± 0.32	2.20 ± 0.10	1.74 ± 0.13	1.76 ± 0.14	2.00 ± 0.07	0.022	0.007	0.740
FCI (% BW day^{-1})	2.32 ± 0.25	2.23 ± 0.26	2.21 ± 0.23	2.66 ± 0.07	2.86 ± 0.16	2.28 ± 0.27	0.030	0.001	0.058
AFC	1.35 ± 0.27	1.21 ± 0.28	1.12 ± 0.15	1.64 ± 0.11	1.74 ± 0.18	1.24 ± 0.16	0.011	0.001	0.161
PER (%)	204.2 ± 40.4	205.7 ± 49.4	201.1 ± 30.2	163.0 ± 11.7	145.0 ± 15.2	183.0 ± 26.1	0.584	0.006	0.423

Means followed by different letters on each row are different by Tukey's test ($P > 0.05$)

FW final weight, *WG* weight gain, *SGR* specific growth rate, *FCI* feed consumption index, *AFC* apparent feed conversion, *PER* protein efficiency ratio

Table 3 Whole-body composition and nutrient retention in *Pseudoplatystoma reticulatum* fed diets with different levels of protein and carbohydrates

	Initial	170CHO			300CHO			ANOVA ($P < 0.05$)		
		360CP	400CP	440CP	360CP	400CP	440CP	CP	CHO	CPxCHO
Whole-body composition										
Dry matter	21.7 ± 1.2	20.7 ± 1.1	21.6 ± 1.2	22.1 ± 1.0	21.8 ± 0.6	21.5 ± 1.5	22.1 ± 1.3	0.265	0.467	0.554
Crude protein	16.8 ± 0.5	17.0 ± 1.1	17.5 ± 0.3	18.2 ± 0.8	17.8 ± 0.4	17.7 ± 0.7	17.6 ± 0.8	0.407	0.647	0.164
Crude lipid	2.19 ± 0.62	2.03 ± 0.40	2.50 ± 0.87	2.43 ± 0.54	2.23 ± 0.17	2.29 ± 0.67	2.81 ± 0.47	0.179	0.561	0.488
Ash	2.46 ± 0.38	1.82 ± 0.42	1.75 ± 0.39	1.73 ± 0.25	1.87 ± 0.13	1.73 ± 0.33	2.01 ± 0.43	0.689	0.424	0.588
GE (MJ kg^{-1})	4.75 ± 0.20	4.74 ± 0.18	4.90 ± 0.48	5.04 ± 0.31	4.97 ± 0.13	4.96 ± 0.38	5.16 ± 0.30	0.183	0.217	0.828
Nutrient retention										
REcp (%)	–	35.2 ± 6.9	36.2 ± 9.6	37.5 ± 5.9	28.3 ± 1.0	26.4 ± 2.2	32.0 ± 4.9	0.486	0.009	0.762
REge (%)	–	19.7 ± 4.9	24.2 ± 8.0	25.5 ± 4.6	16.5 ± 0.3	16.3 ± 1.9	22.4 ± 3.3	0.129	0.016	0.593

Means followed by different letters on each row are different by Tukey's test ($P > 0.05$)

GE gross energy, *REcp* retention efficiency of the crude protein, *REge* retention efficiency of the gross energy

Table 4 Blood metabolites, hepatic glycogen and lipids, mesenteric fat index (MFI), and hepatosomatic index (HSI) in *Pseudoplatystoma reticulatum* fed diets with different levels of protein and carbohydrates

	170CHO			300CHO			ANOVA	($P < 0.05$)	
	360CP	400CP	440CP	360CP	400CP	440CP	CP	CHO	CPxCHO
Triglycerides (mg dL^{-1})	124.0 ± 33.2	124.6 ± 14.1	125.1 ± 25.1	120.7 ± 24.8	151.3 ± 56.9	130.6 ± 22.1	0.411	0.313	0.436
Total Protein (mg mL^{-1})	57.1 ± 2.4	57.4 ± 2.2	55.8 ± 1.9	56.7 ± 3.7	54.1 ± 1.9	56.5 ± 2.7	0.190	0.470	0.109
Glucose (mg dL^{-1})	63.4 ± 15.0^{b}	48.6 ± 7.4^{b}	49.0 ± 10.3^{b}	48.4 ± 12.5^{b}	97.7 ± 32.7^{a}	63.7 ± 17.0^{b}	0.016	0.004	<0.0001
Hepatic glycogen (g 100 g^{-1})	9.1 ± 2.6	10.9 ± 1.9	10.8 ± 1.7	11.9 ± 2.9	10.2 ± 1.9	11.5 ± 2.5	0.663	0.159	0.097
Hepatic lipids (g 100 g^{-1})	3.88 ± 0.58	3.09 ± 0.41	3.83 ± 0.25	4.43 ± 1.07	3.81 ± 0.44	3.65 ± 0.73	0.015	0.068	0.153
MFI (%)	1.07 ± 0.12^{ab}	1.25 ± 0.33^{a}	1.15 ± 0.35^{ab}	1.13 ± 0.19^{ab}	0.85 ± 0.26^{b}	1.26 ± 0.10^{ab}	0.267	0.324	0.015
HSI (%)	1.18 ± 0.18	1.26 ± 0.30	1.22 ± 0.19	1.13 ± 0.23	1.09 ± 0.28	1.17 ± 0.15	0.889	0.175	0.620

Means followed by different letters on each row are different by Tukey's test ($P > 0.05$)

MFI mesenteric fat index, *HSI* hepatosomatic index

(*Ctenopharyngodon idella*) fed diets with more than 400 g kg^{-1} wheat starch (Tian et al. 2012) and in golden pompano receiving more than 224 g kg^{-1} CHO (Zhou et al. 2015). However, diets with little or no CHO can also result in this effect (Dabrowski and Guderley 2002).

In this study, the high amount of CHO in the diet directly influenced the growth of *P. reticulatum*, and fish that consumed diets with 300 g kg^{-1} CHO showed lower growth performance than fish that consumed diets with 170 g kg^{-1} CHO. In juvenile *Pseudoplatystoma corruscans*, another South American catfish, diets with up to 250 g kg^{-1} CHO and 300 g kg^{-1} CP, given for 28 days, did not influence growth performance (Lundstedt et al. 2004). In other study, *P. corruscans* juvenile fed diets with up to 290 g kg^{-1} of non-structural CHO showed no impairment in growth and feed conversion, which can be attributed to the utilization of CHO as an energy source, which spared proteins for use in growth, leading to weight gain (Takahashi and Cyrino 2006).

A better nutrient balance is essential, since lower dietary CHO level (170 g g^{-1}) with an appropriate level of protein (440 g kg^{-1}) and fat (66 g kg^{-1}) resulted in a more efficient retention of CP and better retention of GE, as demonstrated in previous studies with other carnivorous fish, suggesting that these diets provided CHO for use as an energy source and allowed proteins to be spared for growth (Shiau and Lin 2001; Enes et al. 2006; Amin et al. 2014; Zhou et al. 2015).

The fish fed the highest protein level, the most expensive component in the diet, showed an increased on hepatic AST activity, suggesting that amino acids were metabolized and contributed to providing energy; thus, glucose was derived from amino acids (Enes et al. 2009). In addition, it was observed that the 300CHO/400CP diet also stimulated the AST activity, a key enzyme for the metabolism of amino acids for energy production, triggering this metabolic pathway at the cost of using amino acids for animal growth. It means that amino acids acquired from a high protein diet were used, rather than CHO, for energy production (Enes et al. 2006; Kumar et al. 2009). In brown trout fed with a low protein and high CHO diet (*Salmo trutta*), there was not observed negative effect in protein synthesis, although protein accretion decreased, suggesting an increase in protein degradation. However, the principal components of the main tissues (liver, muscle, and gut) were not affected (Viaplana-Marín et al. 2006).

In European sea bass (*Dicentrarchus labrax*) fed diets with 200 g kg^{-1} CHO, metabolic activities suggested that the CHO significantly improved protein use due to the increased activity of glycolytic enzymes, such as GK and HK, along with decreased gluconeogenesis (Enes et al. 2006). In addition, in golden pompano fed diets with up to 168 g kg^{-1} CHO, increased glycolysis and decreased amino acid catabolism, however, the higher levels worsened the growth performance (Zhou et al. 2015). High dietary CHO or lipid contents have been shown to significantly depress growth and feed utilization and influence the glycolytic pathway in another carnivorous fish, the yellow croaker (*Larmichthys crocea*) (Zhou et al. 2016). Accordingly, in juvenile *P. reticulatum*, the high levels of dietary CHO, although promoted greater hepatic HK activity, resulted in higher plasma glucose levels with higher dietary CP.

One of the major functions of the liver is to extract glucose from the bloodstream and store it as glycogen and, if necessary, to mobilize it through glycogen hydrolysis (Higuera and Cardenas 1984). A previous study on the effects of glucose, dextrin, and starch on the growth and body composition of starry flounder (*Platichthys stellatus*) showed an increase in the HSI, with elevated levels of starch, which was directly related to the deposition of hepatic glycogen (Lee and Lee 2004).

The use of CHO by various species depends on the animal's ability to oxidize glucose and store the excess as glycogen or lipids (Xiao et al. 2014). In this study, hepatic lipids showed no significant differences between fish fed diets with different CHO levels. However, fish that

Table 5 Hepatic activity (μmol mg of protein^{-1}) of glucokinase (GK), hexokinase (HK), glucose 6 phosphate dehydrogenase (G6PD), and aspartate aminotransferase (AST) in *Pseudoplatystoma reticulatum* fed diets with different levels of protein and carbohydrates

	170CHO			300CHO			ANOVA ($P < 0.05$)		
	360CP	400CP	440CP	360CP	400CP	440CP	CP	CHO	CPxCHO
GK	1.02 ± 0.91	1.27 ± 0.46	1.27 ± 0.42	1.68 ± 0.02	1.62 ± 1.02	0.83 ± 0.52	0.426	0.509	0.250
HK	3.11 ± 1.33	3.42 ± 1.01	3.19 ± 1.67	5.98 ± 1.28	5.64 ± 1.30	3.48 ± 1.11	0.053	0.0003	0.064
G6PD	271.5 ± 61.6	214.7 ± 47.3	402.8 ± 144.5	279.7 ± 96.6	297.9 ± 35.4	311.0 ± 97.0	0.023	0.998	0.069
AST	16.8 ± 1.8^{c}	16.3 ± 2.1^{c}	22.5 ± 2.8^{ab}	18.1 ± 3.0^{bc}	22.9 ± 2.2^{a}	16.7 ± 1.9^{c}	0.081	0.441	< 0.0001

Means followed by different letters on each row are different by Tukey's test ($P > 0.05$)

GK glucokinase, *HK* hexokinase, *G6PD* glucose 6-phosphate dehydrogenase, *AST* aspartate aminotransferase

consumed diets with 360 g kg^{-1} CP showed higher hepatic lipid levels. In mammals, a protein deficiency can induce non-alcoholic hepatic steatosis (Castro et al. 2009). Currently, there are several other known routes of induction, such as deficiencies of choline and methionine (Kirsch et al. 2003), a fat-rich diet (Gauthier et al. 2003; Lieber et al. 2004), and a simple CHO-rich diet (Zivkovic et al. 2007).

Normally, the growth of fish is not further improved when dietary protein exceeds the optimal requirements. For example, tilapia fed an extruded diet containing 310 g kg^{-1} CP showed growth similar to that of fish fed an extruded diet containing 310 g kg^{-1} CP (Ma et al. 2015). Despite the carnivorous feeding habits of *P. reticulatum*, a CP concentration of 440 g kg^{-1} appears to be excessive because it did not improve growth performance relative to fish fed diets with 360 g kg^{-1} CP, and it increased levels of hepatic lipids through the activation of lipogenesis.

Lipogenesis was detected by measuring hepatic G6PD activity (Pérez-Jiménez et al. 2007). Hepatic G6PD activity increased only in fish fed diets with 440 g kg^{-1} CP, the highest protein level tested, even though an increase in fat content was not found in the carcasses of these fish. In the other hand, an interaction between CP and CHO levels was observed for the MFI. The 300CHO/400CP diet resulted in the least amount of mesenteric fat, which shows that the CHO were used efficiently as an energy source, without impairing growth performance, resulting in the efficient use of protein for growth.

There was an imbalance in glucose homeostasis according to the data, and this imbalance resulted in a malfunction in the absorption of hepatic glucose (glycolysis) and glucose production (gluconeogenesis) (Panserat et al. 2001; Enes et al. 2009). Thus, further investigation involving metabolic enzymes must be carried out to elucidate these mechanisms.

Conclusion

Using 300 g kg^{-1} CHO in the diet of *P. reticulatum* should be considered excessive because it affects the glycolytic pathway and decreases productive indices. Despite the carnivorous feeding habits of *P. reticulatum*, 440 g kg^{-1} CP was excessive, suggested by the activation of the lipogenic pathway. These findings indicate that the balance of nutrients leads to their differential use in the regulation of fish metabolism.

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