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Metabolic responses to dietary carbohydrate/lipids ratios in neotropical hybrid catfish (♂*Pseudoplatystoma corruscans*×♀*Pseudoplatystoma fasciatum*)

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Abstract: The purpose of this study was to assess the effect of different carbohydrate and lipid (CHO/L) ratios on the performance and energy metabolism of hybrid catfish (♂Pseudoplatystoma corruscans × ♀ Pseudoplatystoma fasciatum). One hundred and sixty-eight fish (104.9±16.5 g BW) were distributed into 24 100-L tanks. The experiment was performed using a randomised block design with four CHO/L ratios (0.45, 1.00, 1.66 and 2.85) and six replications. Growth performance, protein content, glucose and triglycerides in plasma, muscle and liver, and the hepatic activity of the malic and glucose-6-phosphate dehydrogenase (G6PD) enzymes were measured after 60 feeding days. No significant differences were observed in growth performance, protein and triglycerides of plasma and muscle, hepatic protein or malic enzyme activity. CHO/L: 2.85 provided the highest plasma and liver glucose, while CHO/L: 0.45 showed high liver triglycerides. A linear effect was found for the hepatic activity of G6PD and dietary starch levels (R2=0.93). The hepatic activity of G6PD was greater in CHO/L=2.85 than for CHO/L: 0.45. In conclusion, the limit of dietary starch inclusion and lipids were 15% and 9.00%, respectively (CHO/L: 1.66), and this level did not affect the energy metabolism of the juvenile hybrid catfish.

Key words: enzymes activity, hepatic function, intermediary metabolism, hybrid catfish, Surubim.

INTRODUCTION

Carbohydrate-containing feedstuffs are less expensive than other nutrients in diet and directly influence economic fish production viability (Papoutsoglou and Lyndon 2006, Kamalam et al. 2017). Although no nutritional requirements of carbohydrates exist in these animals' diets, their inclusion at appropriate levels can ensure better efficiency in utilising other nutrients by contributing positively in the diet extrusion process, and acting

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as an abundant energy source for lipids and proteins (Krogdahl et al. 2005, NRC 2011, Zhou et al. 2015, Kamalam et al. 2017). However, carbohydrate use in a carnivorous fish diet is complex and not well-known for digestion, absorption and metabolism processes (Krogdahl et al. 2005). Such processes depend on the relationships between fish species and carbohydrate type or content (Krogdahl et al. 2005, Kamalam et al. 2017). These species are more efficient in using lipids as an energy source given their metabolism's limited capacity to reduce glycaemia (Caseras et al. 2007, Hemre et al. 2002).

The metabolic profile and its pathways are the reflex from diet, regardless of performance or muscle and liver function. Carbohydrate/ lipids (CHO/L) ratios of around 1.3:1 produce the best growth performance in hybrid catfish (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*) (Bernardes et al. 2016). One of the statements about fish growth variation is the relation between carbohydrate levels and their effects on diet viscosity and digestion which can, in turn, lead to a change in nutrient use (Tran-Tu et al. 2017). Moreover, increased dietary protein and fewer carbohydrates and lipids attenuate glycolytic activity and induce hepatic gluconeogenesis in South American catfish (*Rhamdia quelen*) (Melo et al. 2016). The liver histology was normal in the yellow catfish (*Pelteobagrus fulvidraco*) fed the 2.45, 3.63 and 5.58 CHO/L diets. The hepatic steatosis and hepatocyte apoptosis rates increased in the yellow catfish fed with 1.11 and 1.67 CHO/L than 2.45, 3.63 and 5.58 CHO/L (Wang et al. 2014). Thus it is imperative for modern aquaculture to define the optimum dietary ratio between non-protein energy sources such as carbohydrates and lipids (Wang et al. 2016).

Pseudoplatystoma corruscans and *Pseudoplatystoma fasciatum* are carnivorous fish and important species with commercial characteristics produced in South America (Martino et al. 2002a, Leonardo et al. 2004), along with their hybrids (♂*P. corruscans*×♀P. *fasciatum*) (Beelen et al. 2003). *Pseudoplatystoma* spp. is able to adjust amylase activity in accordance with dietary carbohydrate levels (Lundstedt et al. 2004). Both plasma glucose and plasma protein concentrations lower when corn gluten meal levels increase in *P.fasciatum* (Bicudo et al. 2012). All these South American catfish are species of the same family (Pimelodidae). However, very little is known about this hybrid metabolism function in relation to the nutrition effect. In some carnivorous species, e.g. *Oncorhynchus mykiss*, *Rachycentron canadum* and *Mystus nemurus,* glucose stimulates glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME) activities (Alvarez et al. 2000, Cui et al. 2010, Hamid et al. 2011), which are the main enzymes responsible for producing NADPH, which is required for lipid synthesis. Thus, the quantification of metabolites in different tissues and enzyme activity in relation to energy metabolism are important for understanding the use of dietary carbohydrates to optimise growth, spare proteins and avoid economical waste while farming species.

The present work was conducted to assess the influence of different CHO/L ratio on the energy metabolism of hybrids ♂*Pseudoplatystoma corruscans*×♀*Pseudoplatystoma fasciatum* by estimating performance, protein concentration, glucose and triglycerides in plasma, muscle and liver, and the hepatic activity of both ME and G6PD.

MATERIALS AND METHODS

ANIMAL HOUSING

The growth trial was performed at the Aquaculture Station of the Federal University of Lavras - UFLA, Minas Gerais (Brazil), and was approved by the UFLA's Ethics Committee on Animal Use, and was certified by Protocol 028/12. Juvenile catfish (*Pseudoplatystoma spp.*) were obtained from a farm in Minas Gerais, Brazil. One hundred and sixty-eight fish (initial body weight of 104.9±16.5 g; mean±S.D) were distributed into 24 100-L tanks (7 fish per tank). Fish were fed twice until apparent satiation. The system comprised a 5-µm particle sand filter, an ultraviolet filter and a biological filter. The water flux in the experimental boxes was sufficient to renew water 3 times/hour. During the experiment, the water temperature was maintained at 28±0.1^{o}C. Dissolved oxygen was measured daily by a portable digital oxymeter (F-1550A, Bernauer®, Indaial, SC, Brazil), with average values of 5.1±0.9 mg L-1. Ammonia, nitrite and pH were measured weekly using a commercial kit (Labcon Test, Alcon®, Camboriú, SC, Brazil), with average values of 0.009±0.002 ppm, 0.01±0.001 ppm, and 7.3±0.1, respectively. Fish were submitted to a 15-day adaptation period before the experiment began.

EXPERIMENTAL DIETS

Four isoproteic (460 g kg^{-1} crude protein) and isoenergetic (3,500 kcal kg^{-1}) diets were formulated to contain the dietary carbohydrate and lipid ratios (CHO/L: 0.45, 1.00, 1.66 and 2.85) (Table I). Canola oil was considered herein because the parietal *P. fasciatum*is well able to use plant oils than fish oil to transform linoleate into arachidonate (Arslan et al. 2008). Moreover, plant oil is used by *P. coruscans* (Martino et al. 2002b). Dietary ingredients were mixed in a paddle mixer (Inbramaq®, São Paulo, Brazil) with 20% of water, pelletized (4 mm) and dried for 12 h in an oven at 50°C. Diets were stored in a freezer (5°C) until used.

TABLE I

Composition of the experimental diets.

Ingredients	Inclusion levels			
Fish meal[1]	57.17	52.17	48.00	44.00
Gelatine	5.00	5.00	6.00	7.00
Albumin	7.00	7.00	8.00	8.00
Casein	3.00	6.00	7.00	8.50
Canola oil	11.00	10.00	9.00	7.00
Starch[2]	5.00	10.00	15.00	20.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Mineral andvitamin[3]	0.30	0.30	0.30	0.30
Antioxidant	0.03	0.03	0.03	0.03
Cellulose	5.00	4.00	3.17	2.60
Inert	5.00	4.00	2.00	1.00
Total	100.00	100.00	100.00	100.00
Carbohydrate / lipid ratio	0.45	1.00	1.66	2.85
Approximate composition (g/100 g dry matter)				
CP[4] (%)	46.7	46.3	46.3	46.2
CE[5] (kcal.kg^{-1})	3525	3548	3595	3550

[1]Salmon meal (67% crude protein, Total Alimentos); [2]Carbohydrate source: maize raw starch (Pachá Alimentos); [3]Composition per kilogram of the mineral and vitamin supplement: 1500 IU vitamin A, 20 mg vitamin B1, 15 mg vitamin B2, 1000 IU vitamin B3, 10 mcg vitamin B12, 25 mg vitamin E, 120 mg vitamin PP, 2000 mg choline, 80 mg calcium pantothenate, 2 mg folic acid, 80 mg manganese, 24 mg iron, 50 mg zinc, 8 mg copper, 3 mg iodine, 0.10 mg selenium, and 170 mg BHT; [4]CP: crude protein; [5]CE: crude energy.

EXPERIMENTAL DESIGN

After the adaptation period, fish were fed the experimental diets (4 CHO/L ratios and 6 replies) twice a day at 08:00h and 18:00h. The amount of diet was provided according to apparent animal satiety. The delivered amount of diets was quantified daily by weighing before and after the feeding process. The trial lasted 60 days. At the end of the experimental period, fish were starved for 24h, weighed and then kept in anaesthetic solution until opercular movement had completely ceased (0.8 ml.l^{-1} 2-phenoxyethanol). One millilitre of blood per cardiac punch was also sampled using a heparinised syringe (1 ml; needle size of 21G 1/2" 0.8x40). Blood was centrifuged at 3,000 rpm for 10 minutes before isolating plasma by a Pasteur micropipette. After this procedure, fish were decapitated at the skull insertion point. Then the liver and white dorsal muscle samples (approximately 3g) were collectedfrom each juvenile. The liver was weighed to calculate the hepatossomatic index. Samples were stored at -80ºC for further analyses.

The following parameters were calculated: Hepatosomatic Index (HSI) = [(liver weight/fish weight)×100]; weight gain (WG) = final weight - first weight; feed conversion ratio (FCR) = ingested rate/weight gain; protein efficiency rate (PER) = weight gain/ingested crude protein, and energy efficiency rate (EER) = weight gain/ingested crude energy.

Muscle and liver assessments

The liver and muscle homogenates were obtained according to Paulino et al. (2018) with slight modifications. The white muscle and liver samples were homogenized in ice-cold deionised water and centrifuged (13,400 × g, 10 min; 4ºC).The resultant supernatant was collected and stored at -80ºC. Glucose and triglyceride levels were measured with commercial kits (Glucose HK Liquiform, Cat. 85 and Triglycerides Liquiform, Cat. 87). Soluble protein content was determined according to Bradford (1976), with bovine serum albumin solution (Sigma) used as a standard.

The specific activity of both malic enzyme (ME, E.C.1.1.1.40) and glucose-6-phosphate dehydrogenase (G6PD, E.C.1.1.1.49) was estimated following the methodology outlined in Graeve et al. (1994) and Spina et al. (1970), respectively. Hepatic tissue was macerated with a pistil in an ice-bath. After maceration, the sample was put in an ultrasonic bath for 10 minutes before being centrifuged at 10,000 g and 4ºC for 30 minutes before collecting the supernatant. The homogenate of supernatants was used to estimate specific G6PD activity. All the enzymatic activities are shown as U.mg^{-1} protein.

STATISTICAL ANALYSIS

All the obtained data were analyzed using the SAEG statistical package proposed by Euclydes (1997). A data analysis was done by a one-way analysis of variance (ANOVA) after testing for normality and homogeneity of variances with the Shapiro-Wilk test and the Levene test, respectively. Significant differences among the means were determined by a Student-Newman-Keuls test. A regression equation was obtained to describe the glucose-6-phosphate dehydrogenase pattern.

RESULTS

No fish mortality was recorded during the experimental period. After the 60 days that the trial lasted, no growth performance parameter, e.g. weight gain, feed conversion rate, Hepatosomatic Index, protein efficiency rate and energy efficiency rate, was significantly affected ($P>0.05$) by the dietary carbohydrate/lipid (CHO/L) ratio (Table II).

The concentrations of glucose, triglycerides and protein in the white muscle of juveniles did not change ($P>0.05$) (Table III). However, CHO/L had an effect on glucose in both plasma and the liver, as did triglycerides on hepatic tissue ($P<0.05$). The highest plasma and liver glucose contents were found in the fish fed the diet (CHO/L =2.85). The catfish juveniles fed the diet (CHO/L=0.45) showed higher concentrations of hepatic triglycerides compared to other CHO/L ratios ($P<0.05$). The plasma and liver protein concentrations were not affected by diet ($P>0.05$).

No significant differences were observed for the hepatic activity of ME ($P>0.05$). However, G6PD activity was greater in the treatment (CHO/L =2.85) compared to CHO/L=0.45 ($P<0.05$) (Table IV). A linear effect was found for G6PD activity, which increased with a rising carbohydrate concentration in diet (Figure 1).

DISCUSSION

In this study, the fish fed different CHO/L ratio in their diet did not show any significant differences in their performance. The range within which

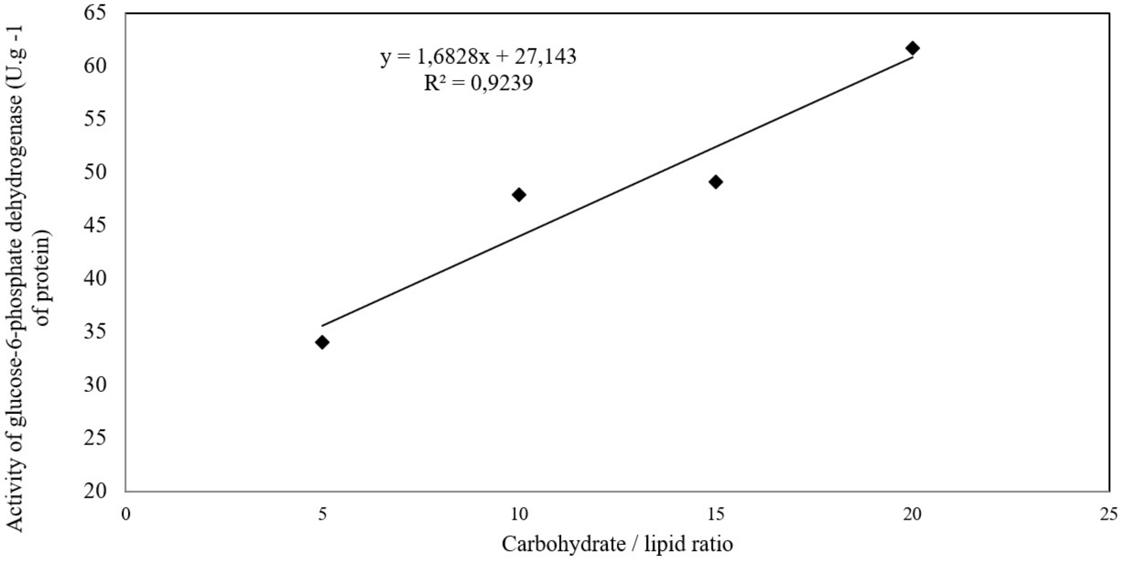

Figure 1 - Linear regression of the hepatic glucose-6-phosphate dehydrogenase activity of the juvenile *Pseudoplatystoma* ssp. fed the experimental diets for 60 days (n=18).

TABLE II
Growth performance, feed utilisation and Hepatosomatic Index of the juvenile *Pseudoplatystoma* ssp fed the experimental diets for 60 days. Lack of superscript letters in the same line implies no differences in data at (P<0.05).

Parameters	Carbohydrate / lipid ratios[1]			
	0.45	**1.00**	**1.66**	**2.85**
WG	117.42 ± 27.49	119.43 ± 24.67	125.43 ± 21.00	132.97 ± 22.81
FCR	1.11 ± 0.27	1.18 ± 0.22	1.11 ± 0.16	1.15 ± 0.15
HSI	0.69 ± 0.09	0.60 ± 0.04	0.72 ± 0.10	0.76 ± 0.11
PER	2.02 ± 0.47	2.06 ± 0.42	2.18 ± 0.36	2.31 ± 0.39
EER	0.26 ± 0.06	0.27 ± 0.05	0.28 ± 0.04	0.30 ± 0.05

[1] Means (n=18) ± standard deviation. Mean in the same row with different superscript letters are significantly different (P < 0.05).WG = weight gain; FCR= feed conversion rate; HSI= hepatosomatic index; PER= protein efficiency rate; EER= energy efficiency rate.

dietary carbohydrates can be used to meet energy requirements vastly varies between, and even within, species, depending on many factors which have been categorised into those also related to biology and experimental conditions (Kamalam et al. 2017). For example, the inclusion of dietary soluble polysaccharides does not affect growth performance, nor the feed conversion ratio, in African catfish (*Clarias gariepinus*). Martino et al. (2005) found that replacing the energy obtained from soya bean oil in diet with corn flour did not affect the specific growth rate, the feed conversion rate, total consumption, protein and the hepatosomatic index of the parietal *Pseudoplatystoma* ssp. On the other hand, the 4%, 8% and 12% increases in soya bean oil led to enhanced nitrogen fixation in a *P. coruscans* carcass (Campos et al. 2006). The feed conversion ratio and daily feed consumption showed a marked drop in the inverse proportion to the lipid level in diet in *P. coruscans* (Martino et al. 2002b). In short, the present study revealed that the hybrid (♂*P. corruscans*×♀P. *fasciatum*)

TABLE III
The plasma (mg.dL^{-1}), liver (mg.g^{-1}) and white muscle (mg.g^{-1}) compositions of the juvenile *Pseudoplatystoma* ssp fed the experimental diets for 60 days. The means in the same row with different superscripts are significantly different at (P<0.05).

Parameters	**Carbohydrate / lipid ratios^{1}**			
	0.45	**1.00**	**1.66**	**2.85**
	White muscle (mg g^{-1})			
Glucose	0.95 ± 0.07	1.12 ± 0.21	1.15 ± 0.18	1.24 ± 0.16
Triglycerides	1.20 ± 0.25	1.13 ± 0.38	1.10 ± 0.28	1.07 ± 0.25
Protein	30.06 ± 5.07	24.80 ± 4.92	27.06 ± 3.75	26.66 ± 4.08
	Plasma (mg ml^{-1})			
Glucose	0.57 ± 0.05^{b}	0.71 ± 0.05^{b}	0.77 ± 0.03^{b}	1.13 ± 0.08^{a}
Triglycerides	1.77 ± 0.10	1.65 ± 0.17	1.62 ± 0.21	1.30 ± 0.18
Protein	3.83 + 0.33	4.10 + 0.53	3.82 + 0.35	4.04 + 0.59
	Liver (mg g^{-1})			
Glucose	19.14 ± 1.98^{b}	19.10 ± 4.16^{b}	22.03 ± 2.71^{ab}	23.59 ± 2.65^{a}
Triglycerides	26.31 ± 1.41^{a}	18.15 ± 2.42^{b}	21.68 ± 2.28^{b}	19.66 ± 1.94^{b}
Protein	90.25 ± 5.71	77.45 ± 6.87	80.15 ± 9.66	82.60 ± 4.85

[1] Means (n=18) ± standard deviation. Mean in the same row with different superscript letters are significantly different (P < 0.05).

TABLE IV
Hepatic enzyme activity of the juvenile Pseudoplatystoma ssp. fed the experimental diets for 60 days. The means in the same row with different superscripts are significantly different at (P<0.05).

Parameters	**Carbohydrate / lipid ratios^{1}**			
	0.45	**1.00**	**1.66**	**2.85**
ME	3.45 ± 1.01	3.89 ± 0.65	4.07 ± 0.98	3.71 ± 0.13
G6PD	34.04 ± 3.01^{b}	47.88 ± 3.96^{ab}	49.09 ± 8.81^{ab}	61.68 ± 0.63^{a}

[1] Means (n=18) ± standard deviation. Mean in the same row with different superscript letters are significantly different (P < 0.05).

was efficient in using different dietary amounts of carbohydrate or lipid ratios as energy sources when considering growth parameters.

In the hybrid catfish, the CHO/L ratios had an effect in energy metabolism terms. The low lipid and high carbohydrateratios increased plasma and hepatic glucose, and also the hepatic triglyceride levels in the studied hybrid. Lundstedt et al. (2004) obtained similar results for *P. corruscans*, according to which the incorporation of corn flour into diet significantly increased glycaemia and hepatic glucose. The liver is the central organ that controls glycaemia via enzymes that exert the main catabolism and synthesis of energy substrates. The metabolic capacity of reducing glycaemia is limited in carnivorous fish (Caseras et al. 2007, Hemre et al. 2002), which can lead to poor carbohydrate absorption, and also to an inefficient metabolism and energy profit from glucose. No influence of the CHO/L ratios was found on the protein content in white muscle, liver and plasma. However, the highest glycaemia and plasma protein rates were low at 0.33, with a high 1.24 protein/CHO ratio, respectively, in the *P. fasciatum* (♀) X *L. marmoratus* (♂)hybrids (Souza et al. 2014). In our study, although the fish fed the diet containing 15%

of corn starch (CHO/L: 1.66) showed high hepatic glucose values, this concentration did not influence glycaemia. The livers of the fish fed this diet were probably able to metabolize circulating glucose after 24 h of fasting. This effect was not found for the treatment that contained 20% corn starch (CHO/L: 2.85), which probably characterises a liver capacity limit to recover glycaemia homeostasis.

Our findings revealed no differences in ME when hybrid catfish were fed at different CHO/L ratios. In agreement with our result, lipogenic enzyme (G6PD) activity was more pronounced than the gluconeogenic enzyme (ME) in catfish (*Mystus nemurus*) (Hamid et al. 2011). G6PD activity was greater at a high CHO/L ratio. Jin et al. (2014) reported an increase in both the G6PD enzyme expression and hepatic glycaemia levels after an intraperitoneal injection of glucose in rainbow trout of a weak lineage. It is the main enzyme in the pentose phosphate pathway to generate NADPH as a reducing power for biosynthesis, such as lipid synthesis, and has been widely assessed to describe animals' adaptation capacity to diets containing a high carbohydrate concentration. G6PD and 6PGDH in the catfish liver did not respond to the dietary concentrations of protein, carbohydrates and lipids. Indeed the only response to be obtained has been found in fasted freshwater South America catfish (*Randia quelen*) (Melo et al. 2016). However, this fish is considered an omnivorous species, which means that the carbohydrate type appeared to influence the activities of liver enzymes to a greater extent. Greater G6PD activity has been observed in freshwater blunt snout breams, *Megalobrama amblycephala,* fed a glucose diet than those fed a dextrin or wheat starch diet at 2 h postprandial (Ren et al. 2015). Thus, our findings suggest that carnivorous hybrid hepatic cells appear to use carbohydrate properly at lower CHO/L ratios. The increased metabolism activity for glucose at the cell level may lead to a drop in not only hepatic G6PD, the rate-limiting enzyme in the pentose-phosphate-pathway, but also in the production of both NADPH and ribose-5-phosphates, the building blocks of nucleic acids, via an initial breakdown of glucose in channel catfish (*Ictalurus punctatus*) (Schroeter et al. 2018).

CONCLUSIONS

By way of conclusion, the present study suggests that a rise in the carbohydrate/lipid ratio (2.85) might lead to increasing glycaemia, but does not improve either growth or the protein efficiency rate in hybrid catfish. However, the glycaemia effect was not observed in the diet with a CHO/L of 1.66, which may be the most suitable carbohydrate and lipid inclusion level for this species. CHO/L=2.85 also affected G6PD activities, which somehow suggests that ♂*Pseudoplatystoma corruscans*×♀*Pseudoplatystoma fasciatum* is unable to efficiently cope with high dietary carbohydrate levels. The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Rosa was the researcher responsible for the elaboration and leadership of the research. Okamura, Araújo and Costa conducted the field assay, and laboratory analyses. Paulino, Fortes-Silva, and Pereira, together with the other authors, participated mainly in the draft paper, statistical analysis and discussion of the results.

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