Revista Varia Scientia Agrárias
v. 05, n.01, p. 77-91

ZOOTECNICA E
ENGENHARIA DE PESCA

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ALTERNATIVE CARBOHYDRATE USE IN RELATION PROTEIN:CARBOHYDRATE IN PERFORMANCE AND METABOLISM OF PACAMÃ (*LOPHIOSILURUS ALEXANDRI*)

ABSTRACT: The inclusion of peeled-mango waste meal as source of carbohydrate in the protein:carbohydrate ratio (CP:CH) on performance and chemical composition of pacamã (Lophiosilurus alexandri) juveniles was evaluated. One hundred and fifty fish (11.31 ± 0.96g) were stocked in sixteen 500 L tanks, fed three times daily (10% of live weight), in a recirculation aquaculture system. The treatments were four experimental diets with decreasing levels of the ratio between crude protein and carbohydrate (1.40, 0.94, 0.56 and 0.29), with four replications. At the end of 60 days, we evaluated animal performance (average weight gain, specific growth rate, total apparent feed intake, carcass yield, survival) and metabolic parameters (glucose, cholesterol, triglycerides, plasma albumin and free total amino acids) and liver enzyme alanine aminotransferase activity (ALT). The protein:carbohydrate ratios affected all performance variables (p<0.05), except for survival. The values of metabolic variables have been changed from the increase of the ratio CP:CH (p<0.05), with the exception of cholesterol and plasma albumin. Mango meal be used in a relation of 1.38 CP:CH without harming the performance and metabolism.

KEYWORDS: nutrition, inclusion, metabolic

Data de submissão: 18-05-2016
Data de aceite: 04-10-2016
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UTILIZAÇÃO DE CARBOIDRATO ALTERNATIVO NA RELAÇÃO PROTEÍNA:CARBOIDRATO NO DESEMPENHO E METABOLISMO DE PACAMÃS (*LOPHIOSILURUS ALEXANDRI*)

RESUMO: Avaliou-se a inclusão do farelo residual de manga sem casca como fonte de carboidrato na relação proteína: carboidrato (PB:CHO) sobre o desempenho e metabolismo em juvenis de pacamãs. Foram utilizados 150 peixes (11,31 ± 0,96g) estocados em 16 caixas de 500L, alimentados três vezes ao dia (10% peso vivo), em sistema de recirculação Os tratamentos consistiram em quatro dietas experimentais com níveis decrescentes da relação entre proteína bruta e carboidrato (1,40; 0,94; 0,56 e 0,29) com quatro repetições. Ao final de 60 dias, foram avaliados o desempenho zootécnico (ganho de peso médio, taxa de crescimento específico, consumo total de ração aparente, conversão alimentar aparente, rendimento de carcaça, sobrevivência), paramentos metabólicos (glicose, colesterol, triglicérides, albumina plasmática e aminoácidos totais livres) e atividade hepática da enzima alanina aminotransferase (ALT). As relações de proteína: carboidrato afetaram todas as variáveis de desempenho ($p<0,05$), com exceção da sobrevivência. Os valores das variáveis metabólicas foram alterados a parti do aumento da relação PB:CHO ($p<0,05$), com exceção do colesterol e albumina plasmática. A farinha de manga pode ser utilizada em uma relação de 1,38 PB:CHO sem prejudicar o desempenho zootécnico e o seu metabolismo.

PALAVRAS-CHAVE: nutrição, inclusão, metabólicos

INTRODUCTION

Mango is cultivated in several regions of Brazil, especially in the northeast, because it presents excellent conditions to its development and production, especially in the region of Vale do São Francisco. The mango fruit is widely available in this region, with a large volume of exports: in 2014, 108.38 thousand tons were exported, representing 90% of all national production (COELHO et al., 2015).

One choice to replace food for energy purposes in fish diets are those from ingredients of fruit growing, to be rich in carbohydrates (ENES et al., 2011). Diverse current searches on fish nutrition pursue alternative forms to supply above 30% glucose utilization in the form of starch (FERREIRA et al., 2015; LIU et al., 2015; VIDAL et al., 2015). However, other sources of carbohydrates such as (fructose, sucrose and galactose), possess different digestibility and metabolism and, at appropriate levels, could limit the use of protein as energy source and utilize them specifically their energy for growth (AZAZA et al., 2009; HONORATO et al., 2009).

A Large variety of regional ingredients have been studied and included in diets for fish, in order to reduce costs in feed and expand regional socio-economic sustainability, like bran licuri(CAMPECHE et al., 2014),.coconut meal (SANTOS et al., 2009), bran mango residue (MELO et al., 2012; SOUZA et al., 2013; LIMA et al., 2011), cassava leaves, mesquite pods (JESUS et al., 2011), coffee residue (PIMENTA et al., 2011), date-palm and (AZAZA et al., 2009) .

Pacamã (*Lophiosilurus alexandri*), a fish of the family Pseudopimelodidae (order Siluriformes), native to the São Francisco river basin. This fish is highly marketable because its meat is devoid of intramuscular spine, and its taste is highly appreciated by the consumers (LUZ et al., 2011).

Given the importance of determining the ratio between protein and carbohydrate in diets for fish, along with the marketability and performance potential of this species to intensive fish farming in the semi-arid region, the objective of this study was to determine the ideal proportion between protein and carbohydrate in the feeding of pacamã juveniles using mango waste meal as source of carbohydrate, evaluating animal performance and metabolism under different diets, thereby proposing the ideal protein level in the composition of their diet.

MATERIAL AND METHODS

Ethics statements and protocols

All procedures were approved by the Ethics Committee on Human and Animal Studies (CEEHA – UNIVASF, protocol no. 0022/26091).

Fish, fish housing and experimental design

The experimental design was randomized, with four treatments and four replications. We used one hundred and fifty pacamãs in the experiment, with initial live weight (ILW) of 11.31 ± 0.96g. They were distributed into 16 circular polyethylene water tanks (500 L), with a density of seven fish per experimental unit, in a water recirculation system with mechanic and biological filter. The animals went through an acclimation period of seven days, fed a diet formulated to have 40% crude protein and 3.800 Kcal gross energy (NRC, 2011, SOUZA et al., 2013). Fishes were fed three times daily, at 08h00, 12h00 and 17h00. The amount of feed supplied daily was 10% of the body weight, which was corrected every 15 days, when biometry was performed.

The water temperature, pH, dissolved oxygen and electrical conductivity were measured; the latter was measured with portable device Hanna Oxy-chek. The experimental units were siphoned in the morning and afternoon, before feeding, with exchange of approximately 10% of the total water so as to remove feces and feed leftovers.

Diets

The treatments consisted of four experimental diets with decreasing levels of the ratio between crude protein and carbohydrate (CP:CH - 1.40, 0.94, 0.56 and 0.29). The CP:CH ratios were calculated based on the proportion of nitrogen-free extracts (NFE) over the proportion of crude protein (CP) (Table 1).

Table 1 Chemical and calculated composition of the experimental diets

INGREDIENTS (%)	CP:CH ratios				
	1.40	0.94	0.56	0.29	Mango meal peeled
Fish meal	64.1	51.6	39.1	26.6	-
Soybean meal	10	7.5	5	2.5	-
Mango meal	15	30	45	60	-
Dicalcium phosphate	0.84	0.84	0.84	0.84	-
Soybean oil	8	7.5	7.1	6.6	-
Inert	-	0.2	0.3	0.5	-
Lysine	-	0.3	0.6	0.9	-
Min. and vit. mix[1]	2	2	2	2	-
Vitamin C[2]	0.05	0.05	0.05	0.05	-
BHT[3]	0.01	0.01	0.01	0.01	-
Calculated values[4]					
CP (%)	40.16	34.40	25.63	16,24	4.50
NFE (%)	28.68	36.63	46.01	56,33	80.39
GE:CP (Kcal. g^{-1})	10.61	12.37	16.57	35.83	89.44
GE (Kcal/kg)	4263	4255	4248	4226	4025
EE (%)	16.03	15.26	14.17	12.58	4.63
CF (%)	1.78	1.99	2.19	2.40	7.34
MM (%)	10.81	8.27	8.26	6.35	3.14
DM (%)	97.46	96.55	96.26	93.22	90.81
Tannins (µg g^{-1})	10.94	20.89	30.83	40.78	66.30

1. Min. and vit. mix (Supremais, Campinas/SP, Brazil): Composition per kg: vit. A = 1,200,000 IU; vit. D3 = 200,000 IU; vit. E = 12,000 mg; vit. K3 = 2,400 mg; vit. B1 = 4,800 mg; vit. B2 = 4,800 mg; vit. B6 = 4,000 mg; vit. B12 = 4,800 mg; folicacid = 1,200 mg; calciumpantothenate = 12,000 mg; biotin= 48 mg; choline = 65,000 mg; nicotinicacid = 24,000 mg; Fe = 10,000 g; Cu = 600 mg; Mn = 4,000 mg; Zn = 6,000 mg; I = 20 mg; Co = 2 mg; and Se = 20 mg. 2. Vit. C (Basf, São Paulo/SP, Brazil): calcium salt, ascorbic acid-2-monophosphate, 42% active ingredient. 3. Butylatedhydroxytoluene. 4. CP = crude protein; NFE = nitrogen=free extract; GE:CP = gross energy:crude protein ratio; GE = gross energy; EE = ether extract; CF = crude fiber; MM = mineral matter; DM = dry matter; CP:CH = crude protein:carbohydrate ratio.

To produce the mango waste meal, we utilized ripe mangos variety Tommy Atkins, originating from discarded fruits. The fruits were washed, peeled, cut in small pieces and had their seeds removed. Afterwards, they were dried in a forced-ventilation oven at 55°C for 86 hours. Subsequently, they were ground in knife mills with 1mm sieves and stored in a freezer at –18°C. All diet ingredients were ground in a knife mill with 0.5mm sieve, and then gathered until the mixture appeared homogeneous. To pelletize the diets, the mixture of ingredients was moistened with water at 50°C and then the diets were processed in a meat grinder and dried in a forced-ventilation oven at 65°C for about 24 hours. The pellets were broken with the aid of a manual mill, and separated into different diameters in sieves of different pores, to have appropriate sizes for the mouths of the animals according to their growth.

Growth parameters

Four biometric analyses were made in order to access fish performance.Every 15 days all fishes were weighted and messured with caliper in order to calculate, the average weight gain (AWG), specific growth rate (SGR), apparent feed intake (AFI), apparent feed conversion (AFC) and survival (S).

Average weight gain (AWG, g) = Final weight – Initial weight
Specific growth rate (SGR, %) = ((nl(Fw) - nl(Iw))/t) × 100;
Apparent feed intake (AFI, g) = (Total feed intake (g))/(N of fish per replicate);
Apparent feed conversion (AFC) =(Feed intake (g))/(Weight gain (g)); and
Survival (S, %) = ((final n of fish))/((initial n of fish))× 100.
Where *W*i and *W*f are the initial and final body weight of fish at the beginning and end of the feeding trial, respectively (g), and ln = logarithm neperian.

At the end of the experimental period, all animals were weighed to measure the growth data, and subsequently stunned with water and ice. The fish were sacrificed by medullar section.

Blood parameters

At the end of experiment, 10 animals were sampled per treatment for blood collection caudal by vase puncture with heparinized syringes. Prior, fishes were anesthetized and following blood samples were collected with heparinized syringes through the vazo flow rate puncture. Then plasma there was obtained by centrifugation at 5,000 x rpm for 5 minutes. Therefore, whole biological material was stored at -20 °C. Were measured in plasma triglyceride, glucose, serum cholesterol and albumin levels (mg/dL) of the reactants second colorimetric methods (Labtest®). Total free amino acids were determined according to Copley (1941). The livers were removed to determine of enzyme activity of alanine aminotransferase (ALT), were determined in this tissue according to reagents colorimetric methods (Labtest®). The total tannins the diets were determined according to the methodology (Steele et al. 2005).

Statistical Analysis

Statistical analyses were performed using the R-Br statistical software. All data collected were subjected to one-way analysis of variance (ANOVA) and the significance of the differences between means was tested using Tukey's ($p<0.05$) and then regression tests were applied for the metabolic variables ($p<0.05$).

RESULTS AND DISCUSSION

The water-quality parameters were: dissolved oxygen 5.4±1.8 standard mg/L, electric conductivity mS 0.13±0.09; pH 7.77±1.8 and temperature 26±3.5 °C. These results are considered acceptable for rearing fish (BOYD, 1990). The mean performance values are described in Table 2. All the analyzed variables, except for survival, showed significant differences ($p<0.05$).

Table 2 Mean values plus standard deviation of the performance variables and carcass characteristics of pacamã juveniles fed different CP:CH ratios

Performance	CP:CH ratios 1.40	0.94	0.56	0.29	CV (%)
IW (g)*	12.4 ± 2.98	10.45 ± 1.93	12.11 ± 2.08	10.27 ± 2.24	20.72
WG (g)	46.5 ± 0.88[a]	17.93 ± 2.07[b]	16.32 ± 1.34[b]	3.17 ± 0.84[c]	18.01
SGR (%/day)	0.96 ± 0.11[a]	0.46 ± 0.12[b]	0.39 ± 0.10[b]	0.01 ± 0.04[c]	12.64
AFI (g)	78.3 ± 5.42[a]	47.01 ± 6.94[b]	54.47 ± 7.38[b]	46.19 ± 8.33[b]	33.48
AFC	1.68 ± 0.03[c]	2.62 ± 0.05[b]	3.34 ± 0.01[b]	14.57 ± 0.02[a]	13.65
S (%)*	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	0.00

IW = initial weight; WG = weight gain; SGR = specific growth rate; AFI= apparent feed intake; AFC = apparent feed conversion; S = survival.
*Mean values followed by the same letter in the row do not differ according to the Tukey test (p>0.05).

The performance of pacamã fed with the diets relations PB: CHO (0.94, 0.56 and 0.29) were dramatically reduced ($p < 0.05$), this fall in fish weight is related to inefficiency use of carbohydrate source as energy substrate growth . Whereas, the mango meal in its total composition of the carbohydrates (fructose, glucose, gacturônico acid, arabinose and xylose) respectively (59.20; 26.36; 10.12; 5.06; 3.54) (RAO and AJILA, 2013; MUIR et al. 2009) has a predominance by fructose. Figueiredo et al. (2014) to test conventional carbohydrate source in diets with respect CP:CH ratio to pacamãs, attained a decrease in performance while decreasing the relation PB:CHO, a fact that proves the inefficiency of using carbohydrates as the main source of energy for the species.

LIMA et al. (2011) included the levels 0, 5, 10 and 15% mango waste meal in diets for tilápia do Nilo and did not observe significant differences in weight gain. MELO et al. (2012) evaluated the inclusion of peeled-mango waste meal replacing corn and concluded that substitution of 100% of corn by mango does not impair the tilapia performance. Whereas, these cited works are composed in different types of delineation, none of them with nutrients ratio. According to HILTON et al. (1987), a tolerable level of carbohydrate in diets for fish is a level which does not compromise growth or results in increased mortality, whereas an optimal carbohydrate level in diets for fish is what enables glucose to be totally oxidized to produce energy, and thereby spare protein for the use of its energy in muscle growth.

For the apparent feed intake (AFI), intake was observed to increase along with the protein concentration of the diet, and consequently with the CP:CH ratio. There was no significant differences between the ratios of 0.94, 0.56 and 0.29, but they presented lower results than the 1.40 CP:CH. AKSNES (1995) verified that feed intake decreased as the carbohydrate level of the diet for Atlantic salmon was elevated, which corroborates the results of the present study, indicating that the energy from the carbohydrate more readily available in the diet might have influenced the satiety of carnivore fish, limiting their appetite. SAMPAIO et al. (2000) obtained results for feed intake that followed the same trend, and the higher AFI followed the higher CP levels of the diet.

With respect to survival, was not affected by the different CP:CH ratios ($p>0.05$). The elevated survival rates indicate that the diets containing mango meal as source of carbohydrate did not have a harmful effect on the health of fish.

Another factor that compromises performance of pacamã, was the gradual Tannin in diets relations CP:CH (0.94, 0:56 and 0:29) observed in (Table 1) due to higher concentrations of the residual of bran mango. Second LEENHOUWERS et al. (2007), the high viscosity of the digestion reduces the mixture of digestive enzymes with substrates and increases the layer thickness of the intestinal taking decrease of digestion and nutrients absorption, and reduce digestibility. Tannins as well combine together with proteins from the outer layer of the interstitial cells leading to reduced in the absorption of nutrients MUELLER-HARVEY AND MCALLAN (1992) what can be observed the reduction of plasma amino acids in relations (0.94, 0.56 and 0.29).

These values show that the efficiency of mango waste meal as source of carbohydrate in diets depends on the species its eating habit, whereas the pacamã has higher harnessing protein for its growth, characterization of sources of carbohydrate and the presence of anti-nutritional factors.

The mean values for the studied metabolic variations are described in (Table 3). With the increase in CP:CH ratio was no alteration in metabolic responses ($p< 0.05$), with increased glucose and triglyceride levels.

Table 3 The mean values more standard deviation of metabolic responses pacamã juveniles fed different CP:CH ratio

Metabolic responses[1]	CP:CH ratios				
	1.40	0.94	0.56	0.29	CV (%)
GL (mg/dL)	104±16.66^{c}	195±23.03^{bc}	220±32.00^{ab}	324±43.25^{a}	41.78
CHL (mg/dL)*	44.95±10.95	34.05±5.46	42.88±5.40	41.35±4.62	22.79
TG(mg/dL)	151.82±36.00^{b}	310.54±34.76^{a}	389.45±43.38^{a}	297.45±42.76^{a}	24.31
ALB(g/dL)*	0.48±0.07	0.50±0.02	0.56±0.05	0.53±0.04	11.89

[1]GL - glucose; CHL - cholesterol; TG - triglycerides; ALB - albumin.
*Mean values followed by the same letter in the row do not differ according to the Tukey test ($p>0.05$).

The metabolic responses were changed when with the increase in CP:CH ratio ($p< 0.05$). Alterations in plasma glucose (GL) may occur due to several factors, e.g., the conversion of surrounding glucose to liver glycogen and lipids in the form of reserve, for metabolizing other metabolite intermediates, energy catabolism, renal filtration, and excretion in the urine (JAING et al. 2014).

SOUZA (2012), studying the inclusion of mango meal in tilapia, obtained the same ¯increasing¯effect, including 100% of mango meal. This effect may be related to the type and use of the sugar present in the carbohydrate source utilized. When the absorbed carbohydrates are not used for energy purposes they may be deposited in the liver as glycogen or converted to lipids (CHEN et al. 2012).

The triglyceride concentration in the plasma was significantly higher ($p<0.05$) in the treatment containing the highest CP:CH ratio. Increase in triglyceride levels were reported by BRAUGE et al. (1995) in *Oncorhynchus mikyss* due to the high levels of carbohydrates and lipids contained in the diet. Well as concentration of hepatic triglycerides in *European seabass* increased with dietary carbohydrates (MOREIRA et al., 2008). The increased triglycerides still occurs due to the increased of dietary energy (LUNDSTEDT, 2003). In this work, increased triglycerides is related to type or amount of carbohydrate used. A ripe mango has significant amounts of fructose and sucrose (BERNARDES-SILVA et al., 2003). Increased triglycerides has been touted as a result of excessive fructose consumption (GABY, 2005).

Fructose is phosphorylated in the oxidative route of glucose does not have specific hormonal regulation, that is, not dependent on insulin and glucagon levels, having an increased control only by their hauliers, the GLUT's. With increase of fructose through the lower ratios CP:CH possibly occurred more activity of the enzymes of the pentose deviations, favoring the production of triglycerides. The mean values of plasma amino acids were significant ($p<0.05$) and these have a linear effect to increase the CP:CH relations, illustrated in (Figure 1).

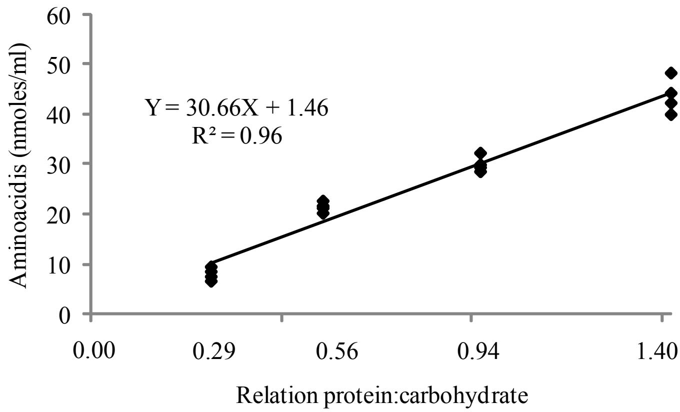

Figure 1 Mean concentration of total plasma amino acids at different CP:CH ratios.

The fishes performing maintenance of the glucose levels through alternative metabolic pathway, for use as substrates amino acid residues in a process called gluconeogenesis for maintaining circulating levels of glucose and transfer energy from amino acids ENES et al. (2011). In the present study has been found that a couple of metabolites may have contributed to maintain glycemia, primarily free total amino acids for use in gluconeogenesis (Figure 1). The reductions in plasma free total amino acid concentrations reflect on their possible utilization by the action of the liver enzyme alanine aminotranferase (Figure 2), which has the ability to capture glucogenic amino acids in the Krebs cycle and convert them to alpha-ketoacid through a process called transamination, targeting the use of these molecules in the formation of blood glucose. To which can be observed in (Figure 2) linear decrease in liver ALT activity. This same effect was described by (CHEN et al. 2012) to test protein and starch in ratios diets for carp.

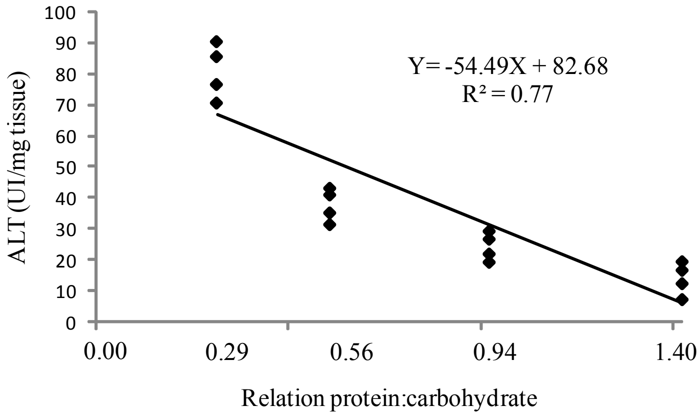

Figure 2 average activity of the liver enzyme alanine aminotransferase in different CP:CH ratios.

The mean values of total plasma cholesterol were no different significantly ($p>0.05$) are described in (Table 3). These data differ from encountered by SEABRA et al. (2009), who used mango meal levels in substitution of corn, observed a reduction in the levels of total plasma cholesterol tambaquis. According to these authors one explanation for reducing these levels due to the presence of soluble fibers present in the fruit which increase the excretion of bile acids by inducing liver in the removal of cholesterol in the blood. MELO (2012) states that diets high lipid levels elevate cholesterol levels. The results of cholesterol found in this study was expected since all feed had similar amounts of lipids demonstrating that the sources of carbohydrates used did not affect the absorption.

The concentrations of albumin (ALB) not presented significant differences between the treatments ($p<0.05$). PÁDUA et al. (2009) reported that hypoalbuminemia has effects on the nutritional state resulting from insufficient protein intake or diseases that affect the synthesis of ALB. SANTOS et al. (2010) evaluated the performance and physiological responses of tambaqui (*Colossoma macropomum*) fed increasing levels of Brazil nut meal and did not observe alterations in the concentrations of plasma ALB.

CONCLUSIONS

In conclusion, this study has demonstrated CP:CH ratios at 1.40% in diets for pacamã juveniles without impairing the fish performance and metabolism. Considering the variations tested with the diets, we can assume that a perfect adjustment of the levels may optimize the growth potential of pacamã.

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