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Energy and Protein in Diet: Effects on Zootechnical and Biochemical Parameters of Piavuçu *Leporinus macrocephalus* (Actinopterygii, Anostomidae)

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Abstract: The objectives of this study were to evaluate the effects of different levels of digestible energy (DE) and crude protein (CP) in feed on zootechnical and biochemical parameters in juvenile piavuçu, *Leporinus macrocephalus*. Juvenile piavuçu (n = 180; 22.23 ± 0.31 g) were distributed in tanks (250 L) and fed two times a day which different experimental diets containing a DE/CP ratio of 10 kcal DE g^{-1} CP (2300/230, 2800/280, 3300/330 and 3800/380) for 60 days. Fish fed the 3300/330 and 3800/380 diets showed better growth results, but the later yielded the best feed conversion and protein efficiency rates. Protein deposition was lower with the 2300/230 feed. The ash content in the carcass of animals fed the 3800/380 diet was lower than in the other treatments, and it was lower with the 3300/330 feed than the 2800/280 feed. Carcass moisture was lower while lipid deposition was higher in animals treated with the 3800/380 feed, and the ammonia excretion rate was higher in those treated with the 3300/330 and 3800/380 rations compared to the other treatments. The deposition of glycogen in the muscle was significantly greater in animals treated with the feed containing 2.300 kcal DE / 230 g CP while in the liver, was significantly higher in animals treated with 3800 kcal DE /380 g CP treatment. We can conclude that 3800 kcal DE / 380 g CP diets promoted better performance in juvenile piavuçu.

Keywords: aquaculture, diets, fish, *Leporinus macrocephalus*, nutrition.

Resumo. Energia e Proteína na Dieta: Efeitos sobre os parâmetros zootécnicos e bioquímicos em piavuçu *Leporinus macrocephalus* (Actinopterygii, Anostomidae). Os objetivos deste estudo foram avaliar os efeitos de diferentes níveis de Energia Digestível (ED) e Proteína Bruta (PB) na ração sobre os parâmetros zootécnicos e bioquímicos de juvenis de piavuçu *Leporinus macrocephalus*. Juvenis de piavuçu (n=180; 22,23 ± 0,31 g) foram distribuídos tanques de 250 L e alimentados duas vezes ao dia, com rações experimentais elaboradas com a relação ED/PB =10 kcal ED g PB^{-1} (2300/230, 2800/280, 3300/330 e

3800/380) durante 60 dias. Os peixes alimentados com as rações 3300/330 e 3800/380 apresentaram melhores resultados para crescimento, porém a ração 3800/380 proporcionou melhores resultados de conversão alimentar e taxa de eficiência proteica. A deposição de proteína foi menor com a ração 2300/230. O teor de cinzas na carcaça dos animais tratados com a ração 3800/380 foi menor que os demais tratamentos, enquanto que com a ração 3300/330 foi menor que a ração 2800/280. A umidade na carcaça foi menor e a deposição de lipídios foi maior nos animais tratados com a ração 3800/380. A taxa de excreção de amônia foi maior nos tratados com as rações 3300/330 e 3800/380 em relação aos demais tratamentos. A deposição de glicogênio no músculo foi significantemente maior nos animais alimentados com a ração 2300/230 enquanto que no fígado, foi significantemente maior nos animais alimentados com a ração 3800/380. Podemos concluir que rações com níveis de 3800 kcal ED / 380 g PB promoveram melhores resultados para desempenho de juvenis de piavuçu.

Palavras-Chaves: aquicultura, dietas, peixe, *Leporinus macrocephalus*, nutrição

Introduction

The piavuçu, *Leporinus macrocephalus* (Anostomidae), which is found in the Paraguay River Basin (Garavello 1979), is considered to have the potential for commercial aquaculture (Soares *et al*. 2000; Rodrigues *et al*. 2006). The fish has a fusiform and elongate body, exhibits rapid growth in its early stages (Garavello & Britski 1988) and presents good performance parameters and high resistance to management (Feiden *et al*. 2009). Another feature that favors its potential cultivation is its omnivorous feeding habit, which allows for the use of feeds with a variety of ingredients, both animal and plant (Rodrigues *et al*. 2006; 2008).

Efficient feeds must meet the nutritional needs of the animals without impairing the water quality in the rearing systems (Fracalossi & Cyrino 2013). According to Cyrino *et al*. (2010), the biological value of a diet can vary depending on factors from the quality and sources of the ingredients to the processing techniques to the interactions between the nutrients and supplements being used. The nutrients present in the diet can influence the growth performance of fish, and their concentrations, especially energy and protein, must be proportionally balanced to avoid harmful deficiencies or excesses (Nogueira *et al*. 2005; Navarro *et al*. 2006; Furuya & Portz 2013). Similarly, the amount of energy should not promote rapid satiety, which can reduce the intake of protein and other nutrients that are essential for maximum growth or lead to the undesirable deposition of large amounts of body fat (Colin & Young 1993; Ribeiro *et al*. 2007).

Therefore, diets with digestible energy and crude protein concentrations based on the requirements of the different species with commercial rearing potential are fundamental to the development of fish farming. Therefore, the objective of this study was to evaluate the effect of diets with different amounts of digestible energy and crude protein on the performance and proximate composition of juvenile piavuçu.

Materials and Methods

The juvenile piavuçu were purchased from a commercial aquaculture operation (Fish Farming Águas do Vale®, MatoLeitão, RS) and transported to the Continental Aquaculture Laboratory (LAC) (32 ° 5 '11 "S, 52 ° 13' 9" W) of the Oceanographic Institute (IO) of the Federal University of Rio Grande (FURG). For this study, 180 juvenile piavuçu (22.23 ± 0.31 g) were selected and randomly placed in 12 tanks (250 L each; 15 animals/tank; 1.33 g L^{-1} stocking density) in a recirculation system. Each tank was equipped with a mechanical-biological filter and maintained under constant aeration, a controlled temperature and an artificial photoperiod of 12 h light: 12 h dark. The animals underwent an acclimation period, during which they were fed an experimental diet with lower levels of digestible energy and crude protein (2300 kcal DE and 230 g CP) for 15 days before the start of the experiment, which lasted for 60 days. The tanks were siphoned daily after the first feeding to remove solid waste, and the water volume was replenished (10%). The supplementary water was stored in tanks in the same environment and under the same conditions as those containing the animals.

The experimental diets were prepared at LAC and formulated to achieve the desired treatment levels based on the available ingredients and nutrients (Table I). The ingredients used to compose the diets were analyzed by estimating their proximate composition in the Food Technology Laboratory (LTA) and their energy in the Physical Chemistry Laboratory (LFQ), both located at FURG.

The ingredients were mixed manually in accordance with the formulation of each diet, and after homogenization, the diets were pelleted and dried at 50 °C in a forced-air circulating oven for 24 h. The pellets were subsequently stored in a freezer (-20 °C), and only the quantity corresponding to the expected consumption was defrosted each day.

Fish were subjected to three replicates of four different treatments, which were defined by the different levels of digestible energy (DE) and crude protein (CP) in the feed (2300 kcal / 230 g, 2800 kcal / 280 g, 3300 Kcal / 330 g and 3800 kcal / 380 g), so the amount of energy relative to protein was maintained at 10 kcal DE / g CP. Feeding was performed twice a day (09 and 16 h) until apparent satiation of the animals. The recirculation was turned off during feeding, so only the aeration remained in operation. Every day before the first feeding, the temperature (27.33 ± 0.71 °C) was verified as well as the amount of dissolved oxygen (6.88 ± 0.47 mgL^{-1}) with a YSI EcoSense® DO200A digital oximeter and the pH (6.94 ± 0.23) with a HI 8424 digital pH meter by Hanna Instruments®. Twice a week, concentrations of the following were also analyzed: total ammonia (TAN) (0.22 ± 0.15 mg L-1) according to Unesco (1983), nitrite (0.19 ± 0.08 mg L^{-1}) according to Bendschneider & Robinson (1952) and total alkalinity (37.87 ± 13.44 mg CaCO3 L^{-1}) according to Eaton et al (1995). The water quality parameters measured during the experiment remained adequate for the species according Boyd (1997) and Pinto *et al*. (2016).

Biometrics data were collected at 0, 30 and 60 days. The animals were fasted for 24 hours and anesthetized with benzocaine hydrochloride (50 ppm), and their weights (g) were obtained with a digital electronic scale (BIOPRECISA® - JH2102; accuracy of 0.01 g); total lengths (cm) and standard lengths (cm) were measured with a graduated ictiometer.

At the end of the experiment, nine fish from each treatment were anesthetized and measured, and blood was collected by puncturing the tail vein. Immediately after blood collection, blood glucose analysis was performed using a digital glucometer (Accu-Check - Roche®). The hematocrit (%) was measured using a microhematocrit centrifuge (Microspin® MH24) and a specific ruler.

Table I. Formulation and composition of the experimental diets with different levels of digestible energy (DE) and crude protein (CP), with a consistent DE:CP ratio of 10 kcal DE/ g CP, supplied to juvenile piavuçu, *Leporinus macrocephalus.*

	Composition of experimental diets			
Ingredients	T1(2300/230)	T2(2800/280)	T3(3300/330)	T4(3800/380)
Inert (cellulose) (%)	33.49	20.06	10	0
Soybean meal (%)	10.18	10.3	20.1	32.3
Fish Meal (%)	27.74	36.39	35.2	33
Wheat bran (%)	5.07	9.07	8	5
Defatted ricebran (%)	9.13	9.07	8.99	5.99
Ricebran (%)	1.8	2.6	5	9.9
Ground corn (%)	10.15	10.08	10	10
Canola oil (%)	0.3	0.3	0.7	1.7
Common salt (%)	0.1	0.1	0.1	0.1
Premix (%)[1]	0.01	0.01	0.01	0.01
Limestone (%)	0.7	0.4	0.2	0
Molasses (%)	2.03	2.02	1.9	2
DigestibleEnergy(kcal/kg)	2337	2800	3300	3800
Crude protein (g/kg)	229.995	280	330	380
DE/CP ratio (kcal/g)	10.16	10	10	10
Total (%)	100	100	100	100

1 -Premix M. Cassab, SP - Brazil:(vit. A (500,000 UI kg^{-1}), vit. D3 (250,000 UI kg^{-1}), vit. E (5,000 mg kg^{-1}), vit. K3 (500 mg kg^{-1}), vit. B1 (1,000 mg kg^{-1}), vit. B2 (1,000 mg kg^{-1}), vit. B6 (1,000 mg kg^{-1}), vit. B12 (2,000 mcg kg^{-1}), niacin (2,500 mg kg^{-1}), calcium pantothenate (4,000 mg kg^{-1}), folic acid (500 mg kg^{-1}), biotin (10 mg kg^{-1}), vit. C (10,000 mg kg^{-1}),colin (100,000 mg kg^{-1}), inositol (1,000 mg kg^{-1}). Trace elements: selenium (30 mg kg^{-1}), iron (5,000 mg kg^{-1}), copper (5,000 mg kg^{-1}), manganese (5,000 mg kg^{-1}), zinc (9,000 mg kg^{-1}), cobalt (50 mg kg^{-1}), iodine (200 mg kg^{-1}).

Blood samples (approximately 1.0 ml) were placed in microtubes and centrifuged at 2500 rpm for 5 min to separate the plasma, which was used to quantify of plasma levels of total protein, cholesterol and triglycerides.

These parameters were analyzed by a commercial laboratory (LUNAV-Análises Clínicas) using spectrophotometry equipment for the automatic analysis of clinical chemistry (Metrolab 2300 Autoanalyzer PlusWiener Lab-Group®). Soon after blood sample collection, the fish were euthanized with a lethal dose of benzocaine (250 mg L^{-1}) followed by a spinal cord section to collect the liver and muscle. The liver, viscera and the carcass of each animal, without the head, were weighed in an accurate electronic balance (MARTE® AD200; precision 0.001 g) to determine the hepatosomatic index (HSI), viscerosomatic index (VSI) and carcass yield (CY). Liver and muscle samples were placed into microtubes and frozen (-20 °C) for glycogen analysis (Van der Vies, 1954) at the Laboratory of Cyanobacteria and Phycotoxins (LCF) at FURG. The carcasses were placed in a universal collector and frozen (-20 °C) for further analysis of proximate composition (AOAC 1995), in LTA-FURG.

The parameters used to estimate growth performance were weight gain = final weight - initial weight; total growth = final total length - initial total length; standard growth: = final standard length - initial standard length; body condition = [weight x (total length^3)$^{-1}$] x 100; specific growth rate = [(ln final weight - ln initial weight) x experimental period^{-1}] x 100; protein efficiency ratio = weight gain x protein intake^{-1}; apparent feed conversion = provided ration x weight gain^{-1}; feed consumption = total feed supplied daily; hepatosomatic index = (liver weight x total weight $^{-1}$) x 100; viscerosomatic index = (weight of the viscera x total weight^{-1}) x 100; and carcass yield = (carcass weight x total weight^{-1}) x 100.

To analyze the total ammonia excretion rate (TAN mg L^{-1}) after feeding, a second experiment was conducted using 45 juvenile piavuçu (18.34 ± 1.26 g). These animals were also placed in 250 L tanks in a room with a controlled temperature and photoperiod, in which they acclimated for 2 weeks (temperature in 27 °C, photoperiod of 12 h light: 12 h dark, and feed corresponding to each treatment). After acclimation, the fish (n = 9) were placed into individual aquaria (6 L) and divided into 5 treatments (no feed and the 4 previously tested diets). At the beginning of the experiment, temperature (27 °C) and dissolved oxygen (6.58 mg L^{-1}) were checked with a YSI DO200A digital oximeter by YSI DO200A EcoSense®, and the pH (7.34) was monitored with a digital pHmeter (HI 8424 - Hanna Instruments®). Throughout the experimental period, water samples were collected in triplicate to quantify ammonia and estimate the ammonia excretion rate (UNESCO, 1983). The total ammonia excretion rate (TANex) mg TAN kg^{-1} h^{-1} was estimated as follows: TANex = (TANf - TANi) x V / w x h), where TANf = final concentration of TAN (mg L^{-1}); TANi = initial concentration of TAN (mg L^{-1}); V = volume of water in the aquaria (L); w = the weight of the fish (kg) and h = hours. To determine the mean basal TANex, water was collected at the start (time zero) and end of the experiment (24 h). The animals that were fed only received feed at the beginning of the experiment at a proportion of 3% biomass. The ratio between the total ammonia excreted and crude protein intake (TAN mg L^{-1} g ICP^{-1}) was estimated as TAN (mg L^{-1}) ICP(g)$^{-1}$ = (TANf x V) / gICP, where: TANf = the final TAN concentration; V = volume of water in the aquaria (L); and gICP = grams of crude protein intake.

All of the experimental data (mean ± SD) were subjected to tests of normality (Kolmogorov-Smirnov) and homogeneity (Levenne) and, when normal and homogeneous, were subjected to a one-way analysis of variance (ANOVA). Means were compared by Tukey's test ($p < 0.05$).

Results and Discussion

The growth performance results of juvenile piavuçu indicate a significant difference between treatments in the growth parameters, except for the body condition factor (K). The morphometric rates (HSI, VSI and CY) of the animals were not significantly different, indicating that the diets did not promote the undesirable accumulation of fat in the liver and viscera and did not affect carcass yield (Table II).

The diets with digestible energy and crude protein levels greater than 3300 kcal and 330 g CP per kg of feed yielded the best results for FW, FTL and SGR, and the values observed for FLS, PER and FCR were higher in animals that were fed the 3800 kcal DE and CP 380 g per kg of feed diet. These results indicate that the highest experimental levels of ED and CP were more efficient, and the weight gain results (WG and DWG) were similar

Table II. Zootechnical parameters (means ± standard deviation) of juvenile piavuçu fed diets with different levels of digestible energy (DE) and crude protein (CP) with a consistent ED:CP ratio of 10kcal DE/ g CP.

Observed Parameters	Treatments (kcal DE/g CP) T1 (2300/230)	T2 (2800/280)	T3 (3300/330)	T4 (3800/380)
K	1.12 ± 0.04	1.14 ± 0.12	1.24 ± 0.04[a]	1.26 ± 0.03
HSI (%)	0.713 ± 0.07	0.714 ± 0.17	0.778 ± 0.11[a]	0.790 ± 0.16
VSI (%)	29.01 ± 7.10	31.31 ± 1.89	30.52 ± 4.02[a]	32.24 ± 2.72
CY (%)	70.99 ± 7.11	68.69 ± 1.89	69.48 ± 4.02[a]	67.75 ± 2.72
FW (g)	50.33 ± 12.44[b]	59.61 ± 17.41[b]	80.39 ± 20.42[a]	90.65 ± 22.06[a]
FTL (cm)	16.48 ± 1.38[b]	17.07 ± 1.41[b]	18.66 ± 1.52[a]	19.32 ± 1.61[a]
SGR (%)	1.38 ± 0.09[b]	1.66 ± 0.18[b]	2.18 ± 0.08[a]	2.35 ± 0.04[a]
FSL (cm)	13.62 ± 1.06[c]	14.26 ± 1.08[c]	15.51 ± 1.17[b]	16.19 ± 1.37[a]
PER (%)	1.75 ± 0.27b	1.72 ± 0.31b	1.64 ± 0.09[b]	1.53 ± 0.07a
FCR	2.54 ± 0.43[c]	2.11 ± 0.39[b]	1.85 ± 0.14[b]	1.72 ± 0.12[a]
WG (g)	27.81 ± 2.98[a]	36.79 ± 6.36[a]	57.74 ± 2.55[b]	67.55 ± 3.89[b]
DWG (cm)	0.48 ± 0.05[a]	0.63 ± 0.11[a]	0.99 ± 0.04[b]	1.16 ± 0.07[b]
FI (g/day)	18.04 ± 0.93[b]	19.67 ± 0.64[b]	27.59 ± 1.99[a]	30.01 ± 0.39[a]
S (%)	100%	100%	100%	100%

K = body condition factor; HIS = hepatosomatic index; VSI = viscerosomatic index; CY = carcass yield; FW = final weight; FTL = final total length; SGR = specific growth rate; FSL = final standard length; PER = protein efficiency ratio; FCR = feed conversion rate; WG = weight gain; DWG = average daily weight gain; FI = feed intake; S = survival. Different letters in the same line indicate significant differences according to a Tukey's test ($p < 0.05$).

to the results observed in the growth parameters (FW, FTL and PER). Feed intake was lower at levels below 3300 kcal DE and CP 330 g per kg of feed. Another relevant factor was the survival of the animals, which was 100% in all treatments. It was observed that animals treated with diets with 3300 kcal and 330 g CP and 3800 kcal and 380 g CP per kg showed weight gain (WG) of 261.95% and 299.87%, respectively, which was significantly higher than that observed for the animals treated diets with 2300 kcal DE and 230 g CP (128.66%) and 2,800 kcal DE and 280 g CP (170.71%). Therefore, better performance was demonstrated with the diets with the highest levels of digestible energy and crude protein. The WG and DWG values were significantly higher in fish fed diets containing levels above 3300 kcal DE and 330 g CP, but significant differences in gains were not observed between the two lower levels tested (2300 kcal DE / 230 g CP and 2,800 kcal DE / 280 g CP) or between the two higher levels (3300 kcal DE / 330 g CP and 3800 kcal DE / 380 g CP).

Pezzato *et al*. (2000) and Bittencourt *et al*. (2010) studied the influence of DE/CP ratio on the performance of fingerling piavuçu and reached different conclusions regarding the levels used in the diets (2800 kcal DE / 280 g CP and 3500 kcal DE / 350 g CP, respectively). However, they reached the same conclusion regarding the constant DE/CP ratio of 10 kcal DE / g CP. The performance differences may be related to the environment because the temperatures in these studies were approximately 23 °C (Pezzato *et al*. 2000), which is considered unsuitable, and 27 °C (Bittencourt *et al*. 2010), which is within the standards for the development of tropical fish according to Boyd (1997). Freitas *et al*. (2011) concluded that the DE/CP ratio must be 10 kcal DE/g CP, and 3.500 kcal DE / 350 g CP was the best for FCR. However, these authors emphasized that at least 3250 kcal DE / 300 g CP should be used to promote improvements in weight gain. Feiden *et al*. (2009), when defining the protein needs for piavuçu fry, found better performance results using a ratio of 9.41 kcal DE / g CP (3200 kcal DE / 340 g CP) and concluded that the minimum protein content should be 34%. Thus, the results suggest that protein levels above 33% promoted improvement in the growth of the animals.

A lower feed intake was observed to result in a similar carcass protein concentration in animals fed experimental diets with 2800 kcal DE / 280 g CP and 3300 kcal DE / 330 g CP, which present higher FCR rates compared to those fish treated with a 3800 kcal DE / 380 g CP diet (Table III). This is extremely important, when we considerate that protein is the most expensive nutrient in the manufacture of feed. This 3800 kcal DE / 380 g CP feed provided greater growth in CP, which is of particularly great interest in the commercialization of the fisheries along with improved FCR. Since higher protein levels result in better growth, this result may be associated with the interaction between the levels of DE and CP, as observed by De Freitas *et al*. (2010).

Rodrigues *et al*. (2010) found negative results for WG, SGR, FCR and PER, which correlated negatively with the increased inclusion of cellulose content in diets for juvenile pacu *Piaractus mesopotamicus* (Characidae, Holmberg, 1887) as well as a reduction in feed intake. Similarly, Braga *et al*. (2014) concluded that the inclusion of more than 2% cellulose in the feed can negatively impact the WG, FCR and CY and thus impair the development of juvenile tambacu (hybrid *Colossoma macropomum* x *Piaractus mesopotamicus*). Seixas-Filho (2004), mentioned that fiber levels between 10 and 20% in the diets of carnivorous and omnivorous fish can cause development changes due to reduced intake. This was observed in this study in the 2300 kcal DE / 230 g CP and 2800 kcal DE /280 g CP feeds with 33.49% and 20.06% cellulose, respectively, which resulted in a 39.88% and 33.45% reduction in feed intake compared with the 3800 kcal DE / 380 g CP feed, which did not include cellulose. This can be justified by the results obtained by Meurer *et al*. (2003), perceiveding a decrease in the transit time of alimentary boluses when cellulose was included in the diet of reversed Nile tilapia fry (*Oreochromis niloticus*, Cichlidae, Linnaeus, 1758) which can influence in decrease the absorption of dietary nutrients in the gastrointestinal tract. In this experiment, the body condition factor (K) did not differ between treatments, indicating that the feed cannot change the physiological condition of animals kept in good health, as all of the fish showed positive growth and weight gain.

The results of the proximate composition (table III) demonstrate that, as DE and CP levels increased, the carcass lipid concentration grew while the humidity decreased. However, independent of the diet consumed, the energy levels in the carcasses were not indicative of fat animals, according to the classification by Jacquot (1961), the lipid content in natural matter remained below 10%. The protein concentration in piavuçu carcasses was significantly lower when fish were fed the 2.300 kcal DE /230 g CP diet compared to other treatments. The variation found in the moisture content of piavuçu carcass, according to the tested diets, depended on the variation in the lipid concentration. When fish increase the concentration of lipids in their bodies, moisture decreases, and this fact is related to the amphiphilic behavior that occurs between lipids and water. This same behavior was observed by several authors for Nile tilapia (Abdel-Tawwab *et al*. 2010), piavuçu (Bittencourt *et al*. 2010), blunt snout bream *Megalobrama amblycephala* (Li *et al*. 2010) , tambaqui *Colossoma macropomum* (Santos *et al*. 2010) and pacific threadfin *Polydactylus sexfilis* (Deng *et al*. 2011).

Table III. Proximate composition (means ± standard deviation) of juvenile piavuçu fed diets with different levels of digestible energy (DE) and crude protein (CP) with a consistent ED:CP ratio of 10kcal DE/ g CP.

Observed	Treatments (kcal DE g^{-1}CP)			
Parameters	T1 (2300/230)	T2 (2800/280)	T3 (3300/330)	T4 (3800/380)
Humidity (%)[1]	73.83 ± 1.19 [a]	74.02 ± 0.83 [a]	72.37 ± 0.81 [a]	70.65 ± 0.33 [b]
Lipids (%)[1]	5.07 ± 0.34 [c]	6.41 ± 0.41 [b]	6.61 ± 0.32 [b]	8.34 ± 0.48 [a]
Proteins (%)[2]	67.86 ± 3.99 [b]	71.19 ± 3.61 [a]	70.28 ± 0.57 [a]	69.21 ± 2.26 [a]
Ash (%)[2]	10.78 ± 0.12 [ab]	11.59 ± 0.46 [a]	10.41 ± 0.42 [b]	9.23 ± 0.14 [c]

[1]Composition in natural matter; [2]composition on a dry basis; different letters in the same line indicate significant differences according to a Tukey's test ($p < 0.05$).

The carcass composition analysis in the present study indicated that diets with 2300 kcal DE /230 g CP resulted in lower protein content. In contrast to these results, Feiden *et al*. (2009), who defined a protein requirement range for piavuçu of 22-38% CP, found no differences between treatments.

In this study, the high concentration of cellulose in the 2300 kcal DE /230 g CP diet (Table I) may have negatively interfered with the digestibility of nutrients, including protein, thus worsening the protein efficiency ratio and corroborating the results of Lanna et al. (2004) for Nile tilapia and Rodrigues *et al*. (2010) for pacu. In diets with lower levels of DE and CP, it was necessary to fix the calcium, using limestone (Table I), which may have been reflected in the proximate composition of the carcasses as ash content was inversely proportional to the tested levels of DE and CP.

The glycogen levels found in the liver and muscle of the tested animals (Table IV) presented a significantly higher deposition in the 3800 kcal DE /380 g CP treatment (193, 87% more than the lowest value observed in the treatment with 2300 kcal DE /230 g CP), while the deposition of glycogen in the muscle was significantly greater in animals treated with the feed containing 2.300 kcal DE / 230 g CP, which was formulated with small amounts of DE and CP (61.26% higher than the average of the other treatments).Variation in the glycogen content in the liver was also observed by Melo *et al*. (2006), who varied the protein content in the diet of *Rhamdia quelen* (Pimelodidae, Quoy & Gaimard, 1824) and recorded a lower value in the treatment with a low protein level (20% CP, at a ED/CP ratio = 22.81 kcal/g). Debnath *et al*. (2007), using different protein levels (25 to 40% CP) in diets for *Labeo rohita* (Cyprinidae, Hamilton, 1822), found a higher concentration of glycogen in the liver of animals treated with 25% CP at a ED/CP ratio of 16.03 kcal /g, and the concentration was inversely proportional to the tested levels of muscle CP. The highest value was recorded at a ED/CP ratio of 10.01 kcal /g. Apparently, the fish in this experiment, which were fed a 2300 kcal DE / 230 g CP diet, reduced their metabolic activity and, due to the rapid feeling of satiety caused by the high cellulose content in the diet, reduced their foraging activity. These results in a greater accumulation of muscle glycogen, so liver glycogen reserves must be used to meet the energy needs of the fish. On the other hand, the higher liver glycogen content in other animals may be related to the energy and protein supplied in the diet, which may have provided higher levels of glucose that were converted to glycogen by the enzyme glycogen synthase, promoting a directly proportional increase in liver reserves.

The results of the blood biochemical parameters of the animals that were subjected to the experimental treatments did not differ among treatments and indicates that the tested feed did not promote changes that might affect these parameters (Table IV), so the health of all of the animals was maintained.

Treatment with 2800 kcal DE and 280 g CP per kg of feed yielded higher average daily ammonia excretion, which is compared to both the basal metabolism of the animals and the other diets studied. The diet with 3800 kcal DE and 380 g CP per kg resulted in an 82.23% increase in total ammonia excretion levels (TAN) compared to the

Table IV. Glycogen content in the muscle (MGC) and liver (LGC) and Hematological parameters (means ± standard deviation) of juvenile piavuçu fed diets with different levels of digestible energy (DE) and crude protein (CP) with a consistent DE:CP ratio of 10kcal DE/ g CP.

Observed	Treatments (kcal DE/g CP)			
Parameters	T1 (2300/230)	T2 (2800/280)	T3 (3300/330)	T4 (3800/380)
MGC (%)	1.174 ± 0.095 [a]	0.714 ± 0.079 [b]	0.752 ± 0.061 [b]	0.718 ± 0.065 [b]
LGC (%)	1.094 ± 0.161 [c]	2.343 ± 0.783 [b]	2.02 ± 0.265 [b]	3.215 ± 0.719 [a]
Hematocrit (%)	45.44 ± 4.03 [a]	43.22 ± 5.56 [a]	44.12 ± 3.01 [a]	43.89 ± 3.10 [a]
Glucose (mg/dl)	52.44 ± 8.51 [a]	60.33 ± 10.78 [a]	57.88 ± 11.92 [a]	57.89 ± 6.97 [a]
Cholesterol (mg/dl)	205.22 ± 19.70 [a]	201 ± 22.06 [a]	197.44 ± 20.57 [a]	182.11 ± 18.24 [a]
Total Proteins (g/dl)	3.32 ± 0.41 [a]	3.29 ± 0.29 [a]	3.39 ± 0.28 [a]	3.17 ± 0.24 [a]
Triglycerides (mg/dl)	199.33 ± 33.29 [a]	201.66 ± 23.66 [a]	244.66 ± 58.80 [a]	233.66 ± 38.96 [a]

Different letters in the same line indicate significant differences according to a Tukey’s test ($p < 0.05$).

Table V. Average daily excretion of ammonia (TAN. mg L^{-1}) and ratio of ammonia excretion per g of CP (TAN mg L^{-1}g CP^{-1}) by juvenile piavuçu fed diets with different levels of digestible energy (DE) and crude protein (CP) with a consistent DE:CP ratio of 10kcal ED/ g CP.

Observed parameters	Treatments (kcal DE / g CP)				
	Basal	T1 (2300/230)	T2 (2800/280)	T3 (3300/330)	T4 (3800/380)
TAN (mg L^{-1})	5.91 ± 0.65 ^{c}	8.38 ± 1.57^{b}	7.58 ± 1.31^{b}	9.94 ± 0.58^{a}	10.77 ± 1.29^{a}
TAN (mg L^{-1}) g CP^{-1}	-	11.49 ± 3.21^{a}	10.31 ± 1.99^{a}	12.68 ± 0.88^{a}	11.91 ± 1.36^{a}

Different letters in the same line indicate significant differences ($p <0.05$); the absence of letters indicates no significant differences ($p> 0.05$) according to a Tukey's test.

basal metabolism and 45.17% in relation to the diet with 2800 kcal DE and 280 g CP (Table V). The ratio of TAN (mg L^{-1}) to protein intake (g) did not differ significantly, corroborating the PER and FCR results (Table II) and demonstrating that, regardless of the total protein intake, piavuçu are able to acquire the protein for needed growth and maintain a constant total ammonia excretion to total protein intake ratio up to 380 g CP kg^{-1} feed. The relationship between the diet supplied to fish and the ammonia excretion rate has been studied in several species, and this relationship can be modulated by food composition or even the speed of flow through the gastrointestinal tract (Cheng *et al*. 2003; Tgn *et al*. 2008; Abdel-Tawab *et al*. 2010; Luo *et al*. 2012). Protein levels influenced the results of this study, and similar results were found by Abdel-Tawab *et al*. (2010), who used protein levels between 25 and 45% CP for Nile tilapia. Ruales *et al*. (2014), with protein levels between 25 and 35% CP for Pirapitinga *Piaractus brachypomus* (Serrasalmidae, Cuvier, 1818); and Silva *et al*. (2015), with protein levels between 43 and 64% CP for Pampo *Trachinotus marginatus* (Carangidae, Cuvier, 1832). Therefore, ammonia excretion increases in direct proportion to the tested protein levels. Yang *et al*. (2002) also found an increase in ammonia excretion rate with protein levels between 13 and 55% CP for Silver perch *Bidyanus bidyanus* (Terapontidae, Mitchell, 1838) and were able to establish a positive regression between the protein levels in feed and the accumulation of the total ammonia in the water. Fish fed diets with 38% CP exhibited the same rate of excretion observed using diets with 33% CP, which can be considered the highest level of feed efficiency as reflected in the PER and FCR performance parameters (Table II). On the other hand, the diet containing 23% CP resulted in an excretion rate similar to that of fish fed the 28% CP diet, which may be related to the lower concentration of protein observed in the proximate composition of the carcasses (Table III).Based on these observations, it can be concluded that the increase in the ammonia excretion rate reflects an increase in protein catabolism when protein levels were increased from 23% CP to 33% CP. However, protein catabolism likely remained constant when the content was increased to 38% CP due to the use of supplied energy (3800 kcal DE), which optimized the use of protein for performance and reduced the excretion of nitrogen compounds, as demonstrated by the results for PER, FCR (Table II) and TAN (mg L^{-1}) /g CP (Table V).

Conclusions

According to the results obtained, we conclude that, diets formulated with a fixed energy / protein ratio of 10 kcal DE / g CP at a level of 3800 kcal of DE and 380 g CP yielded better performance results in juvenile piavuçu, promoting a better use of the ingested protein for utilization In muscle tissue construction.

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Ethical disclaimer

Authors declare that general experimental procedures and the manipulation of animals during the investigations reported in this paper complied with ethical protocols and all applicable national and institutional regulations. All the procedures performed were approved by the Ethics Commission for Animal Use – CEUA, FURG (process 23116.000632 / 2015-66, pq001 / 2015).

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