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GROWTH AND BLOOD CHEMISTRY OF JUVENILE OF *Arapaima gigas* (SCHINZ, 1822) IN RELATION TO DIETARY PROTEIN CONCENTRATION

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ABSTRACT

This study evaluated the growth performance, hematological and biochemical profiles of paiche fed diets with 36, 40, 44, 48 and 52 % crude protein content (CP) and a constant energy to protein ratio (37.7 kJ g^{-1}). One hundred and fifty *Arapaima gigas* (68.75 ± 3.11 g) were randomly divided into 15 tanks and fed for 75 days. Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), daily feed intake (DFI), daily protein intake (DPI), protein efficiency ratio (PER), hepatosomatic index (HSI), visceral somatic index (VSI), chemical composition of fillet and liver and feed apparent digestibility coefficient (ADC) were evaluated. Fish blood samples were analyzed for hemoglobin [Hb], hematocrit (Ht), red blood cells, white blood cells, thrombocytes, corpuscular index, glucose (GLY), total plasma protein (TPP), cholesterol (CHOL) and triglycerides (TRY). Feed protein and energy ADCs were the highest at 36, 40 and 44 % CP and 40, 44 and 48 % CP, respectively. Best FCR, WG and SGR were observed for fish fed 44 and 48 % CP. In turn, fish fed 48 and 52 % CP had the highest HSI, VSI and liver lipids. Fish fed 36 and 40 % CP presented the highest DFI, while DPI was lower in fish fed 44 %. The highest PER values occurred at 44 and 48 % CP. Dietary protein levels had no influence on tissue protein and

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lipid concentrations. The highest values of Ht and Hb, CHOL, TPP and TRY were observed at 48 and 52 % CP. Best performance and health of juvenile *A. gigas* were achieved with 45.8 % CP.

KEYWORDS: Digestibility, fish farming, nutrition, physiology.

CRECIMIENTO Y QUÍMICA SANGUÍNEA DE JUVENILES DE *Arapaima gigas* (SCHINZ, 1822) EN RELACIÓN CON LA CONCENTRACIÓN DE PROTEÍNA DIETARIA

RESUMEN

Se evaluó el crecimiento así como los perfiles hematológicos y bioquímicos de paiches alimentados con dietas conteniendo 36, 40, 44, 48 y 52 % de proteína bruta (PB) y una proporción constante de energía proteína (37,7 kJ g-1). Ciento cincuenta paiches *Arapaima gigas* (68,75 ± 3,11 g) se dividieron al azar en 15 tanques y se alimentaron durante 75 días. Se evaluó la ganancia de peso (WG), índice de conversión alimenticia (FCR), tasa de crecimiento específico (SGR), ingesta diaria de alimento (DFI), ingesta diaria de proteínas (DPI), índice de eficiencia proteica (PER), índice hepatosomático (HSI), índice somático visceral (VSI), así como la composición química del filete e hígado y el coeficiente de digestibilidad aparente (ADC) del alimento. Se analizaron muestras de sangre para evaluar los niveles de hemoglobina [Hb], hematocrito (Ht), glóbulos rojos, glóbulos blancos, trombocitos, índice corpuscular, glucosa (GLY), proteína plasmática total (TPP), colesterol (CHOL) y triglicéridos (TRY). Los ADC de proteína y energía del alimento fueron los más altos con las dietas con 36, 40 y 44 % de PB y 40, 44 y 48 % de PB, respectivamente. Se observaron mejores FCR, WG y SGR para peces alimentados con 44 y 48 % de PB. A su vez, los peces alimentados con 48 y 52 % de PB tuvieron los niveles más altos de HSI, VSI y lípidos hepáticos. Los peces alimentados con 36 y 40 % de PB presentaron el mayor DFI, mientras que el DPI fue menor en los peces alimentados con 44 %. Los valores de PER más altos se produjeron a 44 y 48 % de PB. Los niveles de proteína dietaria no influyeron en las concentraciones de lípidos y proteínas de los tejidos. Los valores más altos de Ht y Hb, CHOL, TPP y TRY se observaron a 48 y 52 % de PB. El mejor desempeño y estado sanitario de los juveniles de paiche se logró con 45,8 % de PB.

PALABRAS CLAVE: Digestibilidad, piscicultura, nutrición, fisiología.

INTRODUCTION

The species *Arapaima gigas* (SCHINZ, 1822), also known as paiche in Perú and pirarucu in Brazil, is among the most representative and emblematic freshwater fish in South America (Castello *et al*., 2011; Chu-Koo *et al*., 2012). This species is a fast-growing (up to 10 $kg \cdot yr^{-1}$), air-breathing, and has high market value and a great potential for tropical aquaculture in Brazil, Perú and worldwide (Ono *et al*., 2008). The production of this species is also of environmental interest because its natural stocks have been overexploited; thus, increasing market supply through farm production may alleviate the pressure on natural populations.

As a potential aquaculture species, the lack of knowledge regarding its nutritional requirements is evident, and similar to other carnivorous fishes under intensive culture (Kim & Lee, 2009; Deng *et al*., 2011), *A. gigas* requires feeds with high protein concentrations (>40 %), which are costly and represent a limiting factor for their large-scale production (Ono *et al*., 2008; Minjarez-Osorio *et al*., 2012). Several studies have attempted to determine the appropriate level of protein for *A. gigas*. However, most of them were only based on body growth and body composition (Ituassú *et al*., 2005; Del Risco *et al.*, 2008; Lopez, 2017), resulting in crude protein level diets between 40 and 50 % and an energy:protein (CE:CP) ratio of 48.5 $kJ \cdot g^{-1}$ (Ituassú *et al*., 2005) for the best growth of this species. Despite the differences in these results, which are probably due to variations in the experimental conditions, the consensus is that the dietary protein level has a great influence on the growth performance of fishes. However, there were few authors who assessed *A. gigas* health conditions using blood parameters, an assessment that can completely change the outcome of a nutritional study based on growth performance (Oliva-Teles, 2012; Pohlenz *et al*., 2014; Gonzales *et al*., 2016; Sanchez *et al*., 2017). Furthermore, it is important to determine digestibility values because that information can contribute to the creation of more accurate diets that maximize protein synthesis and reduce nitrogen excretion into the aquatic environment (Jobling, 2001).

Numerous studies have noted the importance of evaluating fish blood parameters while assessing nutritional requirements to better understand the role of the nutrients in animal health. The physiological responses in studies to determine the protein requirements of species such as the pacu *Piaractus mesopotamicus* (Abimorad *et al*., 2007), *Rhamdia quelen* (Lazzari *et al*., 2011) and rainbow trout *Oncorhynchus mykiss* (Ahmed & Ahmad, 2020), have indicated that variations in dietary protein level promote alterations in metabolism that are reflected in fish blood parameters.

Arapaima gigas was introduced to intensive culture systems after the development of weaning techniques in the last decade and after determining its nutritional requirement, which had become of great importance for improving its performance and health (Velasquez *et al*., 2007; Del Risco *et al*., 2008; Magalhães-Junior *et al*., 2017). In this context, the aim of this study was to evaluate the growth performance and physiological responses of juvenile *A. gigas* fed diets with increasing levels of crude protein.

Material and methods

Experimental diets

Five practical diets were prepared in individual batches and formulated to contain increasing levels (36, 40, 44, 48 and 52 %) of crude protein (CP) with an energy:protein ratio of 37.7 $kJ \cdot g^{-1}$, according to Ono *et al*. (2008) and using conventional feedstuffs (Table 1). Considering that there is no information regarding the energy requirement for *A. gigas*, uniform energy to protein ratio of 37.7 kJ·g^{-1} was applied to all diets, following the results of Ono *et al*. (2008), which was an indicator of the most adequate balance for this species with the aim of using spare protein as an energy source. Thus, in maintaining a constant dietary energy to protein ratio, the objective was to evaluate the capacity of the animal to use high concentrations of protein with no limitation to non-protein energy sources. This effect has been described in the literature for other carnivorous fish species (Santinha *et al*., 2001; Skalli *et al.*, 2004).

Fish meal, meat and bone meal and soybean meal were used as major protein sources. Wheat flour was the primary carbohydrate source and

Table 1: Formulation and proximate composition of the experimental diets.

Ingredients (%)	Dietary protein (%)				
	36	40	44	48	52
Fish meal[a]	28.5	34.7	43.0	56.5	64.6
Meat meal[b]	15.0	14.0	11.0	-	-
Soybean meal[b]	16.0	14.5	12.0	9.0	5.0
Wheat flour[c]	28.0	26.5	25.4	23.7	18.0
Cellulose[d]	6.5	3.0	-	-	-
Animal fat[e]	5.2	6.5	7.8	10.0	11.6
Vitamin and mineral Premix[f]	0.8	0.8	0.8	0.8	0.8
Proximate composition (%)					
Dry matter	92.9	93.3	94.1	93.9	93.2
Crude protein	36.7	40.0	44.9	49.6	52.3
Crude fat	12.2	13.6	15.1	17.8	19.1
Ash	10.3	10.2	9.1	4.8	4.7
Nitrogen free extract (NFE)[1]	33.7	29.5	25.0	21.7	17.1
Gross energy (kJ.g^{-1})[2]	19.38	20.01	21.00	22.63	23.00
Digestible energy (kJ.g^{-1})	13.59	14.69	15.79	16.96	17.93

[a] Fish meal, Brazil. Chemical composition: 77.6% crude protein, 17.4% lipid.
[b] Agrominas, Brazil.
[c] Ocrim S.A., Manaus, Brazil.
[d] Rhoster Industria e Comercio Ltda, Sao Paulo, Brazil.
[e] Bonna Vitta, Marker Macro. Manaus, Brazil.
[f] Microminerals and vitamins premix (mg.kg^{-1} of mixture): manganese (26); zinc (140); iron (100); copper (14); cobalt (0.2); iodo (0.6); selenium (0.6), Vit. A (10,000 UI); Vit D3 (4,000 UI); Vit E (100); Vit K (5); Vit B1 (25); Vit B2 (25); Vit B6 (25); Vit B12 (30); niacin (100); folic acid (5); pantothenic acid (50); biotin (0.8); choline (2,000); inositol (50); Vit C (350).
[1] Nitrogen free extract= 100 – (%moisture + %protein + %lipid + %ash).
[2] Gross energy: values of protein, fat and carbohydrate (23.8; 39.7 e 17.1 kJ.g^{-1}), (NRC, 1993).

feed binder. Cellulose was used to balance the crude energy levels in the diets with 36 and 39 % CP, and animal fat was used as a lipid source. All ingredients were finely grounded, mixed and pelleted using a mincer (CAF Model 22-S, Rio Claro, SP, Brazil) at a 3 mm diameter. For feed digestibility evaluations, 0.5 % chromium oxide (Cr_2O_3) was added as an inert marker to separate the batches of the experimental diets (Ng & Wilson, 1997). Pellets were dried at 40°C for 24 h and stored at -20°C until used. The chemical compositions of the diets used are presented in Table 1 and were determined according to AOAC (1997). The digestible energy of the diets was calculated based on the digestible energy of ingredients according to NRC (1993) and Sullivan & Reigh (1995). Determining chromium oxide concentration in the feed and feces was carried by using the colorimetric method described by Jobling (2001).

EXPERIMENTAL DESIGN

This study was conducted at the Laboratory of Physiology Applied to Fish Farming (LAFAP), Department of Technology and Innovation (COTI), National Institute of Amazonian Research (INPA), Manaus-AM. This work was undertaken with approval from the Committee for Ethics in Research in the Use of Animals at the National Amazonia Research Institute, INPA, CEUA (Process number 007/2013). One hundred and fifty juveniles of *A. gigas* with a mean weight of 68.8 ± 3.1 g and a total length of 20.7 ± 0.4 cm were obtained from a local commercial fish farm. Following a randomized design, fish were randomly distributed to fifteen (five treatments in triplicate) 200-L conical fiberglass tanks at a density of 10 fish per experimental unit and fed four times a day (07:30, 10:30, 13:30 and 16:30 h) to apparent satiation for a period of 75 days for growth evaluations. The experimental tanks were adapted for fecal collection using a modified Guelph system according to Cho (1990). Fish were fed four times daily as previously described, and feces were collected for 20 days to meet a sufficient sample for analysis. The feces were collected 30 minutes after each feeding and stored frozen until analysis.

EVALUATION OF FEED NUTRIENT DIGESTIBILITY

The apparent digestibility coefficient (ADC) of nutrients was determined by using the method described by Jobling (2001): $A = 100 - 100[(X_A / X_B) \times (Y_B / Y_A)]$, where A = apparent digestibility coefficient; X_A = concentration of inert marker in the feed; X_B = coefficient of inert marker in the feces; Y_A = concentration of nutrient in the feed; and Y_B = concentration of nutrient in the feces. Total feed digestibility was calculated by using the following equation: ADC = 100 – 100 x (% inert marker in the feed / % inert marker in the feces). Digestible energy (DE) of the experimental diets was calculated as DE = feed gross energy – ((energy in the feces x % inert marker in the feed) / % inert marker in the feces).

GROWTH PERFORMANCE AND FISH BODY COMPOSITION

Arapaima gigas juveniles were weighed and measured every 15 days for growth evaluations. The following parameters were calculated: weight gain (WG): final mean weight (g) – initial weight (g); feed conversion ratio (FCR): feed consumed (g) / increase in biomass; specific growth rate (SGR) = 100 x (ln final body weight (g) – ln initial body weight (g)) / number of days; daily feed intake (DFI) = feed intake per fish (g) / days; daily protein intake (DPI) = 100 x (feed intake x crude protein) / number of days; condition factor (CF) = body weight (g) / total body length $(cm)^3$ x 100; protein efficiency ratio (PER) = weight gain (g) / protein consumed; hepatosomatic index (HSI %) = liver weight /

fish body weight x 100; visceral somatic index (VSI %) = weight of visceral / fish body weight x 100. At the end of the experimental period, three fish per unit were euthanized for fish fillet and liver proximate analysis. Samples were frozen in plastic bags and subsequently lyophilized for analysis of dry matter, crude protein, lipids and ash according to the A.O.A.C (1997).

HEMATOLOGICAL AND BIOCHEMICAL PROFILES

Blood samples were taken both at the beginning (basal; n = 10) and the end of the study (n = 6, experimental unit). Blood samples were taken by puncture of the caudal vein using syringes with 10 % anticoagulant EDTA (ethylenediaminetetraacetic acid). These samples were used to determine hemoglobin concentration ([Hb]) by using the cyanmethemoglobin method and hematocrit percentage (Ht) by using a microhematocrit. Red blood cell count (RBC) was determined in a solution by Natt and Herrck (1952) using a hemocytometer, and white blood cell count (WBC) and thrombocytes (THR) were performed using the May Grünwald-Giemsa Wright (MGGW) fast staining method. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined. Glucose concentration (GLY) was determined by the enzymatic colorimetric method without deproteinization (GOD-PAP), total plasma protein (TPP) was determined by the Biuret method, and cholesterol (CHOL) and triglycerides (TRY) were determined by the enzymatic colorimetric method with lipid clearing factors (LCF).

WATER QUALITY

Water temperature and dissolved oxygen were measured daily with a oxymeter (Yellow Springs Instruments Model 85, Yellow Springs, OH, USA), and pH was measured with a pH meter (Yellow Springs Instruments, Model 60 digital, Yellow Springs, OH, USA). The ammonia and nitrite concentrations were determined once a week using the colorimetric method of Verdouw *et al*. (1978) and Boyd & Tucker (1992), respectively. All water quality variables analyzed in the present study did not differ among treatments during the experiment and were within the limits described by Oliveira *et al.* (2012) as follows: 28.2 ± 0.15°C temperature; 4.8 ± 0.12 mg·L^{-1} of dissolved oxygen; pH 4.9 ± 0.2; 0.5 ± 0.12 mg·L^{-1} of total ammonia; and 0.12 ± 0.01 mg·L^{-1} of nitrite.

STATISTICAL ANALYSIS

The results of growth performance, fish body composition and hematological and biochemical profiles were evaluated by analysis of variance (one-way ANOVA) at 5 % probability. When data were significantly different, means were compared using Tukey's test (5 % probability). All percentage data were arcsine transformed prior to analysis. A polynomial regression analysis was used to establish relationships between protein level in the experimental diets with ADCs of nutrients and energy, weight gain, protein efficiency and fish body composition in order to estimate the optimal level of dietary crude protein for *A. gigas*. All data were presented as the means ± SD (standard deviation) of three replicates. Analyses were conducted using PASW 18 software (SPSS, Inc. version 18.0, Quarry Bay, Hong Kong).

RESULTS AND DISCUSSION

FEED NUTRIENT AND ENERGY DIGESTIBILITY

The apparent digestibility coefficients of nutrients and energy are presented in Table 2. Fish fed diets with 36.% CP presented lower ADC for dry matter among the experimental diets, probably influenced by low ADC of NNE. Similar results (ADC of DM at 59.3 to 68.3 %) were observed by

Table 2: Apparent digestibility coefficient (ADC) of nutrients and energy of feeds containing increasing levels of protein for juvenile *Arapaima gigas*.

Crude protein (%)	[1]Apparent digestibility coefficients (ADCs) of nutrients and energy of feeds (%)					
	DM	EE	CP	NNE	[2]GE	[2]GE
36	66.45±0.51[a]	95.14±1.19[a]	84.07±0.42[a]	40.75±1.08[a]	73.92±0.46[a]	65.79±0.84[a]
40	73.14±1.72[b]	92.04±0.36[ab]	85.48±1.87[ab]	51.42±1.37[b]	78.96±1.60[b]	72.41±1.69[b]
44	80.25±0.91[c]	95.12±1.48[a]	87.99±1.12[b]	61.68±0.45[c]	84.66±1.08[c]	79.29±1.05[c]
48	75.02±0.42[b]	90.55±1.01[b]	82.72±1.04[a]	48.97±1.60[b]	79.61±0.58[b]	75.75±0.39[d]
52	77.71±0.27[c]	81.74±1.16[c]	81.73±0.02[a]	63.92±1.64[c]	79.46±0.18[b]	78.10±0.09[cd]
P-value	< 0.001	< 0.001	0.0013	< 0.001	< 0.001	< 0.001

Table 3: Mean value ± SD of the weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), daily feed intake (DFI) and daily protein intake (DPI) of juvenile *Arapaima gigas* fed increasing levels of crude protein (CP).

Growth Parameters	CP (%)				
	36	40	44	48	52
WG (g)	82.94±16.73[a]	109.64±4.93[ab]	167.62±13.92[c]	141.93±17.96[bc]	115.68±21.38[ab]
FCR	3.80±0.68[b]	2.84±0.16[ab]	1.85±0.14[a]	2.23±0.30[a]	2.58±0.47[a]
SGR ($\%.day^{-1}$)	1.19±0.18[a]	1.41±0.09[ab]	1.77±0.04[c]	1.63±0.08[bc]	1.37±0.17[ab]
DFI (%)	2.79±0.25[c]	2.35±0.10[bc]	1.74±0.11[a]	2.0±0.22[ab]	2.08±0.25[ab]
DPI (%)	1.03±0.09[ab]	0.94±0.04[ab]	0.78±0.05[a]	0.99±0.11[ab]	1.09±0.13[b]
PER (%)	2.26±0.46[a]	2.74±0.12[a]	3.73±0.31[b]	2.86±0.36[ab]	2.21±0.41[a]
VSI (%)	0.83±0.03[a]	1.18±0.23[a]	1.63±0.58[ab]	2.57±0.36[b]	2.43±0.39[b]
HIS (%)	1.9±0.07[a]	1.9±0.04[a]	1.9±0.2[a]	2.5±0.2[b]	2.4±0.2[ab]

Ono *et al*. (2008), who evaluated the digestibility of feeds with different energy to protein ratios for *A. gigas*.

For the lipid fraction, increasing levels of dietary protein influence the ADC of EE, which presented the lowest ADC among the experimental diets. The energy level of diets could affect the digestibility of nutrients in fish when there is an excess of lipids with a high degree of saturation of fatty acids (Wang *et al*.; 2016). In this study, animal fat was used as the source of lipids, which could have influenced the ADC of EE in higher protein diets (52 %) containing the highest levels of lipids (19.1 %). The ADC value of crude protein for the diet containing 44 % CP was significantly higher than those of the other diets, except for the 40 % CP diet, while the highest ADC of GE was observed in fish fed 44 % CP. Similar values (80 to 85 %) were observed by Rios (2010), who evaluated the protein digestibility of diets with 35, 40, 45 and 55 % CP for juvenile *A. gigas.* This author concluded that the best ADC of CP was observed for the diet with 45 % CP, corroborating the results of the present study. The highest ADC values of gross energy (GE) and total digestibility (TD) were observed for fish fed a diet with 44 % CP (Table 2). Rodrigues *et al*. (2019) found better digestibility with soybean than with animal by-products, demonstrated that the nutritional quality of the animal by-products as feedstuffs for *A. gigas*, could be improved, especially by enhancing the processing methods.

GROWTH PERFORMANCE AND FISH BODY COMPOSITION

Fish growth performance, weight gain (WG) and the specific growth rate (SGR) of *A. gigas* juveniles fed diets containing 36, 40 and 52 % CP were significantly lower compared to those fed diets containing 44, except with 48 % CP (Table 3). A polynomial regression analysis showed a quadratic effect between CP and WG, indicating, according to break-point, that optimum growth was obtained with 45.8 % CP in the diet. The uniform energy to protein ratio of 37.7 $kJ \cdot g^{-1}$ was maintained in the experimental diets according to Ono *et al*. (2008) in order to ensure that fish growth was not influenced by an excess or lack of energy in the diets.

These results are corroborated with the highest values of daily protein intake (DPI) of fish fed 36, 40 and 48 % CP diets, while fish fed 44 % CP presented lower DPI, but not significant with the rest of the diets, except with 52 %. Fish fed 44 % CP presented better WG, FCR and PER (Table 3). Similar results were obtained by Del Risco *et al*. (2008), who determined that the level of protein suitable for A. *gigas* was 45 % in extruded diets, based only on the best growth performance. Moreover, Ituassú *et al*. (2005) determined that the level of crude protein for the proper performance of this species was above 48.6 %, but the authors concluded that the protein requirement for juvenile *A. gigas* could not be determined because the leveling of weight gain, which is a typical situation when nutritional requirements are met, did not occur. However, Magalhães-Junior *et al*. (2017) determinated the optimal dietary protein content for juvenile *A. gigas* is about 36 % but of digestible protein on diet based on zootechnical performance.

The high dietary protein concentration used in this study was also observed for other carnivorous species, such as the sixfinger threadfin *Polydactylus sexfilis* at 41 % CP (Deng *et al*., 2011), golden dorado *Salminus brasiliensis* from 45.08 to 57.63 % CP (Teixeira *et al*., 2010), juvenile rockfish *Sebastes schlegelii* at 50 % CP for growth and 45 % CP for feed utilization (Cho *et al*., 2015) and for the omnivorous species, the larvae of *Anabarilius graham*, from 45 to 55.5 % CP (Deng *et al*., 2013).

In general, fish have a better ability to use protein as an energy source than terrestrial animals (NRC, 1993), especially carnivorous species. This group of fish presents a limited ability to use carbohydrates, therefore requiring lipids and protein to meet energy demand. In contrast, lower levels of dietary protein are usually seen in omnivores, which can use lipids and carbohydrates as an energy source (Lupatsch, 2007). These species include *Piaractus mesopotamicus*, 26 % CP (Klein *et al*., 2014) and *Oreochromis niloticus,* 30 % CP (Gutierrez *et al.*, 2013).

In this study, the daily feed intake (DFI) in fish fed diets with protein levels between 36 and 40 % was significantly higher ($p < 0.05$) than the others, with except 48 and 52 % with 40 %, which resulted in the worst FCR for these treatments (Table 3). When higher amounts of feed were consumed to meet protein requirements, the FCR was worse, reducing the utilization of the supplied feed. The FCR is considered one of the most important parameters for evaluating fish performance because of its great impact on production cost and the water effluents that may reach the environment (Asche *et al*., 2008).

The amount of crude protein in the diet that has been converted to weight can be measured by the protein efficiency ratio, PER. Generally, PER reaches its maximum value when fish protein requirements are met (Lee *et al*., 2006). In the present study, the PER value of the diet with 44 % CP was significantly higher than those with 36, 40 and 52 % CP ($p < 0.05$), showing a quadratic effect with protein levels ($r^2 = 0.89$). A negative linear relationship was observed with other species, indicating that higher levels of protein lowered the PER (Li *et al*., 2010; Deng *et al*., 2011).

Furthermore, fish fed diets containing 44 % crude protein consumed less protein than those fed with 52 %, but it did not affect weight gain. Similar results were recorded by Abimorad *et al*. (2007), who evaluated the energy to protein ratio for *Piaractus mesopotamicus*. These authors concluded that the lower protein intake and the better performance of fish were due to the saving effect of protein by lipids, resulting from an adequate energy:protein ratio in the diet. However, in the present study, fish fed 52% CP in the diet showed low WG and PER despite the increase in protein intake, probably because of the usage of this nutrient for the formation of non-protein compounds (lipids and carbohydrates) by excessive protein intake and the lack of non-protein energy sources.

In the current study, experimental diets containing different protein levels did not influence the fish condition factor ($p > 0.05$), which is an important parameter in determining fish welfare (Rocha *et al*., 2005) and which is directly related to the nutritional intake (Ratz & Lloret, 2003). Thus, the levels of crude protein were apparently adequate to ensure normal fish conditions.

In contrast, in addition to lower WG, the high-protein diets may have caused higher VSI and HSI in fish fed diets containing 48 and 52 % CP, respectively. VSI and HSI presented positive correlations with increasing levels of protein diets (r^2 = 0.95 and r^2 = 0.71, respectively), which also confirmed a higher energy intake by fish fed diets with higher levels of protein. A similar relation was observed for the lipid levels in the liver (r^2 = 0.96), where higher levels of dietary protein increased fat accumulation and reduced liver moisture (Table 4), resulting in increasing metabolism disorder by HSI (Portz & Furuya, 2013). Since the liver is one of the major organs involved in protein synthesis and is targeted mainly for the renewal of body protein in fish (Carter *et al*., 2001), excessive

Table 4: Mean ± SD of liver moisture, crude protein and lipid of juvenile *Arapaima gigas* fed increasing levels of crude protein (CP).

CP (%)	Proximate composition (%)		
	Moisture	Crude protein	Lipids
36	68.07±1.18^{a}	10.38±0.60	8.94±0.78^{a}
40	66.17±3.13^{a}	10.36±0.79	10.77±3.54^{a}
44	65.17±1.86^{a}	10.73±0.71	13.66±5.39^{a}
48	58.30±2.48^{b}	11.40±4.18	19.88±2.78^{b}
52	62.37±8.21^{ab}	9.21±2.71	19.54±1.00^{b}

Values followed by different letters in the same column differ significantly by Tukey test (p<0.05).

dietary protein can increase energy production or non-protein nutrient formation, such as lipids and carbohydrates, while accumulating fat and inhibiting protein production in the liver (Jobling, 2001; Ullah-Khan *et al*.; 2019). In the present study, no significant differences were found among liver protein concentrations related to dietary protein levels ($p > 0.05$).

The higher lipid accumulation in the livers of fish fed the experimental diets containing higher levels of protein may have been caused by the lack of ability of fish livers to synthesize the protein part of the lipoproteins, which are necessary to carry fat from the liver (Xue *et al*., 2017). This metabolic condition represents a danger to the animal and probably compromised the growth of fish fed diets containing 48 and 52 % CP in the present study. According to Portz & Furuya (2013), the balance between the amount of protein and energy during diet formulation is very important because in addition to its effect on animal growth and metabolism, it can compromise meat quality. NRC (1993) and Lovell (1995) recommended dietary energy levels between 12.5 and 15.0 $kJ \cdot kg^{-1}$ DE for major cultivated species. Results of this study concur with this recommendation, considering that the diets containing DE levels between 13.6 and 15.8 resulted in the best *A. gigas* performance and metabolic conditions.

Protein levels of the experimental diets did not influence the composition of the *A. gigas* fillet ($p > 0.05$). Similar results were obtained for other species such as dourado fry *Salminus brasiliensis* (Teixeira *et al*., 2010), pacu *Piaractus mesopotamicus* (Klein *et al*., 2014) and tilapia *Oreochromis niloticus* (De Silva *et al*., 2016) fed different levels of protein and energy. Oliveira (2007) obtained similar values for lipid contents in *A. gigas* fillets (0.62 % on the dorsal and 2.49 % on the ventral portion), suggesting that this species presents significant differences in lipid composition between these areas of the fillet. The composition of the foods largely determines the fat content of fish, and the higher the dietary energy to protein ratio is, the higher the fat content in the tissues is (Portz & Furuya, 2013). The results of this study corroborate this theory because there was low accumulation of fat in the fish fillet, which was guided by the same DE:CP ratio in the diet and which did not influence the composition of the fish fillet.

HEMATOLOGICAL AND BIOCHEMICAL PROFILES

The evaluation of the physiological parameters indicated that levels of crude protein significantly influenced fish physiology after 75 days of feeding ($p < 0.05$). The high value of [Hb] was obtained in the treatment with 48 % CP and high value of Ht in the treatments with 48 and 52 % CP (Figures 1 and 2). For the rest of the blood parameters (RBC, WBC, THR, MCV and MCHC), no significant differences between the treatments were observed in the end of the experiment. However, there was an increase in RBC and decrease in MCV with respect to their basal values (Table 5).

Results suggest that the experimental conditions and the highest protein diets (48 and 52 %) may have induced stress responses, as demonstrated by the increase in Ht and [Hb] values, and required an increase in oxygen-carrying capacity to the tissues (Richards, 2009). Hematological parameters have long been used as important tools for the diagnosis of diseases in animals and are commonly determined as a part of studies evaluating health status (Lim *et al*., 2015). Thus, feeding levels can influence fish nutritional status and affect the levels of hemoglobin and red blood cells under stress conditions (Pohlenz *et al*., 2014). Alterations in the Ht value may indicate hemoconcentration or hemodilution caused by osmoregulatory disorders, which are primarily related to the

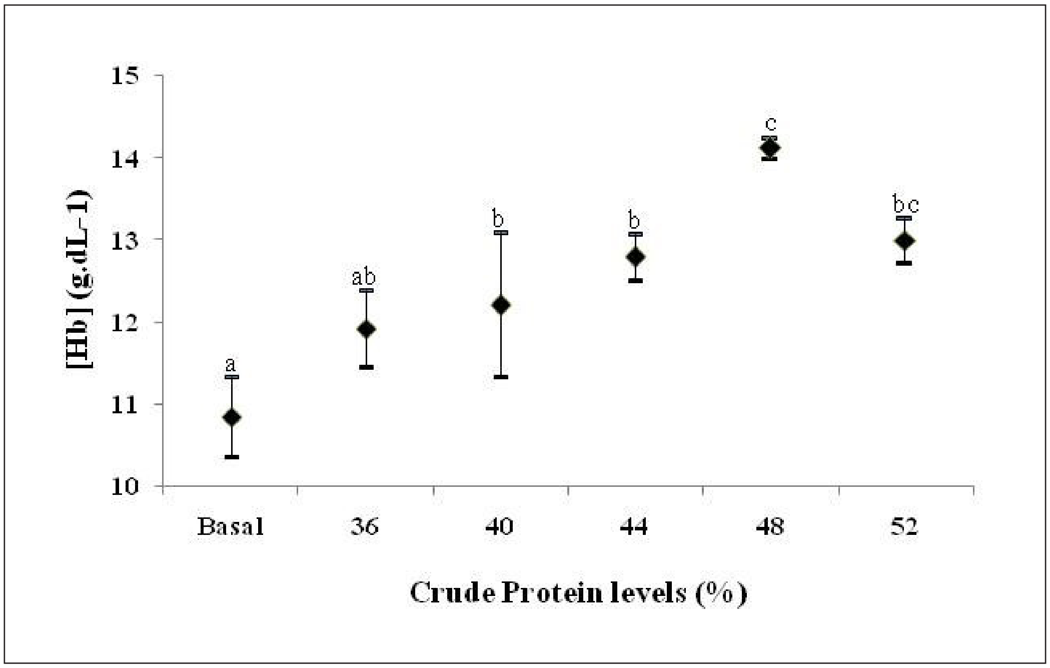

Figure 1. Hemoglobin concentration [Hb] of juvenile *Arapaima gigas* fed different levels of crude protein in the diet (Basal, n = 10).

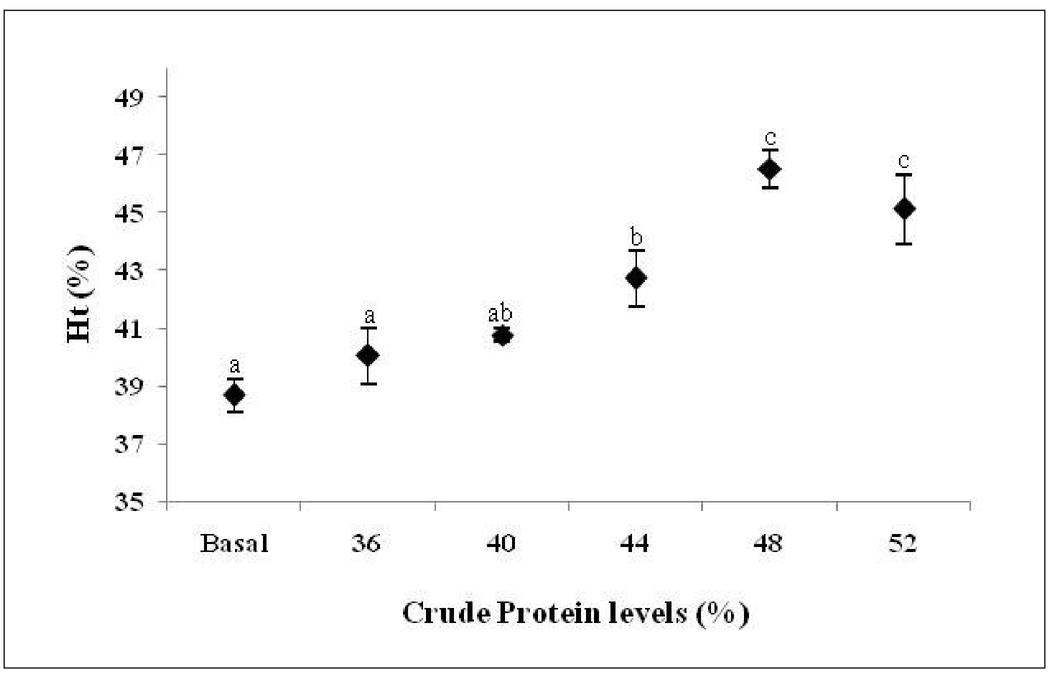

Figure 2. Percentage of hematocrit (Ht) of juvenile *Arapaima gigas* fed different levels of crude protein in the diet (Basal, n = 10).

Table 5: Mean and standard deviation values (±) the red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), thrombocytes (THR), white blood cells (WBC) and glucose (GLY) of juvenile *Arapaima gigas* fed increasing levels of crude protein (CP).

CP (%)	Hematological parameters					
	RBC ($x10^6.\mu L^{-1}$)	MCV (fL)	CHCM ($g.dL^{-1}$)	THR (%)	WBC (%)	GLY ($mg.dL^{-1}$)
Basal	1.42±0.16	274.09±27.60^a	28.02±1.12	8.0±2.0	12.2±1.26^a	29.87±4.46^a
36	2.04±0.30	199.13±24.15^b	29.76±1.16	10.5±1.80	30.3±4.04^{ab}	84.43±11.65^b
40	2.24±0.14	182.70±10.28^b	29.93±1.97	12.5±4.09	35.0±9.54^b	99.64±03.73^b
44	2.23±0.42	181.39±13.10^b	29.91±0.30	10.3±2.02	31.0±2.18^{ab}	96.12±14.34^b
48	2.36±0.31	198.89±24.48^b	30.34±0.18	11.1±2.36	35.5±10.85^b	91.98±03.93^b
52	2.44±0.19	185.69±09.87^b	28.80±0.75	10.8±2.02	33.8±12.69^b	81.56±03.99^b

*Mean ± SD of three replicates. Different letters between the means differentiate the treatments by the Tukey test ($p < 0.05$).

capacity of red blood cells to transport oxygen (Clauss *et al*., 2008).

Differences in the basal and 75 day measurements of the experiment showed that the hematological parameters are influenced by ontogenetic differences in A. *gigas*. According to Hrubec *et al*. (2001), it is difficult to demonstrate the relation between fish growth and hematological parameters, and there is little information regarding this subject for various species. However, Drummond *et al.* (2010) observed that juvenile *A. gigas* (25.9 ± 2.7 g) showed lower values of MCV and higher values of MCHC and RBC compared with fish of greater weight (2350.0 ± 757.1 g). Similarly, in the present study, higher values of RBC and lower values of MCV were found at the beginning (basal) compared with the fish at the end of the experiment or with greater weight. Changes in blood parameters are expected due to the growth of fish hematopoietic tissues (kidney and spleen) (Hrubec *et al.*, 2001). However, more detailed studies on the relation between growth and hematological variables are required.

In contrast, plasma glucose concentrations (GLY) in *A. gigas* fed different levels of protein were significantly higher compared to those of their basal values, but they were not different among treatments after 75 days ($p > 0.05$). Blood glucose can vary depending on fish physiological status (Andrade *et al*., 2007), and hyperglycemia is a characteristic response to chronic stressors (Clauss *et al.*, 2008). Additionally, it is expected that the level of blood glucose decreases with increasing protein concentration and reductions in the dietary carbohydrates. However, it is possible that *A. gigas*, similar to *Salmo salar* (Betancor *et al*., 2018), has the ability to control the degradation and storage of glucose in response to high protein intake.

In this study, the basal concentration of total plasma protein (TPP) of *A. gigas* was significantly lower compared to the values obtained after 75 days of being fed with increasing levels of crude protein ($p < 0.05$). Fish fed diets with 48 and 52 % CP showed the highest TPP, cholesterol and triglycerides values compared to those fed 36, 40 and 44 % CP diets ($p < 0.05$, Figures 3, 4

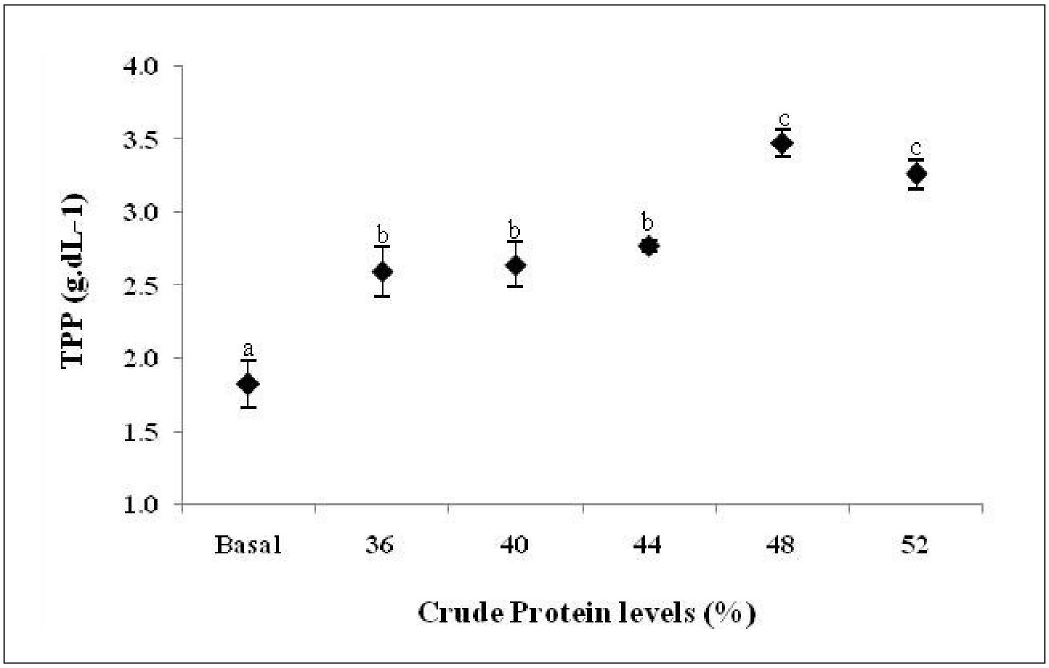

Figure 3. Total plasma protein (TPP) level of juvenile *Arapaima gigas* fish fed with different levels of crude protein (Basal, n = 10).

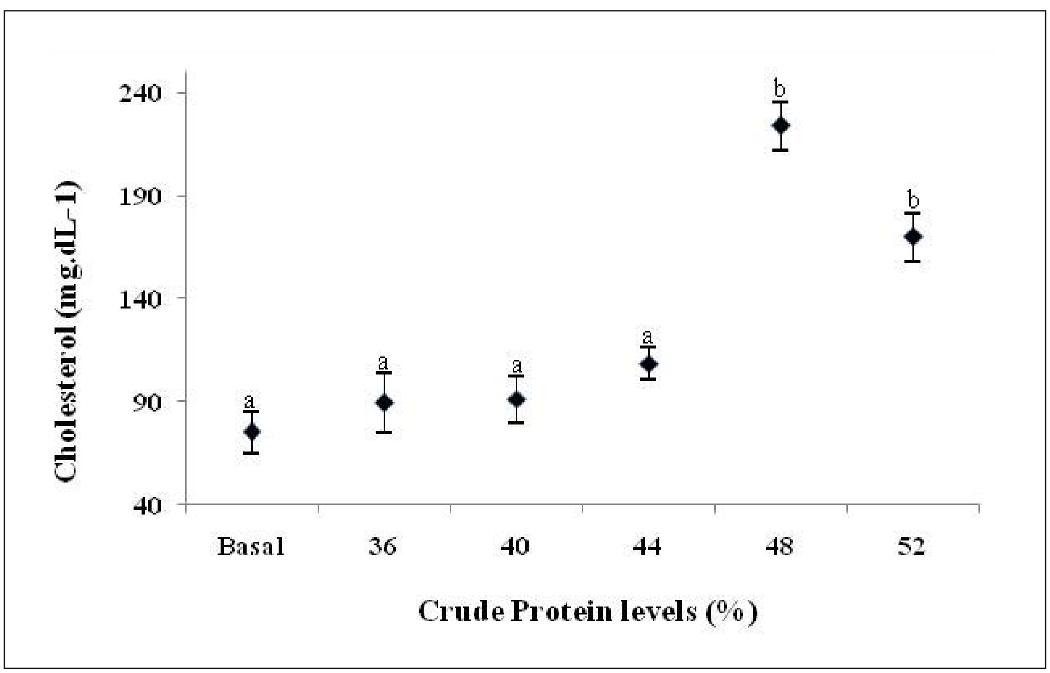

Figure 4: Plasma cholesterol of juvenile *Arapaima gigas* fed with diets containing increasing levels of crude protein (different letters, p<0.05).

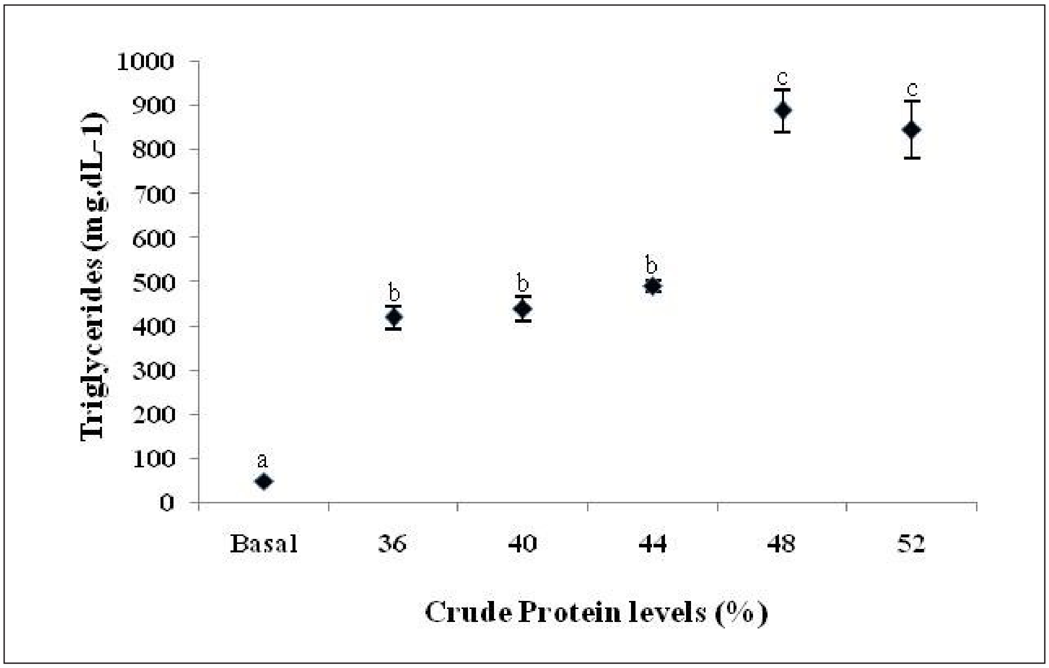

Figure 5. Total plasma protein (TPP) level of juvenile *Arapaima gigas* fish fed with different levels of crude protein (Basal, n = 10).

and 5, respectively). The high values found are consistent with the elevated levels of protein in the diet, indicating that at 48 and 52 % CP, this nutrient was used as an energy source. The lower fish weight gain, elevated concentration of plasma protein, cholesterol and triglycerides and significantly higher liver lipid concentration (Table 4) and VSI corroborate these findings.

According to Tocher & Glencross (2015), excess dietary protein is not accumulated in the muscle nor results in improved animal health and is catabolized. Nitrogen is excreted, and the carbon skeleton of amino acids is transformed into lipids and carbohydrates that are deposited in the tissues of the animal. Like any other nutrient, excess protein in the diet can cause metabolic disorders such as a reduction in the growth rate, liver dysfunction, and an increase in tissue and blood concentrations of ammonia, which leads to poisoning. In addition, carbon skeletons are also used for the synthesis of a number of other compounds such as triglycerides, ketones, and steroids or can be converted into amino acids (Jobling, 2001). Kim & Lee (2009) reported high cholesterol levels in juvenile *Takifugu rubripes* with lower dietary protein levels and mentioned that these values were possibly influenced by non-protein components of the diet. Dias *et al.* (2005) evaluated various sources of protein and their effect on lipid metabolism in the sea bass *Dicentrarchus labrax* and reported lower levels of triglycerides and cholesterol in fish fed diets rich in soy proteins than those fed a fishmeal-based diet, suggesting that the protein sources in the diet can affect fat deposition.

Although growth parameters can provide information on fish performance, the assessment of their physiological state is more of a tool that can contribute to this research by providing important information on the health of farmed animals (Oliva-Teles, 2012). Thus, protein levels below or above what is required by the body, or proteins of poor quality, can result in poor performance of farmed fish and interfere with physiological conditions and result in stress responses that can be evaluated by blood parameters (Pohlenz *et al*., 2014).

In conclusion, the rising levels of crude protein of diets directly influenced *A. gigas* growth and physiological conditions, mainly raising the levels of Ht, [Hb], triglycerides and cholesterol. Thus, the results of the study suggest that the best growth performance and maintenance of physiological homeostasis for *A. gigas* with an initial mean weight of 70 grams is achieved by feeding them a diet with a protein level of 44 % and a readjusted value of 45.8 % CP. These results also contribute to the ontogenetic characterization of hematological parameters of this species.

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