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DIETS FOR GROW-OUT OF PIRARUCU IN NET CAGE: PERFORMANCE, PHYSIOLOGICAL PARAMETERS, FILLET COMPOSITION AND FEEDING COST*

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ABSTRACT

The present study evaluated practical diets with increasing levels of protein and energy on performance, fillet composition, feed cost, and physiological responses of pirarucu (*Arapaima gigas)* juveniles during the grow-out phase in a net cage system. In an on-farm trial for 90 days 225 pirarucu juveniles with initial weight ± standard deviation of 2025 ± 335 g were fed to apparent satiety with extruded diets containing 37.4 (T-37), 40.8 (T-40), 43.9 (T-43), 45.5 (T-46), and 47.1% (T-49) crude protein (CP), increasing lipid levels, and energy:protein ratio fixed in 10 kcal g^{-1}. Protein and lipid concentrations in the diets influenced the cost, fillet composition, and important physiological aspects of the health maintenance and productive performance of the pirarucu juveniles. Fish fed the T-37 diet had lower concentrations of fat in body cavity, fillet and blood, and had a lower cost associated with feeding. The increase in protein and energy levels in the other diets tested reduced the economic return, did not improve the zootechnical performance and caused physiological changes in the fish.

Key words: intensive systems; native fish; nutrition; physiology.

DIETAS PARA A ENGORDA DE PIRARUCU EM TANQUE REDE: DESEMPENHO, PARÂMETROS FISIOLÓGICOS, COMPOSIÇÃO DO FILÉ E CUSTOS DA ALIMENTAÇÃO

RESUMO

O presente estudo avaliou dietas práticas com níveis crescentes de proteína e energia no desempenho, composição do filé, custo com alimentação e respostas fisiológicas de juvenis de pirarucu *Arapaima gigas* durante a fase da engorda em sistema de tanque rede. Em um ensaio em sistema de produção, durante 90 dias, 225 pirarucus com peso médio inicial ± desvio padrão de 2025 ± 335 g foram alimentados até a saciedade aparente com dietas práticas extrusadas contendo 37,4 (T-37), 40,8 (T-40), 43,9 (T-43), 45,5 (T-46) e 47,1% (T-49) de proteína bruta (PB), níveis crescentes de lipídeo e relação energia/proteína de 10 kcal g^{-1}. As concentrações de proteína e lipídeo nas dietas influenciaram o custo, a composição do filé e parâmetros fisiológicos importantes na manutenção da saúde e do desempenho produtivo de juvenis de pirarucu. Os peixes alimentados com a dieta T-37 cresceram com menor volume de gordura visceral, sangue e filé, além do menor custo associado com a alimentação. O aumento nos níveis de proteína e energia nas demais dietas testadas reduziu o retorno econômico, não melhorou o desempenho zootécnico e causou alterações fisiológicas nos peixes.

Palavras-chave: sistemas intensivos; peixes nativos; nutrição; fisiologia.

INTRODUCTION

Most studies on pirarucu *Arapaima gigas*, a large carnivorous species with high productive potential, have been performed using juveniles below 200 g and have obtained contrasting results, indicating ideal protein levels for performance between 400 and 480 g kg^{-1}(Ituassú et al., 2005; Del Risco et al., 2008; Ono et al., 2008). Magalhães-Junior et al. (2017) evaluated the requirements for digestible protein based on the results of Cipriano et al. (2015, 2016) of pirarucus with an average initial weight of 1.8 kg and observed that diets containing 360 g kg^{-1} of digestible protein provided greater weight gain and feed efficiency. Information related to performance and physiological condition in the final stages of grow-out are insufficient, especially in reference to the larger fish (>4 kg). Supplying ideal concentrations of nutrients and energy in the creation could considerably reduce environmental impact and production costs, besides increasing the quality of the fish (Rawles et al., 2018; Diógenes et al., 2019).

In studies carried out with larger fish, a reduction in protein requirements is observed during the grow-out phase compared to requirements in the initial phase. Azevedo et al. (2004) and Einen and Roem (1997) observed this standard for Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus myhiss*); Hatlen et al. (2005) for Atlantic halibut (*Hippoglossus hippoglossus*) and Abdel-Tawwab et al. (2010) for tilapia (*Oreochromis niloticus*). This reduction reflects directly on the economic sustainability of creation, being the protein more onerous macronutrient in a diet (Salze and Davis, 2015). However, the inappropriate use of energy ingredients to replace protein for carnivorous fish may alter pathways related to energy metabolism and muscle growth (Alami-Durante et al., 2019).

Advances in fish nutrition research aim to improve performance, reduce dependence on chemotherapy and reduce economic and environmental losses (Oliva-Teles, 2012; Wang et al., 2017). In intensive systems, fish are frequently subject to high levels of stress and are susceptible to many adverse events, which can lead to physiological changes (Tu et al., 2015; Zhang et al., 2017). Fish nutrition experiments with purified ingredients are not effective in verifying the interaction between diet components and processing, so they are usually validated in research using practical diets (Rawles et al., 2018; Davies et al., 2019). On-farm experiments using practical diets may have greater applicability due to their similarity to production reality (Kabir et al., 2019).

Knowledge about nutrition and cost of food are among the key factors that will limit future fish production, especially when for carnivorous fish (FAO, 2018). For pirarucu production to be more competitive and important in world aquaculture, it is necessary to develop a food program that meets the nutritional requirements throughout the species life cycle without excess. Thus, the objective of this study was to evaluate the effect of increasing levels of protein and lipids on performance, physiological parameters, feed costs, and body composition of pirarucu during the grow-out phase in net cage system.

MATERIALS AND METHODS

The experiment was carried out in Santana Lake, located at the left bank of the Solimões River (S 03°17’40.74” W 060°28’7.8”), Manacapuru, Amazonas, Brazil. In an on-farm trial for 90 days a total of 225 pirarucu juveniles with a mean initial weight ± standart deviation of 2025 ± 335 g were reared in cages with a useful volume of 5.3 m^3, with a mean initial stocking density of 5732 g m^{-3}. Fifteen fish per cage were randomly assigned and fed twice daily to apparent satiety with extruded practical diets (Table 1) with increasing levels of crude protein (CP) and gross energy (GE), respectively: T-37 (370.4 g kg^{-1}, 4036.3 kcal); T-40 (400.8 g kg^{-1}, 4411.8 kcal); T-43 (430.9 g kg^{-1}, 4551 kcal); T-46 (450.5 g kg^{-1}, 4870.2 kcal) and T-49 (470.1 g kg^{-1}, 5021.1 kcal), with three replicates for each treatment. The experiment was carried out according to authorization nº 013/2013 of the Animal

Table 1. Ingredients and proximal composition of practical diets with increasing levels of protein and lipids used in grow-out phase of pirarucu in net cage system.

Ingredient (g kg^{-1})	Practical diets				
	T-37	T-40	T-43	T-46	T-49
Fish meal[1]	280.50	340.70	430.00	560.50	640.60
Meat and bone meal[2]	150.00	140.00	110.00	0.00	0.00
Soybean meal[2]	160.00	140.50	120.00	90.00	50.00
Wheat flour[3]	280.00	260.50	250.40	230.70	180.00
Cellulose[4]	60.50	30.00	0.00	0.00	0.00
Swine fat[5]	50.20	60.50	70.80	100.00	110.60
Vitamin and mineral supplement[6]	8.00	8.00	8.00	8.00	8.00
Proximal composition (g kg^{-1})					
Moisture (MO)	80.50	90.60	90.30	80.30	60.30
Crude protein (CP)	370.40	400.80	430.90	450.50	470.10
Ethereal extract (EE)	80.10	100.60	110.50	140.00	160.60
Ash (AS)	130.60	120.10	140.70	90.80	100.70
Crude fibre (CF)	50.45	50.30	60.80	70.30	70.20
Non-nitrogenous extract (NNE)	260.95	210.60	130.80	150.10	120.35
Gross energy (Kcal)	4036.40	4411.80	4551.00	4870.20	5021.10
GE:CP (kcal g^{-1})[7]	10.80	10.80	10.30	10.70	10.70

[1]Salmon meal Pacific Star®; [2]Ingredients purchased in local market; [3]Wheat flour Dona Maria®; [4]Cellulose Valdequímica®; [5]Swine fat Aurora®; [6]Vitamin and mineral supplement Nutron, [7]Gross energy (kcal)/crude protein (g kg^{-1}).

Research Ethics Committee (CEUA) of the National Institute of Amazonian Research (INPA).

Diets were balanced based on the reference of energy: protein ratio that provided best results in research by Ono et al. (2008) and the interval between protein levels based on studies by Ituassú et al. (2005) and Del Risco et al. (2008), with no other references available on pirarucu nutritional requirement by the time the experiment was carried out. The energy and protein content of the diets were increased with the addition of swine fat and high-quality fishmeal, respectively. This was due to the importance of non-protein energy source in muscle growth mechanisms (Alami-Durante et al., 2019) and to test for the limit of the animal's ability to use higher levels of protein without compromising its assimilation due to lack of amino acids in the diet, besides avoids the use of most expensive nutrient as an energy source (Jiang et al., 2015).

The influence of environmental variations was analyzed throughout the experimental period. Dissolved oxygen (5.44 ± 0.14 mg L^{-1}), temperature (30.22 ± 0.02 ºC), electrical conductivity (25.55 ± 0.09 µS cm^{-2}) and pH (5.80 ± 0.04) were monitored daily, in addition to concentrations of ammonia (0.21 ± 0.01 mg L^{-1}), nitrite (0.002 ± 0.00 mg L^{-1}), alkalinity (10.2 ± 0.14 mg L^{-1}) and hardness (7.78 ± 0.33 mg L^{-1}) were monitored weekly. Samples were collected at three different points located upstream, within, and downstream of the net cage system. The physical and chemical variables of the water of Santana lake, a white-water environment according to Sioli (1985), were within the limits considered acceptable for tropical species and for pirarucu (Oliveira et al., 2012).

Biometric and proximal composition data were collected at the beginning and at the end of the experiment. From these the following response variables were calculated:

- Survival rate – SR (%) = [(Initial fish count − Final fish count) ⁄ Initial fish count] × 100;
- Weight gain - WG (g): WG= Mean final weight − Mean initial weight;
- Feed intake for day – FI (% total weight day^{-1}) = [Intake/ (Mean final weight + Mean initial weight /Number of days/15)] × 100;
- Feed Efficiency - FE = Feed intake ⁄ WG;
- Feeding Cost – FC (R$ Kg $fish^{-1}$) = FE x Diet Price in real (R$);
- Protein Efficiency Ratio - PER = Wet weight gain / Crude protein intake
- Visceral Somatic Index - VSI (%) = (Visceral weight / total weight) × 100;
- Visceral Fat Index – VFI (%) = (Fat weight / total weight) × 100.

Samples of the ingredients, experimental rations and fish fillets at the beginning and at the end of the experiment were analyzed for proximal composition at the Fish Nutrition Laboratory/COTEI/INPA, following the norms of the AOAC (1999). Gross energy was measured by burning the sample in a calorimeter bomb (Parr Instruments 6100©).

At the end of the experiment, a blood collection was performed by puncturing the caudal vessel using the anticoagulant EDTA (10%). The hemoglobin concentration [Hb] was determined by the cyanometahemoglobin method; hematocrit (Ht) by the microhematocrit method; number of erythrocytes (RBC) with formaldehyde-citrate solution using a hemocytometer; and the hematimetric indices of Wintrobe. After centrifugation of the blood, plasma was used to determine glucose (GOD-PAP), total proteins (Biuret), cholesterol (Enzymatic-colorimetric), triglycerides (enzyme-colorimetric), albumin (bromocresol green reaction) and cortisol (ELISA) using commercial kits.

The results of the zootechnical, physiological, and body composition parameters, as well as the physical and chemical variables of the water were tested statistically by analysis of variance ANOVA one way. Tukey test was used when a significant difference was observed (5% probability). To estimate which diet provides the greatest protein efficiency and feed cost per kilogram of fish produced, a linear regression was used. All analyses were performed using the program Statistica 7.1 (STATSOFT®).

RESULTS

Water quality was not altered between the three collection points (upstream, net cage system and downstream) during the three months of the experiment. The survival rate was 100%, except for the T-37 treatment, with 97.7% survival at the end of the experimental period.

Performance results of pirarucu juveniles fed diets with increasing levels of protein and lipids are shown in Table 2. Weight Gain was not significantly different between treatments ($F= 1.83$; $p = 0.19$). Feed Intake for day was higher in fish fed the T-40 diet, which was significantly higher ($F = 4.65$; $p = 0.02$) than T-46. Feed Efficiency was lower in fish fed the T-46 diet (1.46 ± 0.06) and statistically different ($F = 4.18$; $p = 0.03$) from the T-40 diet, which had the worst conversion rates (1.74 ± 0.09).

Protein Efficiency Ratio decreased significantly with increasing protein levels ($F = 12.04$; $p < 0.01$). Feeding Cost was higher in diets with higher protein levels ($F = 17.26$; $p < 0.00$), with the lowest (5.29 ± 0.36) and the highest value (7.69 ± 0.28) observed in T-37 and T-46, respectively. The Protein Efficiency Ratio values showed a negative linear correlation ($F = 54.75$; $R^2 = 0.79$), decreasing significantly with increasing protein and lipid concentrations in the diets. For Feeding Cost, there was a positive linear correlation ($F = 55.00$; $R^2 = 0.79$) between increasing protein levels and cost per kg of live weight produced. Fish fed diets T-37 and T-40 had significantly lower Visceral Somatic Index and Visceral Fat Index values than fish fed other diets ($F = 4.67$; $p = 0.02$ and $F = 6.99$; $p < 0.01$).

The proximal composition of fish fillets (Table 3) was significantly altered by the protein and lipid concentrations of the diet. Dry matter, crude protein, and ash did not differ significantly. Fish fed the T-49 diet had higher lipid concentrations in the fillets ($F= 4.40$; $p = 0.02$) in relation to the T-37 diet; the others did not present a significant difference.

Hematological parameters are represented in Table 4. The values of Hemoglobin Concentration, Red Cell Count, Medium Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean hemoglobin Concentration were not statistically different between treatments. The Hematocrit values obtained in the T-49 diet (33.36 ± 0.34) were statistically different (F = 4.97; p = 0.01) from those of T-37 (28.89 ± 0.80).

The biochemical parameters are shown in Table 5. The cholesterol content in T-49 was higher (F= 12.74; p = 0.00) than the other treatments. The total plasma protein concentration in fish of the T-49 diet was significantly higher (F= 6.68; p = 0.01) than the T-37 diet; the other levels were not statistically different. Glucose, triglyceride, albumin and cortisol concentrations in the blood plasma of pirarucu juveniles did not differ significantly.

Table 2. Performance and corporal indices of pirarucu juveniles fed practical diets during 90 days in grow-out phase in net cage system[1, 2].

Diet	WG[3]	FI[4]	FE[5]	PER[6]	FC[7,11]	VSI[8]	VFI[9]
T-37	4.65 ± 0.53	2.00 ± 0.04[ab]	1.67 ± 0.12[ab]	1.77 ± 0.03[a]	5.30 ± 0.37[c]	7.18 ± 0.29[b]	2.57 ± 0.18[c]
T-40	4.53 ± 0.29	2.08 ± 0.05[a]	1.74 ± 0.09[b]	1.62 ± 0.05[ab]	6.16 ± 0.30[bc]	7.28 ± 0.24[b]	2.64 ± 0.32[bc]
T-43	5.17 ± 0.57	1.95 ± 0.08[ab]	1.53 ± 0.14[ab]	1.51 ± 0.06[ab]	6.09 ± 0.55[bc]	7.44 ± 0.12[ab]	2.82 ± 0.18[abc]
T-46	5.12 ± 0.17	1.86 ± 0.10[b]	1.46 ± 0.06[a]	1.40 ± 0.19[bc]	6.83 ± 0.30[ab]	8.04 ± 0.42[a]	3.41 ± 0.26[a]
T-49	5.15 ± 0.23	1.96 ± 0.04[ab]	1.52 ± 0.06[ab]	1.15 ± 0.15[c]	7.69 ± 0.28[a]	7.52 ± 0.13[ab]	3.23 ± 0.19[ab]
p[10]	0.19	0.02	0.03	<0.01	<0.01	0.02	<0.01

[1]Mean and standard deviation of three replicates. Mean values followed by different letters in the same column presented significant difference (p <0.05).; [2]Initial Mean Weight: 2025.3 ± 335.4; [3]Weight gain (kg); [4]Feed intake for day; [5]Feed efficiency; [6]Protein Efficiency Ratio; [7]Feeding Cost; [8]Visceral Somatic Index; [9]Visceral fat Index; [10]"p" ANOVA 5% probability; [11]Price per pound of ingredients in real: Fishmeal R$6.04; Meat and Bone Meal R$ 1.28; Soybean meal R$ 2.30; Wheat flour R$1.95; Cellulose R$ 0.50; Swine fat R$ 5.61; Vitamin and mineral supplement R$3.50.

Table 3. Proximal composition of juvenile fillet of pirarucu fed practical diets containing increasing levels of protein and lipid in grow-out phase in net cage system [1, 2].

Diet	Proximal composition (%)			
	DM[3]	CP[4]	EE[5]	AS[6]
T-37	24.91 ± 1.24	19.21 ± 0.79	2.68 ± 0.18[b]	1.15 ± 0.07
T-40	25.06 ± 0.66	19.18 ± 0.43	3.56 ± 0.61[ab]	1.15 ± 0.64
T-43	25.98 ± 1.12	19.18 ± 0.67	4.12 ± 0.40[ab]	1.16 ± 0.04
T-46	25.81 ± 1.40	19.22 ± 0.80	4.13 ± 0.78[ab]	1.13 ± 0.07
T-49	25.86 ± 0.74	19.07 ± 0.30	4.42 ± 0.68[a]	1.18 ± 0.15
p[7]	0.64	0.99	0.02	0.97

[1]Mean and standard deviation of three replicates. Values of CP, GE and AS in % in wet matter. [2]Mean values followed by different letters in the same column presented significant difference (p <0.05).; [3]Dry matter; [4]Crude protein;[5]Ethereal extract; [6]Ash; [7]"p" ANOVA 5% probability.

Table 4. Hematological parameters of pirarucu juveniles fed practical diets containing increasing levels of protein and lipid in grow-out phase in net cage system [1 2].

Diet	Hematological parameters					
	[Hb] (g dL^{-1})[3]	Ht (%)[4]	RBC (x10^6 µL^{-1})[5]	VCM (fL)[6]	HCM (pg)[7]	CHCM (g dL^{-1})[8]
T-37	12.80 ± 1.85	28.89 ± 0.80[b]	1.66 ± 0.26	178.07 ± 22.42	78.09 ± 5.26	44.31 ± 5.35
T-40	13.54 ± 1.02	30.92 ± 0.96[ab]	2.05 ± 0.10	153.78 ± 3.27	67.37 ± 6.28	43.92 ± 4.69
T-43	12.67 ± 0.08	29.94 ± 2.22[ab]	1.85 ± 0.04	162.85 ± 8.89	69.24 ± 1.88	42.52 ± 3.19
T-46	13.95 ± 0.85	31.31 ± 1.35[ab]	1.85 ± 0.16	161.51 ± 6.42	72.34 ± 5.15	44.79 ± 4.85
T-49	13.74 ± 0.78	33.36 ± 0.34[a]	1.94 ± 0.14	174.51 ± 15.13	71.67 ± 4.57	41.27 ± 2.39
p[9]	0.53	0.02	0.11	0.22	0.15	0.84

[1]Mean and standard deviation of three replicates. [2]Mean values followed by different letters in the same column presented significant difference (p <0.05).; [3]Hemoglobin Concentration; [4]Hematocrit; [5]Red cell count; [6]Medium Corpuscular Volume; [7]Mean Corpuscular Hemoglobin; [8]Mean Corpuscular Hemoglobin Concentration; [9]"p" ANOVA 5% of probability.

Table 5. Biochemical parameters of pirarucu juveniles fed practical diets containing increasing levels of protein and lipid in grow-out phase in net cage system[1, 2].

Diet	Biochemical parameters					
	Cortisol (g dL^{-1})	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})
T-37	18.44 ± 7.57	61.20 ± 13.01	96.48 ± 6.94[b]	99.81 ± 33.67	2.86 ± 0.32[b]	1.20 ± 0.10
T-40	29.27 ± 2.51	74.83 ± 5.68	110.68 ± 3.62[b]	98.11 ± 15.84	3.16 ± 0.09[ab]	1.26 ± 0.03
T-43	21.68 ± 4.14	73.56 ± 4.30	110.42 ± 8.31[b]	121.22 ± 40.19	3.26 ± 0.02[ab]	1.27 ± 0.03
T-46	20.00 ± 6.28	67.75 ± 9.05	115.24 ± 13.2[b]	150.12 ± 67.49	3.22 ± 0.18[ab]	1.27 ± 0.04
T-49	17.53 ± 7.82	74.61 ± 4.47	142.38 ± 5.22[a]	170.90 ± 37.42	3.58 ± 0.07[a]	1.32 ± 0.02
p[3]	0.20	0.24	<0.01	0.20	0.01	0.16

[1]Mean and standard deviation of three replicates. [2]Mean values followed by different letters in the same column presented significant difference ($p < 0.05$). [3]"p" ANOVA 5% of probability.

DISCUSSION

The diets tested and experimental conditions provided satisfactory growth rates during the grow-out phase of pirarucu in net cage system, with biomass gained surpassing that reported in other studies. Despite the success in zootechnical terms, gradual increases in protein and lipid levels during this developmental phase caused changes in lipid metabolism and higher production costs per unit of biomass. The transport and deposition of excess amino acid carbonic skeletons and unused energy sources resulted in changes in fat content in the blood, muscles and stomach cavity, which was most evidenced in the T- 49 diet.

An elevation of muscle and cavity fat content resulting from diets with high protein and energetic levels was observed in *Epinephelus marginatus* juveniles fed 550 g kg^{-1} of protein and 120 g kg^{-1} of lipids (Tuan and Williams, 2007). Tu et al. (2015) observed the growth, enzymatic and genetic profile of *Carassius auratus gibelio* fed high levels of protein and found an increase in the body lipid content; a pattern also observed by Lee et al. (2001) for giant croaker *Nibea japônica*, Shah-Alam et al. (2008) for black sea bass *Centropristis striata* and Sagada et al. (2017) for the snakehead fish *Channa argus*.

Protein Efficiency Ratio decreased with increasing protein levels a pattern that has been similarly described in the literature for other species (Yang et al., 2002; Jiang et al., 2015). In the two diets with higher protein levels, the excess protein degradation by deamination contributed to the increased total protein level in the blood and consequently the fillet and stomach lipid content, as evidenced by the increase in visceral somatic index and visceral fat index. This biochemical process results in energy expenditure and increases the release of ammonia into the water (Tu et al., 2015), which can damage the quality of the environment.

In this study, no differences were observed in the growth of pirarucus fed increasing levels of protein, suggesting a reduction in the use of high levels of protein throughout the growth phase tested. This contrasts with the work done by Ituassú et al. (2005) e Del Risco et al. (2008), who observed better weight gain between 400 and 480 g kg^{-1} of crude protein in the diet. The differences in the initial mean weight of individuals in this study (<200 g) and the present (>2 kg) may explain the contrasting growth results. Generally, fish protein requirements decrease with increasing size and age, Arnason et al. (2010) reported that protein requirements for maximum growth of Atlantic cod *Gadus morhua* of 40–107 g was 47–52%, while for larger fish (400–900 g) this value was 36% or less. Similar results were also found for salmonids, tilapia and stiped bass *Morone chrysops* × M. *saxatilis* (Einen and Roem, 1997; Abdel-Tawwab et al., 2010; Rawles et al., 2018).

As there is no reference to the feeding rate of pirarucu at this growth stage, food management was performed until apparent satiety. This may have negatively influenced feed efficiency, which was more evidenced in diets with lower protein and energy levels. Even with higher intake, these diets presented lower cost per unit of biomass produced when compared to the others, probably due to the utilization of meat and bone meal, which is a lower cost ingredient. Other studies have shown that the costs of fish production can be reduced by replacing, to the maximum extent possible, the use of high value protein sources such as fishmeal, with alternative energy sources and low-cost protein (Cerdeira et al., 2018; Davies et al., 2019; Diógenes et al., 2019). The inclusion of protein and energy levels above the T-37 diet contributed to the gradual increase in feeding cost without providing a significant increase in biomass, which is undesirable in both productive and environmental aspects (El Sayed et al., 2015; Salze and Davis, 2015).

In addition to economic and environmental impacts, studies have reported that energy-rich protein diets significantly increased energy costs in terms of thermal effects, mean metabolic rates, oxygen consumption and resting metabolic rate in animals (Mikkelsen et al., 2000; Lacroix et al., 2004). The results of the present study corroborate with these authors, in which the T-49 diet presented alterations in the values of Hematocrit, cholesterol and total plasma proteins in relation to T-37.

The observed Hematocrit value was similar to those described by Andrade et al. (2007) and Drumond et al. (2010). The increase in Hematocrit percentage as a response to increased erythrocyte counts, may be a stress response to the excess dietary protein. Fish fed diets rich in protein may exhibit responses to chronic/sub-chronic stresses, and studies suggest that high protein concentrations in diets may cause increased plasma metabolite levels as observed by Wicks and Randall (2002) for rainbow trout *Oncorhynchus* *mykiss*, and Engin and Carter (2001) and Yang et al. (2002) for *Bidyanus bidyanus* and *A. australis australis*, respectively. Excess protein and fat metabolism, especially in the T-49 diet, may have induced increased fish blood activity to improve oxygen transport efficiency.

Protein metabolism is related to energy-producing enzymes and both are linked to the nutritional status of the fish, which can be evaluated by plasma concentrations of free metabolites (Corrêa et al., 2007). The increase in plasma protein concentrations of fish fed increasing levels of protein is a pattern observed in studies with different species, such as *Oncorhynchus mykiss* (Yamamoto et al., 2002), *Brycon cephalus* (Vieira et al., 2005) and *Piaractus mesopotamicus* (Bicudo et al., 2009).

The T-49 diet, due to its high protein and lipid content, caused changes in the plasma protein and cholesterol levels of the pirarucus. In fish, lipids are transported mainly in the form of low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The main function of HDL is to transport cholesterol from peripheral tissues to the liver, while LDL plays a role in transporting cholesterol from liver to body tissues (Luo et al., 2014). The correlation between increased levels of cholesterol and plasma proteins is a pattern observed in other studies (Jiang et al., 2015; Wu et al., 2015), these metabolites are indicative of the mobilization of excess protein as a substrate for hepatic gluconeogenesis, which is undesirable from an environmental and economic point of view.

CONCLUSIONS

The results suggest more viability of T-37 among the tested diets in the grow-out phase of pirarucus weighing 2 to 8 kg in net cage system. In contrast, the T-49 diet had worst cost and protein efficiency, besides increased the concentration of lipid metabolism components in body cavity, blood and fillet. Future studies are recommended to evaluate in factorial designs the optimal protein and energy levels for pirarucu in the final stages of creation.

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