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Biochemical and hematological characterization of pirarucu (*Arapaima gigas* Schinz, 1822) juveniles fed with different nutritional feeding systems

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ABSTRACT. The aimed of this study was to evaluate the influence of dietary protein levels on the biochemical and hematological parameters of pirarucu (*Arapaima gigas)*. A total of 60 pirarucus specimens were used with an initial average weight of 499 ± 36 g and an initial average length of 40.30 ± 10.10 cm, stocked with densities of four pirarucus per fish tank network 48 m^2 of water surface. Feed was provided three times a day (8 am, 1 and 6 pm), with five different levels of crude protein (CP) in diet: 34, 36; 38 and 40 and 45% CP. The fish were euthanized at 310 experimental days with an average of 8.0 ± 1.0 kg of body weight. Blood samples were collected by tail vessel venipuncture, divided into two aliquots 2 mL and used for determination of biochemical levels and blood count. The results were submitted to analysis of variance and regression for CP levels, with the results showed as averages followed by the standard deviation. Orthogonal contrasts were applied to verify linear and quadratic effects at 5% significance. It was observed that the parameters referring to the blood count, given by erythrocytes, hemoglobin, packed cell volume, MCV, HCM, CHCM, glucose and total proteins showed no differences between the different diets. The average values of total proteins were 3.80 ± 0.30 g L^{-1}, glucose and triglycerides shown averages 43.45 ± 5.65 and 56.98 ± 5.80 mg dL^{-1}, respectively. The maximum cholesterol level 120.00 ± 13.33 mg dL^{-1} was obtained at the 35.4% CP level in the growth phase. Protein levels in diet influenced the cholesterol levels in the pirarucu growth phase, however, they did not influence the other biochemical and hematological parameters.

Keywords: srapaimidae; blood count; hematological parameters; fish farming; protein diet.

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Introduction

The Rondônia state produced 90 thousand tons of fish in year 2018, although has experienced consecutive reductions in production due to commercial collapse, saturation of the domestic market and consequent sanitary problems (Pinheiro et al., 2023). However, it became the largest producer of native species even with a considerable reduction in crops due to the high production cost (Dantas Filho et al., 2022; Cavali, Marmentini, Dantas Filho, Pontuschka, & Schons, 2022). In year 2018, the opening of two new refrigerating plants with certification from the federal inspection service took place, even so, production reduced to 57.2 thousand tons of fish in year 2022 (Associação Brasileira da Piscicultura [Peixe BR], 2023). Currently, Rondônia is the 3rd largest fish producer and the largest native species in Brazil, corresponding to 47% of all native fish cultivated in Brazil (Peixe BR, 2023).

Pirarucu *Arapaima gigas* (Schinz, 1822) is a prominent species in Rondônia state and is endemic to the Amazon basin, which mainly inhabits floodplain lakes and flooded forests. It has mandatory air breathing and can reach 200 kg of body mass and 3 m in length (Catâneo et al., 2022). This fish is considered a traditional delicacy of Amazonian cuisine and a sought-after fishing resource (Dantas Filho et al., 2022; Cavali et al., 2023). This fish has a carnivorous habit, and is one of the five most commercialized species in Amazon, has characteristics conducive to cultivation, such as a high growth rate (up to 10 kg in the first year), high rusticity in handling, adaptability to artificial feeding and high carcass utilization (51-57%) (Coutinho et al., 2019). In addition, it is a kind of obligatory air respiration, and this characteristic facilitates its creation, in high storage densities, as well as in low concentrations of dissolved oxygen in the water (Catâneo et al., 2022).

Pirarucu is a fish of great economic importance for the North region and was close to extinction in some regions, due to its intense exploitation. Therefore, environmental authorities have considered that natural populations of pirarucu are in danger of extinction. In order to reduce the effects of overfishing, government authorities created several restrictions on its exploitation, for example, indicating the minimum size allowed for capture (150 cm) and prohibiting fishing during the reproduction period of the species (closed season) (Martins, Franco, Dantas Filho, & Freitas, 2020; Sousa et al., 2022; Pontuschka et al., 2022). Pirarucu has occupied space in world trade, due to its excellent biological characteristics and zoological methods (Dantas Filho et al., 2022), as well as excellent meat quality (Cortegano et al., 2017; Cavali et al., 2023).

In order to assist in the advancement of fish farming, hematology emerges as an important tool, because hematological studies in fish are of ecological and physiological interest, and can help in understanding the relationship between blood characteristics, phylogeny, physical activity, habitat and the adaptability of fish to the environment, be it natural or farmed (Drumond et al., 2010; Batalha et al., 2017). These biochemical tests can used for the diagnosis of diseases, indicators of the physiological and nutritional status and handling stress of the fish (Tavares-Dias, Affonso, Oliveira, Marcon, & Egami, 2008). Because, the study of blood cells has become fundamental for diagnosing infectious diseases, leukemias and environmental stress (Hoshino et al., 2017).

Bearing in mind that knowledge of the relationship between blood parameters, the diet offered and productive development can a fundamental part of successful fish nutrition and disease diagnosis. The aimed of this study was to evaluate the influence of dietary protein levels on biochemical (blood glucose, cholesterol and triglycerides) and hematological parameters (erythrocytes, hemoglobin, packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of pirarucu (*Arapaima gigas*) cultivated in an excavated tank.

Material and methods

The study was conducted at the Carlos Eduardo Matiazze Fish Farm Research Center, Universidade Federal de Rondônia (UNIR), located in Presidente Médici city, Rondônia State, Brazil. The research was approved by the Committee for Ethics in the Use of Animals (Ceua/Unir) under protocol No. 0012/2021. Initially, the fingerlings were stocked in tank network, they had an initial weight of 40.30 ± 10.10 g, were fed with ration 40% crude protein (CP) and 8% of live weight, being fed four times a day, during 310 experimental days.

After the storage period, the initial biometry was performed to start the experiment. A total of 60 pirarucu specimens were used with an initial average weight of 499 ± 36 g and an initial average length of 40.30 ± 10.10 cm. Pirarucus were stocked at a density of four fish per tank network. A total of 15 tank networks used in the study had an area of 48 m^2, made of PVC-coated galvanized screen, in Brazil it is specific for excavated tanks, with a total area for cultivation of 720 m^2. Initial and final biometrics were performed, using an analytical scale, calipers and measuring tape, to verify the productive performance of the fish. The feeding rate occurred three times a day (8 am, 1 and 6 pm), composed of different CP contents, 36, 38, 40, 42 and 45% (Table 1).

Table 1. Centesimal composition[1] of standardized commercial diet (based on dry matter) offered to juveniles of pirarucu (*Arapaima gigas*).

Items	Crude Protein (CP) Levels (%)						
	32	34	36	38	40	42	45
Crude protein (min.g)	32.0	34.0	36.0	38.0	40.0	42.0	45.0
Fibrous matter (max.g)	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Mineral matter (max.g)	2.0	1.5	1.5	1.5	1.5	1.5	1.5
Ethereal extract (min.g)	7.5	7.8	8.0	8.0	8.0	8.0	8.0
Calcium (max.g)	4.5	4.2	4.0	3.5	3.5	3.5	4.0
Calcium (min.g)	2.0	2.5	2.5	2.5	2.0	1.6	1.2
Phosphorus (min.g)	1.0	1.0	1.0	1.0	1.5	1.4	1.2
Moisture (max.g)	9.0	9.0	9.0	9.0	9.0	9.0	9.0

[1]Percentage of nutrients in 100 g, for diets with different levels of CP 36, 38, 40, 42 and 45%. Pantothenic acid (min.) - 3.00; 4.00; 4.00, 45.00 and 7.00 mg; Biotin (min.) - 50; 60; 60; 0.0 and 75 mg; Hill (min.) -290; 295; 300;1200 and 90 mg; Vitamin A (min.) - 28,000; 29,900; 30,000; 9000 and 40,000 IU; Vitamin B_1 (min.) - 2.00; 2.00; 2.00; 15 and 3.00 mg; Vitamin B_{12} (min.) - 4.00 4.90; 5.00; 30 and 6.00 mg; Vitamin B_2 (min.) -3.00; 3.90; 4.00; 25 and 5.00 mg; Vitamin B_6 (min.) - 2.00; 2.10; 2.10; 18 and 3.00 mg; Vitamin D_3 (min.) - 5,000; 6,000; 6,000; 4200 and 8,000 IU; Vitamin E (min.) -45.00; 48.00; 50.00; 180.00 and 35.00 IU; Vitamin K_3 (min.) - 2.00; 2.50; 2.50; 6.00 and 3.50 mg; Vitamin C (min.) - 500; 550; 550.0.0 and 700 mg; Copper (min.) - 10.00; 10.00; 10.00; 9.0 and 8.50 mg; Iron (min.) - 90; 95; 98; 120 and 100 mg; Iodine (min.) - 0.40; 0.40; 0.40; 3.00 and 0.60 mg; Niacin (min.) - 50.00; 50.00; 52.00; 0.0 and 19.00 mg; Manganese (min.) - 10.00; 10.00; 10.50; 15.00 and 10.50 mg; Zinc (min.) - 180; 180; 180; 180 and 30 mg; Selenium (min.) - 0.60; 0.60; 0.60; 0.60 and 0.20 mg, respectively.

During the experimental period, water quality was monitored weekly in terms of parameters: hydrogen potential (pH), dissolved oxygen in water (mg L^{-1}), electrical conductivity (µs cm^{-1}) and water temperature (°C) *in situ*, according to the suggestions by Opiyo et al. (2018). The amount of feed provided to the fish was changed (Table 2) according to the weight during the growth phase (Bezerra, Souza, Melo, & Campeche, 2014).

Blood samples were collected by tail venipuncture, with the aid of 5 mL disposable syringes, blood collection was performed according to the methodology of Drummond, Caixeiro, Tavares-Dias, Marcon, and Affonso (2010) and Paiva (2013). The total volume of blood collected was 6 mL, a total of 2 mL of which were destined for the Erythrogram in a tube containing EDTA, 2 mL for the analysis of Glucose with citrate anticoagulant, and 2 mL in a dry tube for carrying out the plasmatic levels of glucose, triglycerides and proteins totals. With the data obtained from the average erythrocytes, hemoglobin rate and percentage of hematocrit, the hematimetric indices, VCM (Mean Corpuscular Volume) and MCH (Mean Corpuscular Hemoglobin) and biochemical parameters, blood glucose, cholesterol and triglycerides and total protein were calculated (Hoshino et al., 2017). Before blood collection, the pirarucus were fasted for 12 hours and captured with a fishing net, transported in raffia bags and kept in a 250 L tank, under the administration of anesthetic desensitization with 5% Benzocaine. Then, blood collection was carried out at 9 am in two pirarucu per tank network (Figure 1).

After the venipuncture procedure, the samples were homogenized in high precision, aliquots of blood samples were centrifuged in a vortex shaker and shaken for 10 min. at a high speed of 3000 rpm. Subsequently, the aliquots were stored in a cooler with ice to analyzed in laboratory. The method used was that of Coller for hemoglobin and erythrocytes, for the hematocrit where the VCM, HCM and CHCM indices are analyzed, the Golenfarb method was used, Trinder enzymatic methods were used for cholesterol and triglycerides, the God-Trinder method for glucose and the Biuret for total proteins (Andrade et al., 2007; Drumond et al., 2010).

The results were submitted to analysis of variance and regression for crude protein (CP) levels, with the results of biochemical determination and blood count showed as averages followed by standard deviation. Orthogonal contrasts were applied to verify the linear and quadratic effects at 5% significance, using the Genes software (Cruz, 2013).

Table 2. Dietary nutritional differences in relation to crude protein (CP) levels offered to juveniles of pirarucu (*Arapaima gigas*).

Feeding System (CP %)	Body weight 0.4 - 4.0 kg		
	CP (g kg^{-1})	EB (kcal kg^{-1})	EB: PB
A (36-32)	360.0	4000.0	11.0
B (38-34)	380.0	4200.0	11.0
C (40-36)	400.0	4300.0	11.0
D (42-38)	420.0	4400.0	10.0
E (45-40)	450.0	4500.0	10.0

Figure 1. Blood collection procedures, weight and morphometric measurements. (A) juveniles of pirarucu (*Arapaima gigas*) desensitized to avoid stress; (B) and (C) Blood collect by tail vessel venipuncture; (D) Blood collected and immediately will be separated into different aliquots sent to biochemical and hematological analysis.

Results

During this current study, the tank network water quality parameters showed average values 7.65 ± 0.12 pH, 5.15 ± 0.55 mg L^{-1} oxygen dissolved, 108.30 ± 7.06 µs cm^{-1} electrical conductivity and 29, 5 ± 0.5°C *in situ* temperature.

Regarding the blood parameters given by erythrocytes, hemoglobin, packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), glucose, total proteins and triglycerides did not shown differences (p > 0 .05) between treatments. The glucose and triglycerides content did not vary with the different feeding systems in the pirarucu growth phase (p > 0.05), with averages of 43.45 ± 4.65 mg dL^{-1} for glucose and 56.98 ± 5 .80 mg dL^{-1} triglycerides (Table 3). With regard to cholesterol values, there was a statistical difference (p < 0.05) between the different levels of CP tested, it is noteworthy that in the C feeding system, the Ration reduced CP and maintained the EE content.

Table 3. Biochemical and hematological parameters as a function of the different crude protein levels (CP) of pirarucu (*Arapaima gigas*) juveniles feeding.

Variables	CP levels in feed					p value	Regression
	A (36-32%)	B (38-34%)	C (40-36%)	D (42-38%)	E (45-40%)		
Body weight (g)	5571.00 ± 561.00	5256.00 ± 529.30	6030.00 ± 607.22	5128.00 ± 516.39	4688.00 ± 472.08	ns**	Y=5334
Erythrocytes (millions mm^{-3})	4.243 ± 0.18	4.273 ± 0.19	4.321 ± 0.19	4.346 ± 0.19	4.222 ± 0.18	ns	Y=4281
Hemoglobin (g dL^{-1})	12.45 ± 0.62	12.65 ± 0.63	12.80 ± 0.64	12.86 ± 0.64	12.35 ± 0.62	ns	Y=12.62
Packed volume (%)	37.00 ± 1.79	37.33 ± 1.80	37.66 ± 1.83	38.00 ± 1.84	36.56 ± 1.77	ns	Y=37.31
Mean corpuscular volume (MCV)	87.00 ± 0.60	87.00 ± 0.60	87.00 ± 0.60	87.16 ± 0.60	86.50 ± 0.60	ns	Y=86.93
Mean Corpuscular Hemoglobin (MCH)	29.30 ± 0.44	29.53 ± 0.45	29.56 ± 0.45	29.56 ± 0.45	29.16 ± 0.44	ns	Y=29.42
HCM concentration (g dL^{-1})	33.33 ± 0.59	33.66 ± 0.60	33.66 ± 0.60	33.50 ± 0.59	33.43 ± 0.59	ns	Y=33.51
Cholesterol (mg dL^{-1})	104.90 ± 8.21	107.16 ± 8.39	124.06 ± 9.71	97.10 ± 7.60	102.56 ± 8.03	0.01	*
Triglycerides (mg dL^{-1})	47.33 ± 4.87	49.00 ± 5.04	58.33 ± 6.00	56.75 ± 5.83	63.43 ± 6.52	ns	Y=56.98
Glucose (mg dL^{-1})	40.00 ± 7.05	45.46 ± 8.01	49.43 ± 8.71	40.93 ± 7.22	41.46 ± 7.31	ns	Y=43.45
Total proteins (g mL^{-1})	3.70 ± 0.40	4.03 ± 0.44	3.96 ± 0.43	3.76 ± 0.41	3.73 ± 0.40	ns	Y=3.83

Considering the 155 evaluation days; Significant* and not significant (ns)** at 5% significance. Cholesterol= -728.0533+45.430000N-0.669000N2 (R^2 = 63.00).

Discussion

The water quality parameters of the excavated tank administered in the current study remained adequate to the average values recommended for the raised of pirarucu (*Arapaima gigas*) (Tavares-Dias & Moraes, 2007; Cavali et al., 2020). The results for the hematological variables observed in the current study corroborate those obtained by Santos et al. (2010), who evaluated the physiological state of tambaqui (*Colossoma macropomum*) juveniles fed diets containing levels of inclusion of Amazonian nuts as a protein source. These authors concluded that it is possible to add up to a 30% variation in the protein contents in diets for this species, without compromising the physiological homeostasis. On the other hand, they disagree with the results found by Padua, Silva, Pádua, and Urbinati (2009), who found changes in hemoglobin and hematocrit levels in pacu (*Piaractus mesopotamicus*) fed different levels of cassava branches and crude protein (CP) levels in the diets. For the authors, this behavior characterizes an interaction between the CP content and hemoglobin biosynthesis.

The fact that the digestible energy : crude protein (DE: CP) ratio is higher, a ratio of 11:1 in the supply levels of protein A, B and C, regardless of the level of CP supplied reaches 40%. This may have favored an increase in cholesterol with a maximum value 120 mg dL^{-1} at the level 35.4% CP. This result may related to the fact that pirarucu has enough energy (4300 kcal kg^{-1}) available for this growth phase, in addition to the excess CP being a precursor of cholesterol through cholesterol biosynthesis (Drumond et al., 2010). However, although the glucose and triglycerides parameters did not showed statistical differences (p > 0.05) between the nutritional levels, a numerical increase was observed in average values of these variables in the feeding system C. This non-significant result in cholesterol levels can understood that the diet provided was isocaloric, especially in relation to etheral extract.

The evaluation of blood parameters allows a quick detection of the stress that the culture environment can impose on the fish (Tavares-Dias et al., 2008). Furthermore, this information can managed by veterinary inspection to assess and control the physiological fish state, standardizing ideal conditions for the cultivation of each species (Batalha et al., 2017; Pedrosa et al., 2019). One of the main indicators of environmental stress in fish is cholesterol levels (Hoshino et al., 2017). High cholesterol is linked to serious health problems like heart disease, angina pectoris, etc. Remember that coronary disease is caused by narrowing of the arteries (arteriosclerosis) that supply blood to the heart. Fat deposits such as cholesterol and other waste products build up inside the artery.

Average values of total proteins were 3.80 ± 0.30 g L^{-1} and are below those observed by Cavero et al. (2019) for the same species, however, Drumond et al. (2010) found values around 4.3 g L^{-1} in pirarucus larger than 12 kg. The authors ensured that the pirarucus were not parasitized. The same authors found values of 3.5 g L^{-1} in juveniles of pirarucu weighing 2.35 kg. However, triglyceride values remained below that observed by Drumond et al. (2010) in juvenile of pirarucu, with an average value of 397.6 mg dL^{-1}. Drumond et al. (2010) observed that pirarucu fingerlings have significantly lower levels of total proteins (1.99 ± 0.30), triglycerides (73.5 ± 31.3), urea (1.1 ± 0.3) and VCM (159.8 ± 21.3), although higher concentration of glucose (61.9 ± 11.3) and cholesterol (280.2 ± 46.4), erythrocyte count (2.150 ± 0.313), hemoglobin (9.1 ± 1.0), hematocrit (33.8 ± 2.8) and CHCM (27.1 ± 3.3), when compared to juveniles of pirarucu. However, Centeno et al. (2007) found an increase in hematocrit and hemoglobin concentration when comparing fingerlings and juveniles of tambaqui (*Colossoma macropomum*).

Drumond et al. (2010) and Dias et al. (2020) also found that pirarucu fingerlings had naturally elevated cholesterol levels compared to juveniles. The results of this current study were as expected for the species at this stage of life. However, in a hybrid of *M. chrysops x M. saxatilis*, an increase in the levels of total proteins, hematocrit, hemoglobin, MCV and number of erythrocytes was reported with increasing age, although a reduction in levels of glucose and cholesterol (Hrubec, Smith, & Robertson, 2001). In juveniles of carp (*C. carpio*), hematocrit and hemoglobin increased during the first year of life (Pedrosa et al., 2019), when fish metabolism is higher, due to their growth. Therefore, changes in the values of these blood parameters can expected due to the growth of hematopoietic tissues (kidney and spleen), which continue to develop in young fish (Hrubec et al., 2001). Although the glucose concentration of the pirarucu in control group was the highest among all groups in this curret study, it was still below the average glucose values determined by Drumond et al. (2010) for fingerlings (61.9 ± 11.3 mg dL^{-1}) and juveniles (48.5 ± 16.6 mg dL^{-1}).

In the Literature it was not found for juveniles of pirarucu, hematological parameters. The results for plasmatic glucose found in this current study were not altered with the levels of protein in the feed. It is worth noting that the increase in crude fiber content in diets was proportional to the crude protein (CP) content. And yet, there was no decrease in blood glucose levels. This demonstrates the capacity that the fish has to take advantage of fibrous foods, besides the pirarucu is a carnivorous animal. This use is related to the action of the gastrointestinal bacterial flora, which helps in the digestion of difficult-to-digest food components (Inoue, Oliveira, Gusmão, Boijink, & Tavares-Dias, 2016).

Conclusion

Dietary feeding systems influenced cholesterol levels in the growth phase of pirarucu (*Arapaima gigas*) juveniles raised in excavated tanks (into tank network) up to 5.5 kg. However, they did not influence the other biochemical and hematological parameters. Another factor to highlight, including the numerical levels of blood glucose, a possible result of hyperglycemic diet.

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