

**Exigência em proteína bruta do híbrido jundiara (*Pseudoplatystoma fasciatum* x *Leiarius marmoratus*)**

**Crude protein requirements in feeding for hybrid jundiara (*Pseudoplatystoma fasciatum* x *Leiarius marmoratus*)**

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## **Resumo**

Objetivou-se determinar a exigência de proteína bruta para juvenis de jundiara, híbrido de *Pseudoplatystoma fasciatum* X *Leiarius marmoratus*. Foram utilizados 240 juvenis do híbrido, peso médio inicial de  $8,4 \pm 1,41$  g, distribuídos em 15 tanques de fibra de vidro (170

L), em sistema fechado de recirculação de água e aeração constante, com densidade de 16 peixes por tanque. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e três repetições. Os tratamentos consistiram de dietas com 32, 34, 36, 38 e 40% de proteína bruta. Após 70 dias de experimento foram mensurados o peso final dos peixes e o consumo de ração. Dois exemplares de cada unidade experimental foram coletados para a avaliação da composição química da carcaça, eficiência nutricional e avaliação dos parâmetros sanguíneos. O aumento dos teores de proteína bruta promoveu o efeito significativo linear crescente para ganho de peso médio, consumo médio de ração e taxa de crescimento específico, e decrescente para conversão alimentar. Também foram evidenciadas diferenças significativas na composição química da carcaça, eficiência nutricional, proteína plasmática total, glicose, colesterol, triglicerídeos e atividade enzimática da lipase. Com isso, a elevação nos teores de proteína bruta de até 40% nas dietas para o jundiara promove melhoria no desempenho produtivo dos juvenis de jundiara.

**Palavras-chave:** Carnívoros; Conteúdo proteico; Necessidade nutricional; Pintado amazônico.

### **Abstract**

The objective of this study was to determine the crude protein requirements for juvenile jundiara fish, a hybrid of *Pseudoplatystoma fasciatum* X *Leiarius marmoratus*. A total of 240 juveniles of the hybrid, with an initial mean weight of  $8.4 \pm 1.41$  g, were distributed in 15 fiberglass tanks (170 L) in a closed water recirculation and constant aeration system, with a density of 16 fish/tank. The experimental design was completely randomized with five treatments and three replications. The treatments consisted of diets with 32, 34, 36, 38 and 40% crude protein. Seventy days into the experiment the final fish weight and feed intake were measured. Two specimens from each experimental unit were collected for evaluation of carcass chemical composition, nutritional efficiency and blood parameters. The increase in crude protein content promoted the significant, increasing linear effect for mean weight gain, mean feed intake and specific growth rate, and decreasing effect for feed conversion. Significant differences were also observed in carcass chemical composition, nutritional efficiency, total plasma protein, glucose, cholesterol, triglycerides and lipase enzymatic activity. Thus, the increase in crude protein levels of up to 40% in jundiara diets promotes improvement in the productive performance of juvenile jundiara.

**Keywords:** Carnivorous; Nutritional requirement; Protein content; Amazonian pintado.

## Resumen

El objetivo de este estudio fue determinar la exigencia de proteína bruta de jundiara a los juveniles del híbrido *Pseudoplatystoma fasciatum* X *Leiarius marmoratus*, Fueron utilizados 240 juveniles del híbrido, con peso medio inicial de  $8,4 \pm 1,41$  g, que se distribuyeron en 15 estanques de fibra de vidrio (170 L) en un sistema cerrado de recirculación del agua, en la densidad de 16 pez por estanque. El delineamiento experimental fue enteramente demandado, con cinco tratamientos y tres repeticiones. Los tratamientos residen en la inclusión de 32, 34, 36, 38 e 40% de proteína bruta. Después de 70 días de experimento, el peso final y la ingestión de ración de los peces fueron medidos. Dos ejemplares de cada unidad experimental fueron colectados para evaluación de la composición química de los residuos, eficiencia nutricional y parámetros sanguíneos. El aumento de los contenidos de proteína bruta promovió el efecto significativo efecto lineal creciente para el ganancia de peso medio, consumo medio de la ración y índice de crecimiento específico, y decreciente a la conversión alimentar. También fueron evidenciadas diferencias significativas en la composición química de los residuos del pez, eficiencia nutricional, proteína plasmática total, glucosa, colesterol, triglicéridos y actividad enzimática de la lipasa. Con eso, el aumento de los niveles de proteína bruta hasta 40% en las dietas para el jundiara promueve una mejora en el desempeño productivo de los juveniles de jundiara.

**Palabras clave:** Carnívoros; Contenido proteico; Necesidad nutricional; Pintado Amazónico.

## 1. Introduction

In recent years, Brazilian aquaculture has grown its productivity by over 20%. A contributing factor for this growth is the high number of endemic fish species that have the potential for cultivation and marketing (Campeche et al., 2011). These species include *Pseudoplatystoma fasciatum* (Linnaeus, 1766), popularly known as surubim, which is found in the Amazon, Prata, and São Francisco basins. This species has two factors that limit its breeding in a production system: the difficulty of feeding with inert feed and high rates of cannibalism in the larval and post-larval phases (Cornélio et al., 2014; Honorato et al., 2015).

An alternative to mitigate these negative factors and facilitate the raising of *P. fasciatum* in confinement is the use of hybridization (Díaz-Olarte et al., 2009). Among the species, the most suitable for crossing is *Leiarius marmoratus* (Gill, 1870), due to its good adaptation in production systems, ease of consumption of dry diets and absence of cannibalism (Mateo & Rojas, 2005). The hybrid formed between the crossings of the *P.*

*fasciatum* X *L. marmoratus* is called jundiara.

As in the confinement of endemic fish species, the lack of information on the nutritional requirements of hybrids limits the production systems of these animals (De Souza et al., 2014). In most cases, the diets used are inadequate for the nutritional requirements of hybrids, especially regarding crude protein requirements.

The crude protein requirement of carnivorous species and its hybrids, compared to other eating habits species, is higher. However, the excess of this nutrient in the diets for carnivorous species negatively interferes with the productive performance of these animals, leading to increased feeding costs, excess waste generation and environmental impact on aquaculture production systems (Souza et al., 2014).

Determining the amount of protein needed in carnivorous fish diets is necessary to formulate balanced diets that provide positive animal performance responses. (Teixeira et al., 2013). Thus, the objective of this study was to determine the crude protein requirement for jundiara juvenile, a hybrid of *Pseudoplatystoma fasciatum* with *Leiarius marmoratus*.

## 2. Methodology

The study was conducted at the Laboratory of Fish Nutrition and Feeding (AQUANUT) of the State University of Santa Cruz. Fifteen tanks (170 L) were used, mounted in a closed water recirculation system with biological filter, and aeration using a blower. Sixteen juveniles of the hybrid *Pseudoplatystoma fasciatum* X *Leiarius marmoratus*, with a mean initial weight of  $8.4 \pm 1.41$  g, were stored per tank. The experiment had a completely randomized design with five treatments and three replications. The experimental period was 70 days, and the first seven days served as acclimatization of juveniles to the infrastructure and experimental management. During this phase, the animals were fed with commercial feed containing 40% of crude protein. After the acclimatization period, the juveniles began receiving the experimental diets containing five levels of crude protein inclusion, in the proportions of 32, 34, 36, 38 and 40% (Table 1). At the end of the acclimatization period, five juveniles were removed per treatment, which was anesthetized, euthanized, and then individually weighed, lyophilized and stored in plastic bags identified for later use in carcass chemical composition analysis.

**Table 1.** Composition of experimental diets for jundiara fingerlings, hybrids of *Pseudoplatystoma fasciatum* x *Leiarius marmoratus*.

Ingredients (kg)	Crude protein inclusion levels (%)				
	32	34	36	38	40
Soybean meal 45%	6.75	9.25	10	16.20	18.50
Corn bran	30.00	26.00	24.00	13.25	12.15
Wheat bran	21.00	20.00	20.00	19.00	18.50
Corn gluten 21%	10.95	8.75	8.00	12.25	8.00
Poultry offal flour 50%	3.40	7.00	2.00	9.40	10.00
Fish meal 55%	11.70	13.00	13.00	11.90	11.90
Meat and bone meal 45%	3.13	3.13	3.13	3.13	3.13
Hydrolyzed feather flour 70%	8.00	8.00	15.00	11.00	14.25
Antioxidant <sup>1</sup> (BHT)	0.02	0.02	0.02	0.02	0.02
Premix <sup>2</sup>	0.60	0.60	0.60	0.60	0.60
Common salt	0.25	0.25	0.25	0.25	0.25
Fish oil	4.20	4.00	4.00	3.00	2.70
Total	100.00	100.00	100.00	100.00	100.00
	Composition analyzed				
Gross energy (kcal kg <sup>-1</sup> )	4333	4327	4379	4341	4323
Crude protein (g kg <sup>-1</sup> )	321.9	338.8	359.2	378.9	410.3
GE:CP Ratio (kcal EB g <sup>-1</sup> PB)	13.46	12.77	12.19	11.48	10.53
Ethereal extract (g kg <sup>-1</sup> )	86.39	83.92	74.93	79.86	74.21
Dry matter (g kg <sup>-1</sup> )	927.4	930.9	926.5	959.3	952.3
Mineral matter (g kg <sup>-1</sup> )	88.25	94.32	85.39	101.38	99.83
ENN	426.0	391.0	375.9	333.8	310.8
CP:CHO Ratio	0.76	0.86	0.96	1.13	1.32

<sup>1</sup> BHT = Butyl hydroxy toluene; <sup>2</sup> Mineral vitamin premix (Composition / kg of product): vit. A = 6,000,000 IU; vit. D3 = 2,250,000 IU; vit. E = 75,000 mg; vit. K3 = 3,000 mg; vit. thiamine = 5,000 mg; riboflavin = 10,000 mg; vit. pirodixin = 8,000 mg; biotin = 2,000 mg; vit. C = 192,500 mg; niacin = 30,000 mg; folic acid = 3,000 mg; Fe = 100,000 mg; Cu = 600 mg; Mn = 60,000 mg; Zn = 150,000 mg; I = 4,500 mg; Co = 2,000 mg; Se = 400 mg. Source: Authors (2018).

For control and the determination of dietary intake during the experimental period, a container was placed in each experimental unit with a lid that contained previously weighed feed. Feeding was performed until apparent satiety three times a day (8:00, 11:30 and 16:00 hours). Water quality was monitored periodically, with temperature, dissolved oxygen and pH measured daily with the aid of a digital multiparameter, and ammonia (NH<sub>3</sub>) was measured

weekly by photocolorimetric analysis. The water quality parameters were:  $28.34 \pm 0.60$  °C for temperature,  $4.40 \pm 0.45$  mg L<sup>-1</sup> for dissolved oxygen with a saturation of  $56.33 \pm 4.9\%$ , N-ammonia of  $1.3 \pm 0.67$  mg L<sup>-1</sup>, and  $6.05 \pm 0.21$  for pH. Throughout the study, the water and dissolved oxygen temperatures remained within the recommended limits (Baldisserotto, 2014). The pH was below the recommended level; however, this variable did not negatively affect the development of animals. Every ten days, the tanks were siphoned, and three times a week the biological filter was drained to remove solid particles.

At the end of the experimental period, the fish were submitted to a 24-hour fast to empty the gastrointestinal tract. The number of fish and the individual weight in each experimental unit were counted. These data, added to consumption, were used to calculate the following productive performance parameters: Weight Gain (WG) = (Final Body Weight - Initial Body Weight); Mean feed consumption (MFC) = (Total Consumption/ no. Fish); Apparent Feed Conversion (FC) = (Consumption of Supplied Feed/Weight Gain); Daily Specific Growth Rate (SGR) = [(ln Final Weight - ln Initial Weight) x 100 x time (day<sup>-1</sup>)] and Survival (SUR) = [(Number of Fish at the End of the Experiment/Number of Fish at the Beginning of the Experiment) x 100].

At the end of the experiment, 10 juveniles were randomly collected from each treatment, anesthetized and blood samples were taken by cardiac puncture, according to the protocol suggested by Ranzani-Paiva et al. (2013). Then, they were euthanized and fragments of the anterior and middle portions of the intestine were removed for enzymatic and liver activity analysis to determine the hepatosomatic index ( $\% = 100 \times (\text{Liver weight}/\text{Fish weight})$ ). After these procedures, they were lyophilized and stored in identified plastic bags for analysis of the chemical composition of the carcass. The blood samples were taken to the Laboratory of Hematology of the State University of Santa Cruz, where the parameters of glucose, cholesterol and total plasma proteins were quantified. The fragments of the anterior and middle portions of the intestine were sent to the Laboratory of Aquaculture of the University of São Francisco Valley (UNIVASF), where the enzymatic activity of amylase, lipase and alkaline protease was determined via a commercial kit (Labtest Ref. 11, 76, 85, 108, 109).

The fish collected at the beginning and end of the experimental phase were used to determine the dry matter, crude protein, ether extract, mineral matter and gross energy of the carcass. Analyses of these parameters were performed at the Laboratory of Forage Cultivation and Pasture in Itapetinga Campus at the State University of Southwest Bahia and at the Laboratory of Fish Nutrition and Feeding (AQUANUT) at the State University of Santa Cruz. The mean of the initial and final fish chemical composition of carcasses allowed the

determination of the protein retention rate =  $100 \times [(final\ carcass\ protein\ content \times final\ weight) - (initial\ body\ protein\ content \times initial\ weight)] / consumed\ crude\ protein$ .

The collected data were subjected to analysis of variance, with significance at 5%. When significant differences were detected between the variables, the most appropriate regression models for the variables were used, with the aid of the statistical program R Core (2011).

### 3. Results and Discussion

The increase in crude protein content in the diet resulted in significant changes for mean weight gain and feed intake, apparent feed conversion, and fish specific growth rate ( $p \leq 0.05$ ). The variables of the mean weight gain ( $y = 2.729x - 62.563$ ;  $R^2 = 0.61$ ), mean feed intake ( $y = 1.919x - 28.591$ ;  $R^2 = 0.50$ ), and specific growth rate ( $y = 0.097x - 0.885$ ;  $R^2 = 0.67$ ) increased linearly as the level of crude protein in the diets increased, while FC ( $y = -0.032x + 2.308$ ;  $R^2 = 0.61$ ) decreased linearly. Increasing crude protein in the dietary did not influence jundiara survival rate nor hepatosomatic index. These variables had mean values between treatments of 91.11% and 1.78%, respectively (Table 2).

**Table 2.** Average weight gain (AWG), Average feed intake (AFI), feed conversion (FC), specific growth rate (SGR), hepatosomatic index (IHS), survival rate (SUR), and protein retention ratio (PRR) of juveniles of jundiara, hybrid of the cross of *Pseudoplatystoma fasciatum* x *Leiarius marmoratus* fed with diets with different levels of crude protein.

Variable	Crude protein (%)					EPM <sup>1</sup>	C.V.% <sup>2</sup>	P
	32	34	36	38	40			
AWG* (g)	28.74	26.50	31.68	44.65	46.96	2.85	13.82	0.001
AFI* (g)	35.33	34.29	37.17	47.85	47.73	3.35	14.34	0.033
FC*	1.23	1.29	1.17	1.07	1.02	0.04	6.18	0.005
SGR <sup>1</sup> (%)	2.36	2.26	2.47	2.93	2.99	0.10	6.76	0.001
IHS (%)	1.49	1.28	2.39	1.79	1.93	0.33	31.72	0.215
SUR (%)	88.89	82.22	91.11	93.33	100	4.22	8.02	0.123
	<i>Nutritional efficiency</i>							
PRR <sup>1</sup> (%)	25.30	23.83	24.95	34.43	30.87	1.03	6.43	0.000

<sup>1</sup> Average Standard Error; <sup>2</sup> Coefficient of variation; \* Linear effect. Source: Authors (2018).

The average weight gain of jundiaras was positively correlated with increasing levels of crude protein in the diet. According to Moreira & Pozza (2014), animal growth occurs when its protein synthesis (anabolism) is greater than its cellular degradation (catabolism).

Thus, the result suggests that the increase of the crude protein in the diet to the level of 40%, provided a greater protein synthesis, which increased the relationship between the anabolic and catabolic processes of amino acid metabolism dietary, resulting in increased growth and consequently in weight gain of the jundiarias.

According to Mora Sanches et al. (2009), an animal's weight gain increases as the crude protein in the diet increases, but this occurs to a certain level (peak), at which point the animal's growth starts to decrease. The correlation obtained in this study showed an increasing linear effect, and consequently, it shows that the levels used were possibly not able to meet the maximum requirement for jundiara protein since the maximum point of weight gain was not reached. Therefore, it can be deduced that the protein requirement of this hybrid is probably greater than 40% of crude protein. For cachara (*P. reticulatum*), a pure species of *Pseudoplatystoma*, the protein requirement is met with 49.25% crude protein in the diet. (Cornélio et al., 2014).

The calculation of the specific growth rate is based on the weight gain of the animals. According to Silva et al. (2015) the growth rate reaches its peak when the protein requirement of the species is met. Thus, the results for SGR had a similar effect on MWG. Souza et al. (2014) also observed that jundiara SGR increased linearly as a result of the conclusion of increased levels of crude protein dietary.

In this study, the ratio of crude energy to crude protein between diets decreased as protein content increased, thus treatment with lower crude protein had a ratio of 13.46 kcal CR g<sup>-1</sup> CP, and treatment with the highest content had 10.53 kcal CE g<sup>-1</sup> CP. Under normal water and diet quality conditions, fish increase their consumption as its dietary energy-protein ratio decreases, as its initially feed to meet their energy needs (Sampaio et al., 2000). Thus, the linear increase in the jundiara average feed intake (MFI) may be explained by the decrease in the energy/protein ratio as the dietary crude protein content increased. In a study conducted on the energy: crude protein nutrition ratio in tucunaré (*Cicla* sp.), it was observed that animal consumption decreased by 28% as the protein: energy ratio increased from 8 Kcal g<sup>-1</sup> CP to 11 Kcal g<sup>-1</sup> CP (Ibid et al., 2000).

Feed conversion (FC) was another variable that showed correlation with increased inclusion of crude protein in the diets, however, the effect was linear decreasing. In all treatments, the value for FC was less than 1.30. According to Bicudo et al. (2012), a feed conversion rate of up to 2 may be considered adequate for farmed carnivorous fish. Therefore, the FC values found in this study can be considered efficient. This result suggests that the amount of non-protein source energy available in the diet was adequate to meet the energy



requirement of the hybrid, and dietary protein was used to meet the need for growth. In a study where cachara juveniles were fed with five diets with different levels of crude protein (30, 35, 40, 45, and 50%), Cornelio et al. (2014) observed the FC ranging from 1.83 to 1.03. These values, as found in this study, are within the range that Bicudo et al. (2012) classified as efficient. The hepatosomatic index (HSI) can be influenced by two factors: diets rich in non-nitrogenous extracts, as its favor the accumulation of glycogen in the liver (Moro et al., 2010), or by diets with high protein levels that may increase liver size and weight as a result of metabolic overload due to protein degradation for energy purposes (Honorato et al., 2014). In this study, the similarity between the treatments for HSI shows that the protein content of the diets was not used to meet the jundiara's energy needs. Honorato et al. (2015) reported that levels of crude protein up to 40% in the diet did not influence the HSI of surubin (*Pseudoplatystoma* sp).

The similarity of the survival rate among the experimental treatments showed that this variable was not influenced by the different crude protein levels of the diets. According to Honorato et al. (2015), the causes of decreased survival rates of native species in aquaculture systems are generally associated with management errors, environmental factors and the quality of water used in the production process.

For the nutritional efficiency variable, differences were observed among the experimental diets (Table 2). The increase in crude protein level resulted in a positive linear effect ( $p \leq 0.05$ ) in protein retention rates ( $y = 1.088x - 11.269$ ;  $R^2 = 0.50$ ). The increase in dietary protein promoted greater body protein deposition. Levels of crude protein in the diet, higher than the requirement of the fish, may affect the efficiency of protein utilization because excess dietary crude protein may be directed for synthesis of fatty acids and triglycerides (lipogenesis), glucose (gluconeogenesis) or in energy production for the animal (Cotan et al., 2006). Honorato et al. (2014), studying digestible protein levels for *Pseudoplatystoma* sp. raised in cages, reported that diets with up to 40% crude protein did not significantly affect animal PRR.

The increase in dietary crude protein did not influence the chemical composition of the carcass of jundiara, as dry matter, crude energy and mineral matter (Table 3). Honorato et al. (2014) also observed no change in carcass DM composition of *Pseudoplatystoma* sp. grown in net-ponds and rationed with increasing levels of crude protein. On the other hand, the increase in crude protein levels provided a linear increasing effect on crude protein contents ( $y = 14.43x + 54.22$ ;  $R^2 = 0.54$ ) and decreasing response to ether extract ( $y = -15.43x + 928.22$ ;  $R^2 = 0.64$ ) in the carcass of the jundiara juveniles.

**Table 3.** Average values of chemical composition of carcass of juveniles of jundiara, hybrid of the cross of *Pseudoplatystoma fasciatum* x *Leiarius marmoratus* fed with diets with different levels of crude protein.

Variable	Crude protein (%)					EPM <sup>1</sup>	C.V.% <sup>2</sup>	P
	32	34	36	38	40			
Dry matter (g Kg <sup>-1</sup> )	271.7	255.2	270.5	245.0	253.7	0.63	4.20	0.054
Crude protein (g Kg <sup>-1</sup> )*	511.6	569.5	589.2	657.6	611.8	1.60	4.85	0.000
Ethereal extract (g Kg <sup>-1</sup> )*	426.9	388.6	431.0	301.5	316.2	1.42	6.63	0.000
Gross energy (Kcal Kg <sup>-1</sup> )	5305	5797	5468	5711	5545	167	5.21	0.315
Mineral matter (g Kg <sup>-1</sup> )	121.8	134.6	119.2	141.5	137.0	0.65	8.57	0.133

<sup>1</sup> Average Standard Error; <sup>2</sup> Coefficient of variation; \* Linear effect. Source: Authors (2018).

According to Bicudo et al. (2012), the efficiency of the use of dietary nutrients by carnivorous fish is controversial, since animal growth is dynamic and involves protein utilization and energy metabolism, possibly causing changes in body composition. Given this, changes in crude carcass protein among experimental diets can be correlated to the jundiara's protein utilization efficiency. Given that the increase in dietary crude protein promoted an increase in protein synthesis, which possibly favored higher protein decomposition in the hybrid carcass. This justification can also be attributed to the decrease in ethereal extract, since the increase in protein synthesis limits the use of dietary protein for the synthesis of fatty acids and triglycerides through lipogenesis, consequently reducing the EE deposition in the animal carcass.

The administration of diets with different crude protein levels did not influence the hematocrit (HT) volume among treatments ( $p < 0.05$ ) (Table 4). However, due to dietary crude protein levels, the total plasma protein variables ( $y = 0.1714x^2 - 12.386x + 226.23$ ;  $R^2 = 0.80$ ), glucose ( $y = 4.773x^2 - 338.62x + 6067.4$ ;  $R^2 = 0.70$ ), and cholesterol ( $y = 3.220x^2 + 23817x + 45281$ ;  $R^2 = 0.75$ ) had quadratic effect. Triglycerides (TRIG), also had difference, however, it was not possible to estimate a linear model that has a biological interpretation (linear regression,  $p = 0.218$ ; quadratic,  $p = 0.947$ ).

**Table 4.** Mean blood metabolite values of juveniles of jundiara, *Pseudoplatystoma fasciatum* x *Leiarius marmoratus* hybrid fed with diets with different levels of crude protein.

Variable	Crude protein (%)					EPM <sup>1</sup>	C.V.% <sup>2</sup>	P
	32	34	36	38	40			
Hematocrit (%)	22	20	20	19	26	0.02	14.31	0.105
Total plasma protein (g dL <sup>-1</sup> )*	6.80	5.27	4.67	4.20	7.40	0.23	7.17	0.000
Glucose (g dL <sup>-1</sup> )*	111.1	87.8	66.7	72.37	169.3	13.5	23.04	0.002
Cholesterol (g dL <sup>-1</sup> )*	204.7	150.7	129.7	126.3	153	11.5	13.06	0.005
Triglycerides (g dL <sup>-1</sup> )	539.0	207.7	583.7	273.2	298.7	60.3	27.42	0.004

<sup>1</sup> Average Standard Error; <sup>2</sup> Coefficient of variation; \* Quadratic effect. Source: Authors (2018).

In fish, HT values are in the range of 20 to 45% (Weiss et al., 2010). Values lower than this range may be indicative of anemia, while higher values result from stress arising from the capture or use of inadequate anesthetic dose at the time of fish containment (Labarrère et al., 2012). The results for HT of jundiara used in this study were within this range, showing that the animals did not have anemia, indicative of malnutrition or infectious diseases.

Blood plasma protein concentrations result from protein metabolism and animal nutritional conditions (Higuchi et al., 2011), and are altered in response to diet, varying according to the ingredients used and/or the amount consumed (Souza et al., 2014). In this study, experimental diets had the same ingredients in their formulation but in varying proportions. This variation causes changes in the availability of dietary protein. Thus, the difference in jundiara total plasma protein (TPP) concentrations observed may be related to the proportion of each ingredient and its effect on protein availability in experimental diets.

In blood plasma, the variation in glucose levels (GLUC) may be due to the stress generated by factors arising from the metabolism or physiology of the animal (Labarrère et al., 2012). For Melo et al. (2016), increased crude protein levels in the diet causes changes in blood glucose concentrations by two factors: gluconeogenesis and decreased digestibility of ingredients of plant origin, as a measure of how high its participation is in the diet. Thus, the quadratic behavior observed for jundiara plasma glucose levels about the crude protein content of the diets can be explained by the variation of the proportions of the ingredients of plant origin in the experimental diets.

In this study, the variations found in cholesterol concentrations (COL) in fish blood plasma may be associated with the composition and proportion of the ingredients used in the experimental diets. In a study with jundiara fed with different levels of crude protein, Souza et al. (2014) reported that the elevation of crude protein in diets caused changes in plasma COL concentrations, indicating that these changes are metabolic strategies to maintain normal animal growth and survival processes.

The increase in crude protein in the diet did not promote differences in the activity of amylase and protease enzymes ( $p > 0.05$ ). Among the treatments, the following mean values for the activity of these digestible enzymes were 20.46 U mg<sup>-1</sup> for amylase and 6.30 U mg<sup>-1</sup> for protease (Table 5). Protein levels of experimental diets provided quadratic model adjustments ( $y = 2.660x^2 + 185.86x - 3049.1$ ;  $R^2 = 0.43$ ) to lipase activity.

**Table 5.** Activity of digestible enzymes of juveniles of jundiara, hybrid of the cross of *Pseudoplatystoma fasciatum* x *Leiarius marmoratus* fed with diets with different levels of crude protein.

Variable	Crude protein (%)					EPM <sup>1</sup>	C.V.% <sup>2</sup>	P
	32	34	36	38	40			
Amylase (UI)	27.26	23.50	24.91	15.80	10.85	4.49	37.99	0.125
Lipase (UI)*	172.67	192.34	217.0	146.38	139.22	15.8	15.77	0.031
Protease (UI)	5.17	5.28	9.12	6.37	5.54	0.96	26.49	0.077

<sup>1</sup>Mean standard error; <sup>2</sup>Coefficient of variation; \*Quadratic effect. Source: Authors (2018).

According to Seixas-Filho (2003), the composition of the diet may influence the animal's organism to increase the release of the digestive enzyme amylase. However, in this study, the increase in crude protein levels in the diet did not modify the activity of the amylase enzyme in the jundiara intestine. This result can be explained by two factors: the first is that the parental species of jundiara are carnivorous, and they have amylohydrolytic activity in the lower intestinal content when compared to omnivorous and herbivorous species (Moraes et al., 2014). The other factor is that amylase participates in polysaccharide digestion, thus the increase in its secretion is related to the presence of carbohydrates in diets (Ibid et al., 2014). In this study, the participation of carbohydrates in the composition of the experimental diets decreased as the crude protein contents increased, causing a tendency of lower secretion of this enzyme in the jundiara intestine.

The increase in crude protein in the diet did not alter the activity of the protease enzyme in the jundiara intestine. Lundstedt *et al.* (2004) studying four levels (20, 30, 40 and 50%) of crude protein in surubim (*P. corruscans*) feed, found similar results to those found in this study. The authors cited that the variation in the protein level of the diet did not influence the protease activity in the intestine of the animals. For this reason, they classified protease action in this species as specific, when compared to other species, because *Pseudoplatystoma* spp. are adapted to the best use of protein, regardless of its content in the diet. Since jundiara is a hybrid of *Pseudoplatystoma*, the statement by Lundstedt *et al.* (2004) justifies why the increase in crude protein did not interfere with protease activity in the jundiara intestine in this experiment.

Lipase activity was influenced by the increase inclusion of crude protein in the diet. With the increase in crude protein, there is a reduction in dietary ethereal extract, leading to a reduction in lipase activity. Melo *et al.* (2016) studying digestive aspects of juvenile Jundiás (*Rhamdia quelen*) fed with four protein levels (20, 27, 34 and 41%), found that lipase activity was inverse to the crude protein content of the diets. The same trend was observed in the hybrid of *Pseudoplatystoma*, with carnivorous eating habits.

#### 4. Conclusions

Juveniles of hybrid jundiara fed with diets contain up to 40% crude protein have improvements in weight gain and growth rate, efficiency in protein retention, and the protein deposition of carcass increase. Crude protein levels increase in jundiara after fed up to 40% in diets and cause changes in blood plasma protein, glucose, cholesterol, and triglyceride concentrations, and decrease lipase activity.

It is suggested to carry out studies on the digestibility of food of animal and vegetable origin, and subsequent growth tests with dose-response to define levels of digestible protein, amino acids and energy for the hybrids of *Pseudoplatystoma fasciatum* X *Leiarius marmoratus*.

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