# **Research Article**



# Growth and body composition of juvenile curimatã-pacu (*Prochilodus argenteus*) fed diets with different protein: lipid ratios

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**ABSTRACT.** Promising species for aquaculture need to have their nutritional requirements defined. Considering that protein is the most expensive macronutrient in aquaculture diets, the present study aimed to estimate the protein requirement of curimatã-pacu fed with diets containing two lipid levels. Thus, eight experimental diets were formulated to contain 20, 25, 30 and 35% of crude protein (CP) associated to 11 and 15% of crude lipids (CL) were formulated, constituting a completely randomized factorial design (4 CP x 2 CL) with three replicates per treatment. The juveniles  $(3.6 \pm 0.01 \text{ g})$  were fed daily with 6% of the biomass, three times a day (08:00, 12:00 and 16:00 h) for 70 days. The protein requirement was estimated by the combination of the broken line and quadratic regression models. The CP concentration in the diets was the only factor influencing (P < 0.05) the growth and feeding efficiency variables of the fish, without interaction (P > 0.05) with the CL level of the diets. The requirement for weight gain and fish feed efficiency was estimated at 32.5 and 34.5% CP. Protein retention decreased (P < 0.05) in proportion to CP elevation. Fish fed 15% CL had higher (P < 0.05) body lipid deposition. On the other hand, the increase of CP of the diets decreased (P < 0.05) lipid deposition in juveniles. Therefore, it is recommended that young curimatã-pacu between 3 and 27 g in weight should be fed diets containing 32.5-34.5% CP and 11% of CL, equivalent to a protein: energy ratio of 22-23 mg kJ<sup>-1</sup>.

Keywords: *Prochilodus argenteus*; Prochilodontidae; protein requirement; non-protein energy sources; iliophage fish

## **INTRODUCTION**

The curimatã-pacu (*Prochilodus argenteus*) is an iliophagous, rheophilic indigenous species of the São Francisco River basin (Brazil), whose populations in its natural habitat have decreased due to the construction of hydroelectric plants (Pereira-Arantes *et al.*, 2011) and overfishing (Soares *et al.*, 2011). Due to its social and economic importance as a fishery resource, initiatives for its production in aquaculture systems have recently been studied (Almeida *et al.*, 2015; Santos *et al.*, 2016). However, knowledge about the nutritional requirements of species of *Prochilodus* is scarce.

Due to the economic and environmental importance of protein in aquafeeds, the determination of protein requirement is usually the first step in nutritional studies for the aquaculture production of new fish species (Cyrino *et al.*, 2010). Santos *et al.* (2018) estimated the protein requirement of *P. argenteus* feeding isocaloric diets (2,759 kcal digestible energy kg<sup>-1</sup>) at 36% crude protein. However, fish protein requirement is influenced by different factors, such as feeding habit, water temperature, stage of development and presence and levels of non-protein energy sources in diets (NRC, 2011). Thus, to increase lipid in aquafeeds has been an effective strategy to spare dietary protein as an energy source for fish and even for omnivorous species (Khan & Abidi, 2012; Aminikhoei et al., 2015). However, a low dietary protein: lipid ratio can result in early satiety of fish and a decrease in essential nutrient intake, which result in poor growth and high body fat deposition. On the other hand, a higher protein:lipid ratio increases the use of dietary protein as an energy source by fish, and consequently, reducing the economic and environmental efficiency of diets.

Thus, this study aimed to determine the protein requirement and the best protein:lipid ratio for curimatã-pacu juveniles fed with diets containing two lipid levels.

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#### MATERIALS AND METHODS

All experimental procedures were approved by the Animal Ethics Committee of the Federal Rural University of Pernambuco (License N°085/2016).

## **Experimental diets**

Eight diets were formulated with four levels of crude protein (20, 25, 30, and 35%) and two levels of crude lipids (11 and 15%) in a completely randomized factorial design (4×2), with three replicates per treatment. Ingredients and proximate composition of the experimental diets are given in Table 1. The ingredients were homogenized through a hammer mill ( $\leq 1$  mm sieve), mixed, moistened (37% water), pelletized (2 and 4 mm), dried for 24 h in a forcedventilation oven (50°C), and hermetically packed in black plastic bags and stored at 4°C until use.

#### Fish and experimental procedures

Curimatã-pacu juveniles were provided by the Reference Center of Aquaculture and Fishery Resources of Itiúba (Porto Real do Colégio, AL, Brazil) of Companhia de Desenvolvimento dos Vales do São Francisco e Parnaíba (CODEVASF). Before the experiment, fish were submitted to a prophylactic bath (1 g NaCl L<sup>-1</sup>) for one minute and transferred to two circular tanks (360 L) with temperature control, supplementary aeration, and biological filters to allow them to adapt to laboratory conditions. During this adaptation time, fish were fed three times a day (08:00, 12:00 and 16:00 h) for seven days with a commercial diet (40% crude protein) at 5% of the biomass.

At the beginning of the experiment, 20 fish from the initial population were fasted (24 h), euthanized by anesthetic overdose (benzocaine, 500 mg L<sup>-1</sup>), and frozen for whole body chemical analysis. The remaining juveniles (initial weight  $3.6 \pm 0.1$  g) were selected and randomly distributed in 24 glass aquariums with 60 L water at an equal stocking rate of 15 fish per aquarium. The aquariums were provided with water connected into a recirculation system and equipped with biological and ultraviolet filters, supplementary aeration, temperature control, and controlled photoperiod (12:00 h light: 12:00 h dark). The aquariums were cleaned weekly, and approximately 50% of the water was renewed.

Fish were hand-fed daily at 6% of the biomass three times a day (08:00, 12:00, and 16:00 h) for 10 weeks. This feeding rate was determined in a previous experiment (*unpubl. data*), and the ideal feed rate was defined as when no uneaten food was observed 30 min after feeding. Every 14 days, the fish were anesthetized

(benzocaine 50 mg  $L^{-1}$ ) and weighed to adjust the feed supply in each experimental unit.

Water conditions throughout the experiment were temperature  $(28.4 \pm 1.4^{\circ}C)$ ; dissolved oxygen  $(5.6 \pm 0.7 \text{ mg L}^{-1})$ ; pH  $(7.3 \pm 0.3)$ ; N-NH<sub>4</sub>  $(0.9 \pm 0.7 \text{ mg L}^{-1})$ ; N-NO<sub>2</sub>  $(0.4 \pm 0.0 \text{ mg L}^{-1})$ ; alkalinity  $(51.4 \pm 13.6 \text{ mg L}^{-1})$ ; and hardness  $(75.5 \pm 25.3 \text{ mg L}^{-1})$ . All water parameters remained within limits considered adequate for species of *Prochilodus* (Fonseca *et al.*, 2010).

#### Sample collection and processing

After 10 weeks, all fish were fasted for 24 h, euthanized as previously described, counted and weighted. Nine fish from each aquarium were randomly sampled, ground, homogenized and frozen (-20°C) until further analysis. Another five fish from each aquarium were randomly sampled, individually weighted, and dissected to obtain liver, visceral fat, and muscle samples. Liver and visceral fat were weighted to calculate hepatosomatic index and visceral fat index. Due to their small muscle samples were pooled to constitute one sample per experimental unit. Muscle samples were frozen (-20°C) until further chemical analysis.

Fish bodies and muscle samples were freeze-dried in a lyophilizer (Model LD1500, Terroni<sup>®</sup>, São Carlos, SP, Brazil) until reaching constant weight for moisture determination. Crude protein content was determined using the micro-Kjeldahl method, crude lipid by the Soxhlet method, ash by combustion at 550°C for 18 h, and crude dietary fiber by acid-base digestion (AOAC, 2000). The nitrogen-free extract of diets was calculated using the formula (NFE = 100 - (moisture + crude protein + crude fat + ash + crude fiber). The gross energy was estimated using standard values for protein (23.6 kJ g<sup>-1</sup>), lipids (39.5 kJ g<sup>-1</sup>) and carbohydrates (17.2 kJ g<sup>-1</sup>) according to NRC (2011). Digestible energy was estimated using standard physiological values for protein (19.7 kJ g<sup>-1</sup>), lipids (34.7 kJ g<sup>-1</sup>), and carbohydrates (11.3 kJ g<sup>-1</sup>).

#### Calculated variables and statistical procedures

Fish growth parameters and feed utilization were calculated as follows: weight gain [WG = final weight of fish - initial weight of fish]; specific growth rate (SGR =  $100 \times [(\ln \text{ final weight of fish} - \ln \text{ initial weight of fish}) \div \text{experimental time in days}]; feed efficiency ratio (FER = WG / total feed intake); protein efficiency ratio (PER = weight gain / total crude protein intake); protein net value [PNV = (final weight of fish × final whole body crude protein content) - (initial weight of fish × initial whole body crude protein content) / total crude protein intake]; hepatosomatic index [HSI = <math>100 \times (\text{liver weight / fish weight})]; and visceral fat index [VFI = <math>100 \times (\text{visceral fat weight / fish weight})].$ 

**Table 1.** Ingredients and chemical composition (dry matter basis) of experimental diets. Assurance levels (kg<sup>-1</sup> product)<sup>1</sup>: vit. A, 1.000.000 UI; vit. D3, 312.500 UI; vit. E, 18.750 UI; vit. K3, 1.250 mg; vit. B, 2.500 mg; vit. B2, 2.500 mg; vit. B6, 1.875 mg; vit. B12, 4 mg; Vitamin C, 31.250 mg; Nicotínic acid, 12.500 mg; Calcium pantothenate, 6.250 mg; Biotin, 125 mg; Folic acid, 750 mg; Choline, 50.000 mg; Inositol, 12.500 mg; Iron sulphate, 6.250 mg; Copper sulfate, 625 mg; Zinc sulfate, 6.250 mg; Manganese sulfate, 1875 mg; Sodium selenite, 13 mg; Calcium iodate, 63 mg; Cobalt sulphate, 13 mg. BHT: butylated hydroxytoluene, CP: crude protein, DE: digestible energy.

			Γ	Dietary cruo	de l	ipid leve	ls		
In gradiants $(\%)$	11%			15%					
Ingredients (%)	I	Dietary c	rude prot	ein		D	ietary cru	ide prote	in
	20%	25%	30%	35%		20%	25%	30%	35%
Corn	60.27	45.19	30.11	16.07		56.11	41.03	25.95	10.87
Fish meal	16.27	20.34	24.41	28.47		16.27	20.34	24.41	28.47
Soybean meal	16.13	26.67	37.22	47.56		17.03	27.57	38.12	48.68
Soybean oil	4.00	4.00	4.00	4.00		8.00	8.00	8.00	8.00
Cellulose	2.27	2.73	3.19	2.84		1.53	1.99	2.45	2.91
Suppl. Vit./Minerals	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00
Vitamin C	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05
BHT	0.02	0.02	0.02	0.02		0.02	0.02	0.02	0.02
Chemical composition (dry	matter b	oasis)							
Moisture (%)	8.6	7.1	7.6	7.5		7.4	8.4	8.6	7.2
Crude protein (%)	22.1	27.6	32.9	38.1		23.0	28.7	32.8	39.1
Crude lipid (%)	12.3	11.9	12.0	12.0		16.7	15.8	15.5	14.4
Ash (%)	7.1	7.7	7.1	7.3		7.6	8.1	8.4	8.2
Crude fiber (%)	5.1	6.1	6.3	6.2		5.3	5.6	6.1	7.4
Nitrogen-free extract (%)	53.5	46.6	38.4	36.4		47.4	41.8	37.2	30.9
Digestible energy (kJ g <sup>-1</sup> )	14.7	14.9	14.9	15.8		15.7	15.8	16.0	16.2
Gross energy (kJ g <sup>-1</sup> )	18.6	18.4	19.2	19.4		19.7	19.7	19.6	19.5
CP:DE ratio (mg kJ <sup>-1</sup> )	15.1	18.6	22.1	24.1		14.7	18.1	20.5	24.2

All data were previously analyzed for verification of normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test). Two-way ANOVA tested the effects of crude protein and lipid levels, and interactions. When significant (P < 0.05) differences were registered, Duncan's multiple range tests were applied. Regression analysis was performed using quadratic and non-linear models. Protein requirement was estimated at the first intersection of the quadratic curve with the plateau of the broken line model (Baker *et al.*, 2002). All statistical analyses were performed using SAS software version 9.1.

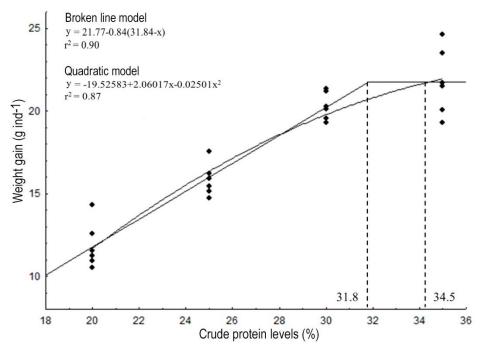
#### RESULTS

Growth performance, feed efficiency, and protein use of curimatã-pacu juveniles were mainly affected (P < 0.05) by dietary crude protein levels (Table 2). The crude protein requirement of *P. argenteus* juveniles was estimated at 32.5% to feed efficiency and 34.5% to weight gain (Figs. 1-2).

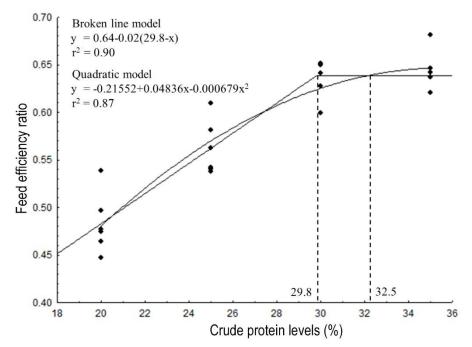
Fish fed with 35% of crude protein showed a low efficiency to use the dietary protein regardless of the lipid level of diets. Indeed, a linear decrease in protein

efficiency ratio of fish (P < 0.05; y = 3.06654 - 0.035x;  $R^2 = 0.72$ ) was recorded due to the increase in the protein levels of diets. On the other hand, increasing dietary crude protein resulted in a cubic model (P <0.05; y = 224.31 - 21.949x +  $0.8287x^2 - 0.01034x^3$ ; R<sup>2</sup> = 0.71) to protein net value of fish, demonstrating that protein retention was similar between fish fed with diets of 25 and 30% crude protein. The hepatosomatic index of fish was negatively influenced by crude protein levels in diets (P < 0.05; y = 1.4332-0.0196x;  $R^2 = 0.53$ ). A significant effect (P < 0.05) of crude lipid levels was registered only on survival and the visceral fat index of fish. Fish fed 35% crude protein: 15% lipid diets showed (P < 0.05) a lower survival than fish fed with other diets. Increasing dietary crude lipid from 11 to 15% proportionally increased (P < 0.05) the visceral fat index in fish. On the other hand, high levels of dietary crude protein resulted (P < 0.05) in the low deposition of visceral fat in fish.

The interaction between crude protein:lipid levels influenced (P < 0.05) only the feed intake of curimatã-pacu juveniles. Fish fed with 11% of dietary lipid increased (P < 0.05) the feed intake proportionally the increasing dietary crude protein from 20 to 35%.



**Figure 1.** The relationship between weight gain and dietary protein level for curimatã-pacu juveniles as described by the second-polynomial and broken line regression model. Protein requirement was estimated at the first intersection of the quadratic curve with the plateau of the broken line model (Baker *et al.*, 2002).



**Figure 2.** The relationship between feed efficiency ratio and dietary protein level for curimatã-pacu juveniles as described by the second-polynomial and broken line regression model. Protein requirement was estimated at the first intersection of the quadratic curve with the plateau of the broken line model (Baker *et al.*, 2002).

However, when lipid level of diets was increased to 15%, there was a similar (P > 0.05) feed intake in fish fed with 30 and 35% crude protein.

The proximate composition of whole body fish from the initial population was 84.2% moisture, 9.7% crude protein, 1.5% crude lipids, and 4.2% ash. Chemical

			D	Dietary crude protein: crude lipid levels	n: crude lipid leve	els				P values	
	20 CP:11 CL	20 CP:11 CL 25 CP: 11 CL	30 CP:11 CL	35 CP:11 CL	20 CP:15 CL	25 CP:15 CL	30 CP:15 CL	35 CP:15 CL	CL	CP	CF×CP
Initial weight (g ind-1)	$3.6 \pm 0.1$	$3.7 \pm 0.1$	$3.6 \pm 0.0$	$3.6 \pm 0.1$	$3.6 \pm 0.1$	$3.7 \pm 0.0$	$3.6 \pm 0.0$	$3.5 \pm 0.1$	0.6249	0.1701	0.5034
Final weight (g ind <sup>-1</sup> )	$15.5 \pm 2.0^{\mathrm{d}}$	$19.7 \pm 1.4^{c}$	$23.9 \pm 1.0^{b}$	$26.8 \pm 1.4^{a}$	$15.4 \pm 0.7^{d}$	$19.3 \pm 0.8^{\circ}$	$23.8\pm0.8^{ m b}$	$23.8 \pm 1.0^{\mathrm{b}}$	0.0860	< 0.0001	0.1568
Weight gain (g ind <sup>-1</sup> )	$11.9 \pm 2.1^{d}$	$16.0 \pm 1.3^{\circ}$	$20.3 \pm 1.0^{b}$	$23.3 \pm 1.5^{a}$	$11.8 \pm 0.7^{d}$	$15.6 \pm 0.8^{\circ}$	$20.3 \pm 0.8^{b}$	$20.3 \pm 1.1^{b}$	0.0976	< 0.0001	0.1685
Specific growth rate (% day)	$2.1 \pm 0.2^{d}$	$2.4 \pm 0.1^{\circ}$	$2.7 \pm 0.1^{b}$	$2.9 \pm 0.1^{a}$	$2.1 \pm 0.1^{d}$	$2.3 \pm 0.1^{\circ}$	$2.7 \pm 0.1^{ab}$	$2.7 \pm 0.1^{ab}$	0.2970	<0.0001	0.4368
Feed efficiency	$0.48 \pm 0.1^{c}$	$0.56 \pm 0.0^{b}$	$0.64 \pm 0.0^{a}$	$0.65 \pm 0.0^{a}$	$0.48 \pm 0.0^{\circ}$	$0.56 \pm 0.0^{b}$	$0.63 \pm 0.0^{a}$	$0.64 \pm 0.0^{a}$	0.6702	< 0.0001	0.9753
Feed intake (g ind <sup>-1</sup> )	$0.35\pm0.0^{ m d}$	$0.40\pm0.0^{ m c}$	$0.45 \pm 0.0^{\mathrm{b}}$	$0.51\pm0.0^{a}$	$0.34\pm0.0^{ m d}$	$0.39 \pm 0.0^{\circ}$	$0.46 \pm 0.0^{b}$	$0.47\pm0.0^{ m b}$	0.1387	<0.0001	0.0457
Protein efficiency ratio	$2.4 \pm 0.2^{a}$	$2.2 \pm 0.2^{ab}$	$2.1 \pm 0.0^{b}$	$1.8 \pm 0.1^{\circ}$	$2.3 \pm 0.1^{ab}$	$2.1 \pm 0.1^{b}$	$2.1 \pm 0.1^{b}$	$1.7 \pm 0.1^{c}$	0.0708	<0.0001	0.7985
Protein net value (%)	$35.3 \pm 2.0^{a}$	$32.3 \pm 1.3^{b}$	$31.7 \pm 0.9^{b}$	$28.5 \pm 1.7^{\circ}$	$32.8 \pm 1.8^{ab}$	$31.5 \pm 1.1^{b}$	$33.2 \pm 0.8^{ab}$	$27.1 \pm 1.2^{\circ}$	0.2047	< 0.0001	0.1464
Survival (%)	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$	$97.8 \pm 3.9^{ab}$	$95.5 \pm 3.9^{b}$	0.0499	0.1817	0.1817
Hepatosomatic index (%)	$1.0 \pm 0.1^{ab}$	$0.9 \pm 0.1^{\text{abc}}$	$0.8 \pm 0.0^{\text{bcd}}$	$0.8 \pm 0.1^{\text{bcd}}$	$1.1 \pm 0.2^{a}$	$1.0 \pm 0.1^{ab}$	$0.8 \pm 0.1^{cd}$	$0.7 \pm 0.1^{d}$	0.8210	0.0010	0.3053
Visceral fat index (%)	$2.2 \pm 0.3^{b}$	$1.5 \pm 0.2^{cd}$	$0.9 \pm 0.1^{e}$	$0.8 \pm 0.1^{\text{bcd}}$	$2.6 \pm 0.1^{a}$	$1.9 \pm 0.4^{\rm bc}$	$1.4 \pm 0.3^{de}$	$1.2 \pm 0.2^{de}$	0.0041	<0.0001	0.5457

dard deviation) of curimatã-pacu juveniles fed diets containing crude protein (CP) and crude	perscripts letters indicate significant differences ( $P \le 0.05$ ) by Duncan's test.
<b>Table 2.</b> Growth, feed utilization and biometric parameters (mean $\pm$ sta	lipid (CL) levels for 70 days. Values in the same column with differe

sition (wet basis) of whole body and muscle (mean $\pm$ standard deviation) of curimatã-pacu juveniles fed diets containing crude protein (CP)	for 70 days. Values in the same column with different superscripts letters indicate significant differences ( $P \le 0.05$ ) by Duncan's test.
Table 3. Chemical composition (wet basis) of whole	and crude lipid (CL) levels for 70 days. Values in the san

5			D	Dietary crude protein: crude lipid levels	n: crude lipid leve	sls				P values	
	20 CP:11 CL	20 CP:11 CL 25 CP:11 CL 30 CP:11 CL		35 CP:11 CL	35 CP:11 CL 20 CP:15 CL 25 CP:15 CL	25 CP:15 CL	30 CP:15 CL 35 CP:15 CL	35 CP:15 CL	CL	CL CP CF×CP	CF×CP
Whole body											
Moisture (%)	$73.74 \pm 1.17^{cd}$	$74.62 \pm 0.12^{abc}$	$75.34 \pm 0.52^{a}$	$74.90 \pm 0.32^{ab}$	$73.45 \pm 0.09^{d}$	$73.94 \pm 0.11^{\text{bcd}}$	$73.41 \pm 0.53^{d}$	$75.15 \pm 0.90^{a}$	0.0148	0.0074	0.0374
Crude protein (%)	$13.60 \pm 0.78^{\circ}$	$13.81 \pm 0.58^{bc}$	$14.27 \pm 0.56^{abc}$	$14.74 \pm 0.42^{ab}$	$13.38 \pm 0.73^{\circ}$	$13.80 \pm 0.75^{bc}$	$15.12 \pm 0.27^{a}$	$15.15 \pm 0.37^{a}$	0.2915	0.0013	0.4220
Crude lipid (%)	$7.49 \pm 0.38^{b}$	$6.45 \pm 0.12^{\circ}$	$5.17 \pm 0.13^{\mathrm{d}}$	$5.64 \pm 0.27^{d}$	$8.10 \pm 0.02^{a}$	$6.75 \pm 0.29^{\circ}$	$6.64 \pm 0.08^{\circ}$	$5.01 \pm 0.51^{d}$	0.0005	<0.0001	0.0002
Ash (%)	$3.82 \pm 0.08$	$3.89 \pm 0.07$	$3.93 \pm 0.12$	$3.81 \pm 0.02$	$3.84 \pm 0.09$	$3.84 \pm 0.05$	$3.89 \pm 0.09$	$3.90 \pm 0.29$	0.9661	0.7366	0.7890
Muscle											
Moisture (%)	$78.21 \pm 0.20$	$78.41 \pm 0.47$	$78.56 \pm 0.38$	$78.56 \pm 0.11$	$78.12 \pm 0.44$	$78.70 \pm 0.37$	$78.51 \pm 0.71$	$78.69 \pm 0.28$	0.6745	0.2472	0.8365
Crude protein (%)	$17.46 \pm 0.80$	$18.05 \pm 1.20$	$18.24 \pm 0.78$	$17.60 \pm 0.19$	$17.28 \pm 0.73$	$17.57 \pm 0.90$	$17.65 \pm 0.60$	$18.30 \pm 0.42$	0.6627	0.5177	0.4639
Crude lipid (%)	$2.77 \pm 0.41^{ab}$	$2.82 \pm 1.15^{ab}$	$2.21 \pm 0.38^{b}$	$2.02 \pm 0.11^{b}$	$3.45 \pm 0.82^{a}$	$2.76 \pm 0.11^{ab}$	$2.32 \pm 0.35^{ab}$	$1.87 \pm 0.33^{\rm b}$	0.5644	0.0169	0.6366
Ash (%)	$1.40 \pm 002$	$1.40 \pm 0.04$	$1.37 \pm 0.02$	$1.38 \pm 0.03$	$1.36 \pm 0.06$	$1.37 \pm 0.03$	$1.36 \pm 0.04$	$1.39 \pm 0.02$	0.2384	0.5831	0.6722

composition data of whole body and fish muscle at the end of the experiment are summarized in Table 3. Whole body lipid of fish decreased linearly (P < 0.05; y = 10.98493 - 0.16744x; R<sup>2</sup> = 0.78) as crude protein in the diet increased. By contrast, an opposite trend was observed for whole body protein of fish, which increased (P < 0.05; y = 11.33841 + 0.10532x; R<sup>2</sup> = 0.51) with increasing dietary crude protein levels. In fish muscle, only the lipid content was affected (P < 0.05) by dietary treatments, decreasing linearly (y = 4.7408 - 0.0805x; R<sup>2</sup> = 0.43) proportional to the increase crude protein of diets.

#### DISCUSSION

In the present study, it was not possible to register the maximum response of growth and feed efficiency by fish in the quadratic model due to the range of dietary protein levels evaluated. Therefore, considering that the quadratic and broken line models could, respectively, over- or underestimate the protein requirement of curimatã-pacu juveniles, we used the methodology proposed by Baker *et al.* (2002). These authors considered it most appropriate to consider nutritional requirement as an intermediate value between the requirements estimated by the broken line and quadratic models. In this case, the requirement is determined at the first point of intersection of the quadratic curve in the broken line response plateau.

The use of dietary protein as an energy source is undesired. Because of this, it is appropriate to express the dietary protein requirement through the protein:energy ratio. Therefore, considering the crude protein requirement estimated for feed efficiency (32.5%) and weight gain (34.5%), the ideal protein: energy ratio ranged 22-23 mg CP kJ<sup>-1</sup> for curimatã-pacu juveniles. These values are within the range (19-27 mg CP kJ<sup>-1</sup>) considered ideal to provide adequate growth for most fish species (NRC, 2011).

Caution is required to compare results among different studies, due to the influence of different factors (*e.g.*, water quality, experimental diets, interspecific differences) on fish performance. However, the interest in aquaculture production of curimatã-pacu is novel and, therefore, data about growth performance of this species in different production systems are scarce. In this study, the fish fed with 35% crude protein: 11% crude lipid diets showed the highest specific growth rate (2.9% per day). This result is 45% (Almeida *et al.*, 2015) and 79% (Santos *et al.*, 2018) higher than the best specific growth rate reported for the same *Prochilodus argenteus* reared, respectively, in ponds and laboratorial conditions. The growth performance of curimatã-pacu juveniles was

also higher than that recorded by Bomfim *et al.* (2005) for *P. affins* with an initial weight of 2.7 g under laboratory conditions. Only juveniles of *P. lineatus* (Galdioli *et al.*, 2002) and *P. mariae* (Visbal *et al.*, 2013) showed specific growth rates higher than those observed for *P. argenteus* in the present study. Thus, it is possible to hypothesize that *P. argenteus* is among the most promising species of the genus *Prochilodus* for aquaculture production.

In general, the increase in dietary lipid results in lower feed consumption, as the fish's energy demand is met quickly and satiety is achieved. Despite this, fish can adjust their feed intake at different ranges of dietary energy, as recorded for pacu (*Piaractus mesopotamicus*) by Bicudo *et al.* (2010) and Nile tilapia (*Oreochromis niloticus*) by Y e *et al.* (2016). The interaction (P < 0.05) between dietary crude protein and lipid levels on feed intake of curimatã-pacu possibly resulted in fish growth through dietary protein increase because controlled feeding (6% of body weight d<sup>-1</sup>) does not allow fish to adjust naturally to the feed intake. This hypothesis is corroborated by the fact that only fish fed with 35% crude protein decrease (P < 0.05) their feed intake when crude lipid increases from 11 to 15%.

Omnivorous fish use energy derived from carbohydrates more efficiently than that derived from dietary lipids, unlike carnivorous species (NRC, 2011). Increasing the lipid content of the diets did not result in an improvement of the curimatã-pacu productive performance (P > 0.05); this suggests that diets with 11% crude lipid were able to supply the energetic requirement of the species. Consequently, the dietary protein sparing effect by curimatã-pacu juveniles due to the increase of the lipid level of the diets was not verified. Aminikhoei et al. (2015) recommend a maximum of 12% lipid for cyprinid diets. For instance, in diets for juvenile grass carp (Ctenopharyngodon idella) and major carp (Catla catla), the maximum recommended lipid levels are 6% (Jin et al., 2013) and 8% (Priva et al., 2005), respectively. Other previous studies with neotropical omnivorous fish also do not register a protein-sparing effect due to the increase in dietary non-protein energy sources (Sá & Fracalossi, 2002; Bomfim et al., 2005; Cotan et al., 2006; Bittencourt et al., 2010).

As reported for Nile tilapia (Koch *et al.*, 2017) and grass carp juveniles (Guo *et al.*, 2015) increasing dietary crude protein reduced the protein net value in curimatã-pacu. In this case, it is assumed that in higher protein diets, dietary energy is mainly from dietary protein (Ebrahimi *et al.*, 2013). Possibly, these diets have a high proportion of amino acids exceeding the minimum requirement of species and because of this were catabolized and used as an energy source and not for protein synthesis (Koch *et al.*, 2017).

In general, cultured fish are fatter than wild fish, and the main sites of lipid body deposition are the coelomic cavity, liver, and muscles (Guo et al., 2015). Curimatãpacu juveniles increased 3-5-fold in body lipid content at the end of the experiment, with approximately onethird being deposited in the muscle. It is known that migratory species such as curimatã-pacu tend to deposit lipid mainly as visceral fat (Bicudo et al., 2010), this explains the high visceral fat and hepatosomatic index due to the increase of dietary lipid. On the other hand, increasing crude protein levels of the diets reduced (P < 0.05) the hepatosomatic index, visceral fat index, and lipid deposition in the whole body and muscle. Somatic indexes are an indirect way of measuring energy retention in fish nutrition studies (NRC, 2011). Therefore, the higher lipid deposition recorded in fish fed with lower concentrations of dietary crude protein is supposed to be associated with the broader availability of digestible carbohydrates in these diets.

In conclusion, it is recommended that curimatãpacu juveniles between 3 and 27 g of weight could be fed with diets containing 32.5-34.5% of CP and 11% of lipids under production scale.

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#### REFERENCES

- Almeida, E.O., Santos, B.R., Coelho-Filho, P.A., Cavalcante-Junior, A., Souza, A.P.L. & Soares, E.C. 2015. Polyculture of curimatã pacu and freshwater prawn. Boletim do Instituto de Pesca, 41(2): 271-278.
- Aminikhoei, Z. Choi, J. & Lee, S-M. 2015. Optimal dietary protein and lipid levels for growth of juvenile Israeli carp *Cyprinus carpio*. Fisheries and Aquatic Science, 18(3): 265-271.
- Association of Official Analytical Chemists (AOAC). 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Maryland.
- Baker, D.H., Batal, A.B., Parr, T.M., Augspurger, N.R. & Parsons, C.M. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poultry Science, 81(4): 485-494.

- Bittencourt, F., Feiden, A., Signor, A.A., Boscolo, W.R. & Freitas, J.M.A. 2010. Protein and energy in rations for piavuçu fingerlings. Revista Brasileira de Zootecnia, 39(12): 2553-2559.
- Bicudo, A.J.A., Sado, R.Y. & Cyrino, J.E.P. 2010. Growth performance and body composition of pacu *Piaractus mesopotamicus* (Holmberg, 1887) in response to dietary protein and energy levels. Aquaculture Nutrition, 16(12): 213-222.
- Bomfim, M.A.D., Lanna, E.A.T., Serafini, M.A., Ribeiro, F.B. & Pena, K.S. 2005. Crude protein and digestible energy in the diets for curimbatá fingerlings (*Prochilodus affins*). Revista Brasileira de Zootecnia, 34(6): 1795-1806.
- Cyrino, J.E.P., Bicudo, A.J.A., Sado, R.Y., Borghesi, R. & Dairiki, J.K. 2010. Fish farming and the environment - the use of environmental friendly feeds in fish culture. Revista Brasileira de Zootecnia, 39: 68-87.
- Cotan, J.L.V., Lanna, E.A.T., Bomfim, M.A.D., Donzele, J.L., Ribeiro, F.B. & Serafini, M.A. 2006. Dietary levels of digestible energy and crude protein for lambari tambiú fingerlings. Revista Brasileira de Zootecnia, 35(3): 634-640.
- Ebrahimi, G., Ouraji, H., Firouzbakhsh, F. & Makhdomi, C. 2013. Effect of dietary lipid and protein levels with different protein to energy ratios on growth performance, feed utilization, and body composition of *Rutilus frisii kutum* (Kamenskii, 1901) fingerlings. Aquaculture Research, 44(9): 1447-1458.
- Fonseca, F.A.L., Ituassú, D.R., Cavero, B.A.S. & Bordinhon, A.M. 2010. Cultivation of curimatã (*Prochilodus* spp). In: Baldisseroto, B. & de Gomes, L.C. (Eds.). Native species for fish farming in Brazil. Editora UFSM, Santa Maria, pp. 57-66.
- Galdioli, E.M., Hayashi, C., Soares, C.M., Furuya, V.R.B.
  & Faria, A.C.E.A. 2002. Replacement of soybean meal protein by canola meal protein in "curimbatá" (*Prochilodus lineatus* V.) fingerling diets. Revista Brasileira de Zootecnia, 31(2): 552-559.
- Guo, X., Liang, X.F., Fang, L., Yuan, X., Zhou, Y., Zhang, J. & Li, B. 2015. Effects of dietary non-protein energy source levels on growth performance, body composition and lipid metabolism in herbivorous grass carp (*Ctenopharyngodon idella* Val.). Aquaculture Research, 46(5): 1197-1208.
- Hernández, G., González, J., Alfonso, E. & Salmeron, Y. 2010. Effects of the relation energy/protein in the larvas of coporo (*Prochidolus mariae*). Zootecnia Tropical, 28(2): 173-182.
- Jin, Y., Tian, L., Zeng, S., Xie, S., Yang, H., Liang, G. & Liu, Y. 2013. Dietary lipid requirement on nonspecific immune responses in juvenile grass carp

(*Ctenopharyngodon idella*). Fish and Shellfish Immunology, 34(5): 1202-1208.

- Khan, M.A. & Abidi, S.F. 2012. Effect of varying proteinto-energy ratios on growth, nutrient retention, somatic indices, and digestive enzyme activities of singhi, *Heteropneustes fossilis* (Bloch). Journal of the World Aquaculture Society, 43(4): 490-501.
- Koch, J.F.A., Barros, M.M., Teixeira, C.P., Carvalho, P.L.P.F., Fernandes-Junior, A.C., Cintra, F.T. & Pezzato, L.E. 2017. Protein-to-energy ratio of 21.43 g MJ<sup>-1</sup> improves growth performance of Nile tilapia at the final rearing stage under commercially intensive rearing conditions. Aquaculture Nutrition, 23(3): 560-570.
- National Research Council (NRC). 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington.
- Pereira-Arantes, F., Santos, H.B., Rizzo, E., Sato, Y. & Bazzoli, N. 2011. Collapse of the reproductive process of two migratory fish (*Prochilodus argenteus* and *Prochilodus costatus*) in the Três Marias Reservoir, São Francisco River, Brazil. Journal of Applied Ichthyology, 27(3): 847-853.
- Priya, K., Pal, A.K., Sahu, N.P. & Mukherjee, S.C. 2005. Effect of dietary lipid sources on growth, enzyme activities and immuno-hematological parameters in *Catla catla* fingerlings. Asian-Australasian Journal of Animal Science, 18(11): 1609-1616.

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- Santos, A.E., Pedreira, M.M., Santos, T.G., Moura, G.D.S., Santos, J.C.E. & Silva, R.C. 2016. Development of the digestive system in larvae of the neotropical fish *Prochilodus argenteus* (Characiformes, Prochilodontidae). Acta Scientiarum, 38(1): 9-16.
- Sá, M.V.C. & Fracalossi, D.M. 2002. Dietary protein requirement and energy to protein ratio for piracanjuba (*Brycon orbignyanus*) fingerlings. Revista Brasileira de Zootecnia, 31(1): 1-10.
- Santos, E.L., Oliveira, W.D.S., Soares, E.C., Lima, M.R., Silva, L.L.A., Machado, S.S., Silva, J.M., Silva, C.F. & Silva, F.F. 2018. Exigência de proteína bruta para juvenis de curimatã-pacu. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 70(3): 921-930.
- Soares, E.C., Bruno, A.M.S.S., Lemos, J.M. & Santos, R.B. 2011. Ictiofauna and shing in the surroundines of Penedo, Alagoas. Biotemas, 24(1): 61-67.
- Visbal, B.T.E., Morillo, S.M., Altuve, P.D., Aguirre, P. & Medina, G.A.L. 2013. Nivel óptimo de proteínas en la dieta para alevines de *Prochilodus mariae*. Revista Chilena de Nutrición, 40: 141-146.
- Ye, J.D., Chen, J.C. & Wang, K. 2016. Growth performance and body composition in response to dietary protein and lipid levels in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) subjected to routine and temporally restricted feeding regimes. Journal of Applied Ichthyology, 32(2): 332-338.