

## Protein to energy ratios and cost, performance, and ammonia excretion in juvenile jundiá (*Rhamdia quelen*), South American catfish

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Edited by: Melissa Izabel Hannas

Received September 15, 2020

Accepted November 04, 2020

**ABSTRACT:** The determination of nutritional requirements, based on diets administered in practice, considers physiological specificities and sustainability are essential to the development of suitable aquaculture technologies for novel fish species. The growth of jundiá (*Rhamdia quelen* Quoy and Gaimard) juveniles (31.54 ± 4.92 g) fed five diets comprising increasing levels of digestible protein (DP: 24, 29, 34, 39, and 44 %) and digestible energy (DE: 12.13, 13.39, 13.81, 14.64, and 15.06 MJ kg<sup>-1</sup>) with DP:DE ratios: 20, 22, 25, 27, and 29 g MJ<sup>-1</sup>, respectively, was evaluated. Each diet was fed to three groups of 25 fish for 75 days. Regression analysis showed that the different DP:DE ratios affected both growth and economic performance as well as excretion of total ammonia nitrogen. Fish fed diets with DP:DE ratios between 22 and 27 g MJ<sup>-1</sup> achieved a higher daily weight gain, superior apparent net protein utilization, feed conversion rates, and lower feed costs. For the economically viable and environmentally friendly farming of jundiá juveniles in the initial grow-out stage (30 to 80 g average weight), the best DP:DE ratio was 25 g MJ<sup>-1</sup> DP:DE (34.05 % DP and 13.81 MJ kg<sup>-1</sup> DE).

**Keywords:** DP:DE, nutritional requirements, nitrogen excretion, practical diets

### Introduction

Protein, a high-cost nutrient in aquafeeds, is a key determinant of fish growth and, consequently, of the profitability of aquaculture systems (Guy et al., 2018). The optimal level of this nutrient in feeds depends on several factors, including its amino acid profile, its digestibility (Glencross et al., 2007; Mo et al., 2019), and the associated availability of non-protein energy sources (Steffens, 1989; Mock et al., 2019; National Research Council, 2011). A delicate balance between the amounts of protein and energy (P:E) in aquafeeds is needed, considering that feed consumption is largely determined by the energy content provided in the diet (NRC, 2011).

Jundiá (*Rhamdia quelen* Quoy and Gaimard) is a fish species with husbandry potential found in subtropical regions such as southern Brazil, Argentina, and Uruguay. However, jundiá farming lacks species-specific technologies and the species is being commercially farmed using aquafeeds developed for other omnivorous fish, such as the Nile tilapia (*Oreochromis niloticus* L.). However, jundiá is not a typical omnivore, presenting a short intestine (Gominho-Rosa et al., 2015; Rodrigues et al., 2012) and having less ability to utilize dietary carbohydrates than Nile tilapia has (Moro et al., 2010; Oliveira Filho and Fracalossi, 2006; Rodrigues et al., 2012).

The protein requirements of jundiá have already been determined by dose-response studies; however, the values obtained differ considerably, ranging from 32 to 51 % crude protein (Melo et al., 2006; Meyer and Fracalossi, 2004; Piedras et al., 2006; Reidel et al., 2010; Salhi et al., 2004; Signor et al., 2004; Tessaro et al., 2012). Among other reasons, this wide range is a factor in the determination of the protein requirement without

a concomitant evaluation of the energy availability on dietary intake and protein utilization. In addition, the few studies that have considered the amount of energy in the diets used semi-purified ingredients (Meyer and Fracalossi, 2004; Salhi et al., 2004), which have limited application in the feed industry as a basis for formulating appropriate aquafeeds.

We aimed to analyze the performance of jundiá juveniles fed practical diets containing different digestible protein/digestible energy (DP:DE) ratios and whose formulation was based on the digestible nutrients needed by this species in the initial grow-out phase. In addition, we evaluated the cost of these diets in relation to weight gain and ammonia production by the fish with the aim of providing recommendations for the aquafeed industry and fish farmers.

### Materials and Methods

This experiment was carried out from May to Aug 2015 in Florianópolis, Santa Catarina, Brazil (27°43' S, 48°30' W', altitude of 2.74 m.).

#### Experimental diets

Five diets with increasing concentrations of DP: 24, 29, 34, 39, and 44 % and DE: 12.13, 13.39, 13.81, 14.64, and 15.06 MJ kg<sup>-1</sup>, yielding DP:DE ratios of 20, 22, 25, 27, and 29 g MJ<sup>-1</sup>, respectively, (Table 1) were formulated. The essential amino acid requirements reported for jundiá by Montes-Girao and Fracalossi (2006) were used in the formulation of these diets. The diet with DP:DE 20 g MJ<sup>-1</sup> was supplemented with synthetic amino acids to ensure the minimum dietary requirements of lysine and methionine.

**Table 1** – Formulation, cost and chemical composition (expressed on a dry matter basis) of five experimental diets fed to jundiá ( *Lepomis microlophus* ) juveniles for 75 days.

Ingredient (%)	Digestible protein (DP, g kg <sup>-1</sup> ): Digestible energy (DE, MJ kg <sup>-1</sup> ) DP:DE ratio (g MJ <sup>-1</sup> )				
	20	22	25	27	29
Soy protein concentrate <sup>1</sup>	10.00	16.50	22.10	27.60	33.20
Poultry by-product meal <sup>2</sup>	10.60	16.40	22.00	27.70	33.20
Salmon by-product meal <sup>3</sup>	18.00	18.00	18.00	18.00	18.00
Corn <sup>4</sup>	58.90	46.40	35.40	23.90	13.40
Soybean oil	0.90	1.70	1.50	1.80	1.20
Vitamin-mineral premix <sup>5</sup>	1.00	1.00	1.00	1.00	1.00
L-Lysine <sup>6</sup>	0.30	–	–	–	–
DL-Methionine <sup>6</sup>	0.40	–	–	–	–
Chemical composition (%)					
Dry matter	89.00	89.57	89.75	89.91	90.25
Digestible protein	24.10	28.96	34.05	39.00	43.87
Digestible energy (MJ kg <sup>-1</sup> )	12.13	13.39	13.81	14.64	15.06
Crude fat	7.45	7.75	8.15	8.71	8.67
Acid detergent fiber	8.66	9.27	8.02	8.45	8.32
Ash	10.53	7.24	9.21	11.80	11.72
Nitrogen-free extract <sup>7</sup>	32.73	28.53	22.43	12.56	7.49
Diet cost (US\$ kg <sup>-1</sup> ) <sup>8</sup>	2.76	2.44	2.67	2.93	3.22

<sup>1</sup>Importação, Exportação e Indústria de Óleos (Paraná, Brazil); <sup>2</sup>Doux-Frangosul (Rio Grande do Sul, Brazil); <sup>3</sup>Tectron Nutrição Animal (Paraná, Brazil); <sup>4</sup>Bunge Alimentos (São Paulo, Brazil); <sup>5</sup>Raguife Indústria e Comércio (São Paulo, Brazil) composition kg<sup>-1</sup>: folic acid 1200 mg, antioxidant 5000 mg, biotin 200 mg, coline 100 000 mg, cobalto 80 mg, copper 3500 mg, iron 20 000 mg, iodine 160 mg, inositol 25 000 mg, manganese 10 000 mg, niacin 20 000 mg, selenium 100 mg, vitamin (vit) A 2400 000 UI, vit B<sub>1</sub> 4000 mg, vit B<sub>2</sub> 4000 mg, vit B<sub>6</sub> 3500, vit B<sub>12</sub> 8000 mg, vit B<sub>3</sub> 4000 mg, vit B<sub>5</sub> 10 000 mg, vit B<sub>9</sub> 3500 mg, vit C 60 000 mg, vit D<sub>3</sub> 600 000 UI, vit E 30 000 UI, vit K<sub>3</sub> 3000 mg, zinc 24 000 mg; <sup>6</sup>Biolys e DL-Methionine (Evoñik Degussa Brazil Ltda, São Paulo, Brazil); <sup>7</sup>Nitrogen-free extract = 100 – (% crude protein + % fat + % ash + % fiber); <sup>8</sup>1 US\$ dollar = 3.25 Brazilian reais

The practical ingredient values for digestible protein, essential amino acids, and energy already defined for jundiá were used to formulate the experiment diets (Freitas, 2015; Oliveira Filho and Fracalossi, 2006). The inclusion ratios of the major protein sources (soy protein concentrate and poultry by-product meal) varied from diet to diet (Table 1) whereas a fixed amount of salmon by-product meal was included in all diets to ensure a balance of essential amino acids, as well as an appropriate n-3: n-6 fatty acid ratio. The amount of soybean oil included in the experimental diets varied slightly (0.9 to 1.8 %) and the DP:DE ratios were maintained mainly by the inclusion of ground corn as a carbohydrate source following the approach used in previous jundiá studies (Gominho-Rosa et al., 2015).

The dry ingredients were ground to 1 mm particles in a hammer mill and screened through a 600 µm mesh. A vitamin-mineral premix was combined with the fine dry ingredients and homogenized for 10 min. Soybean oil was added to the resultant batter which was mixed for a further 10 min. The moisture content of the mixture was adjusted to 20–25 % using a moisture-determining scale. The mixture was then extruded at 100 °C in a 3 mm matrix, and the resulting pellets were dried at 55 °C in a convection oven until the moisture content reached 10 %. The experimental feeds were stored at –20 °C until used.

### Experiment conditions

Approximately 600 jundiá juveniles (average weight

31.54 ± 4.92 g) were acclimatized in 1000 L circular tanks for 10 days. Thereafter, the fish were anesthetized with clove oil and an alcohol solution (80 mg L<sup>-1</sup>) diluted in water (0.4 mL L<sup>-1</sup>) and weighed individually. Groups of 25 individuals were randomly assigned to 15 experimental units (70 L tanks) providing five treatments with three replicates each. The tanks were connected to a closed water recirculation system, equipped with a biofilter, mechanical filter, continuous aeration, and temperature control. The mean water flow rate in each tank was 1 L min<sup>-1</sup> and the salinity of the water was maintained at a concentration of 2 g L<sup>-1</sup> to prevent Ichthyophthiriasis (Baldisserotto et al., 2010). The photoperiod was 12 h light (07h00 to 19h00).

The 75-day feeding trial began after the fish had become acclimatized to the diets and management routines for two weeks. The experiment diets were offered daily at 09h00 and 16h00, and the fish were fed until apparent satiety. Daily feed intake per tank was recorded by calculating the difference in weight of food before the first and after the second feeding.

### Weight monitoring, determination of performance variables, and body composition sampling

At two-week intervals, the fish in each tank were fasted for 24 h, counted and weighed individually. The following variables were determined: (1) daily weight gain (DWG = (final weight – initial weight) / days of experiment), (2) specific growth rate (SGR = (ln (final

weight) - ln (initial weight))  $\times$  100 / days of experiment), (3) feed conversion ratio (FCR = dry feed consumed / (wet final weight - wet initial weight)), and (4) daily dietary intake (DDI = [dry matter consumption / (final weight + initial weight) / 2] / days of experiment  $\times$  100).

At the start of the experiment, two samples of 20 fish were collected for determination of initial body composition and five fish per experiment unit (15 fish per treatment) were collected for analysis of final body composition after 75 days. Apparent net protein utilization (ANPU) was calculated as ANPU = 100  $\times$  (final body protein  $\times$  final weight - initial body protein  $\times$  initial weight) / protein consumption in the dry matter. The remaining fish in each experiment unit (60 fish per treatment) were weighed individually, their viscera removed and weighed, and their livers separated and weighed to determine the viscerosomatic (VSI = (viscera weight / total animal weight)  $\times$  100) and hepatosomatic (HSI = (liver weight / total animal weight)  $\times$  100) indexes, respectively. The fish were euthanized with an overdose of clove oil and alcohol solution. All fish were handled in accordance with the Ethics Committee on Animal Use of the Federal University of Santa Catarina, and fish management and sampling were approved according to protocol No. PP00815 CEUA/UFSC. Animal handling and stress were minimized during the study.

### Diet cost evaluation

The costs of the experiment diets were determined based on the market price of the ingredients. The following indices were used for the economic evaluation of the five experiment diets: (1) average cost of feed (ACF = feed consumed  $\times$  price of diet / gain in weight), (2) economic efficiency index (EEI = 100  $\times$  lowest ACF between diets / ACF of diet assessed), and (3) cost index (CI = 100  $\times$  ACF diet / lowest ACF between diets). These indices were proposed by Bellaver et al. (1985) and Barbosa et al. (1992).

### Diet and water analyses

The experiment diets were analyzed following AOAC (2012) methodologies. Dry matter was determined by oven drying at 105 °C until constant weight (method 950.01) and mineral matter by incineration at 550 °C (method 942.05). Lipid content was determined by ether extraction using a Soxhlet extractor, after acid hydrolysis in the case of the feeds (method 920.39C), and crude protein by Kjeldahl N  $\times$  6.25 (method 945.01). The energy content was analyzed using a calorimeter following the method of Potter and Matterson (1960), and the fiber content by digestion in neutral detergent following the method proposed by Van Soest (1994). Fish body composition was analyzed following the same methodologies, except for the analysis of fiber and the acid hydrolysis prior to ether extraction which were not performed.

Daily monitoring of water quality indicators (temperature, dissolved oxygen, salinity, and pH) was conducted after the 16h00 feed using a multiparameter probe. Hardness, alkalinity, and nitrogen compounds (nitrite and total ammonia nitrogen) were measured every 15 days using a photocolorimeter. The mean temperature ( $26.90 \pm 0.12$  °C), dissolved oxygen, ( $6.40 \pm 0.08$  mg L<sup>-1</sup>), salinity ( $1.90 \pm 0.03$  g L<sup>-1</sup>), pH ( $6.80 \pm 0.04$ ), hardness ( $93.20 \pm 1.46$  mg L<sup>-1</sup> CaCO<sub>3</sub>), total alkalinity ( $22.33 \pm 1.85$  mg L CaCO<sub>3</sub>), and ammonia ( $0.21 \pm 0.02$  mg L<sup>-1</sup>) were within the optimal range for jundiá (Baldisserotto et al., 2010). The nitrite content remained close to zero throughout the feeding trial.

During the final week of the experiment, the concentration of total ammonia nitrogen (TAN) in each tank was measured at short intervals to determine the daily TAN curve for each treatment. For the TAN determination, the recirculating system was turned off during the sampling period; however, water aeration continued. Beginning one and a half hours after each feeding (09h00 and 16h00), water samples (500 mL) were collected from each of the 15 experimental units at half hour intervals for a total of 10 h. The water samples (n = 300) were immediately vacuum filtered (< 10 mm Hg) in a glass fiber filter (1.2  $\mu$ m) for subsequent colorimetric determination using a spectrophotometer following the methodology described by Valderrama (1981).

### Statistical analyses

A one-way analysis of variance (ANOVA;  $\alpha = 0.05$ ) was used to detect differences between the treatments. All studied variables presented normality and homogeneity of variance. Polynomial regressions were plotted to determine the optimal levels for each variable. All statistical analyses were performed in RStudio.

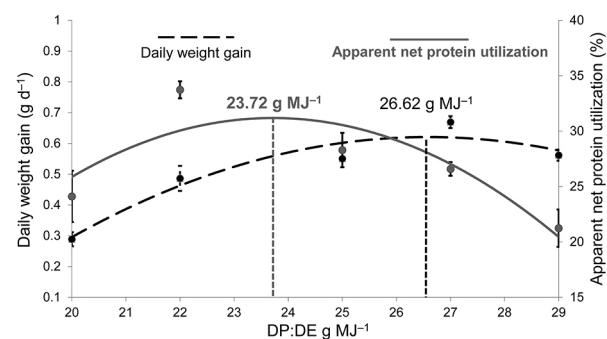
## Results

There was no fish mortality during the study and the different DP:DE dietary ratios affected most of the performance variables and cost indices (Table 2). Final weight, DWG, SGR, and DDI increased, while FCR improved with the DP:DE ratio increasing up to and including 27 g MJ<sup>-1</sup>. The highest ANPU was supported by the diet containing 22 g MJ<sup>-1</sup> DP:DE, and there was a reduction in ANPU values as the DP:DE ratio of the diets increased further. Regression analysis showed that the best daily weight gain was achieved with a ratio of 26.62 g MJ<sup>-1</sup> DP:DE in the diet (38.64 % DP and 14.86 MJ kg<sup>-1</sup> DE). However, the highest ANPU corresponded with a lower DP:DE ratio of 23.72 g MJ<sup>-1</sup>, which equates to a diet containing 32.23 % DP and 13.46 MJ kg<sup>-1</sup> DE (Figure 1). The cost of the diets increased proportionally with the increase in DP and DE (Table 2) except for the 20 g MJ<sup>-1</sup> DP:DE diet, which was supplemented with synthetic amino acids.

**Table 2** – Effects of different digestible protein: digestible energy (DP:DE) ratios on performance and economic variables in culture of jundiá (*Rhamdia quelen*) juveniles for 75 days.

Variables <sup>1</sup>	Digestible protein (DP, g kg <sup>-1</sup> ): Digestible energy (DE, MJ kg <sup>-1</sup> ) DP:DE ratio (g MJ <sup>-1</sup> )					Best DP:DE
	20	22	25	27	29	
Final weight <sup>2</sup> (g)	53.55 ± 2.61	68.18 ± 3.82	71.48 ± 0.71	82.10 ± 2.61	73.66 ± 1.91	26.74
Daily weight gain (g d <sup>-1</sup> )	0.29 ± 0.02	0.49 ± 0.04	0.55 ± 0.01	0.67 ± 0.02	0.56 ± 0.02	26.62
Specific growth rate (% d <sup>-1</sup> )	0.69 ± 0.03	1.02 ± 0.05	1.15 ± 0.02	1.26 ± 0.01	1.13 ± 0.01	26.39
Daily dietary intake (% weight d <sup>-1</sup> )	1.04 ± 0.02	1.16 ± 0.04	1.27 ± 0.03	1.39 ± 0.02	1.36 ± 0.04	29.57
Feed conversion ratio	1.54 ± 0.06	1.20 ± 0.02	1.17 ± 0.03	1.18 ± 0.02	1.28 ± 0.02	25.38
Economic efficiency index (%)	66.84 ± 2.51	97.41 ± 1.29	90.77 ± 2.59	81.88 ± 1.38	69.72 ± 2.69	24.30
Cost index (%)	150.05 ± 5.87	102.69 ± 1.35	110.35 ± 3.20	122.20 ± 2.05	143.85 ± 5.34	24.39

<sup>1</sup>Mean (± standard error) of three replicates. The polynomial regression analysis was significant (< 0.001) for the variables, generating the following equations: final weight:  $y = -0.52x^2 + 27.68x - 292.41$  ( $R^2 = 0.77$ ); daily weight gain:  $y = -0.01x^2 + 0.39x - 4.63$  ( $R^2 = 0.86$ ); specific growth rate:  $y = -0.01x^2 + 0.68x - 7.69$  ( $R^2 = 0.93$ ); daily dietary intake:  $y = -0.004x^2 + 0.221x - 1.891$  ( $R^2 = 0.87$ ); feed conversion ratio:  $y = 0.01x^2 - 0.65x + 9.36$  ( $R^2 = 0.76$ ); economic efficiency index:  $y = -1.28x^2 + 62.31x - 661.84$  ( $R^2 = 0.75$ ); cost index:  $y = 2.07x^2 - 101.14x + 1337.13$  ( $R^2 = 0.77$ ). <sup>2</sup>Initial weight:  $31.54 \pm 4.92$  g.

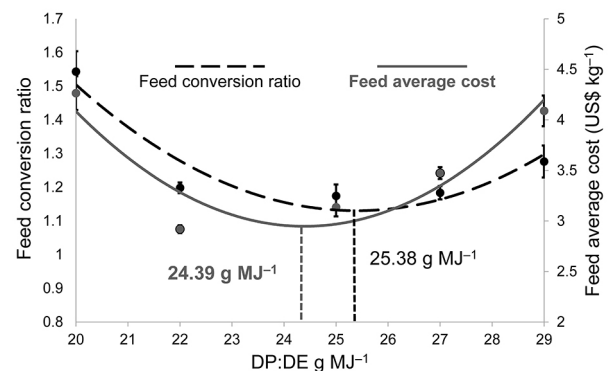


**Figure 1** – Apparent net protein utilization ( $y = -0.38x^2 + 18.24x - 185.17$ ;  $R^2 = 0.59$ ;  $p < 0.0001$ ) and daily weight gain ( $y = -0.01x^2 + 0.39x - 4.63$ ;  $R^2 = 0.86$ ;  $p < 0.0001$ ) in jundiá (*Rhamdia quelen*) juveniles fed diets containing different digestible protein: digestible energy (DP:DE) ratios for 75 days.

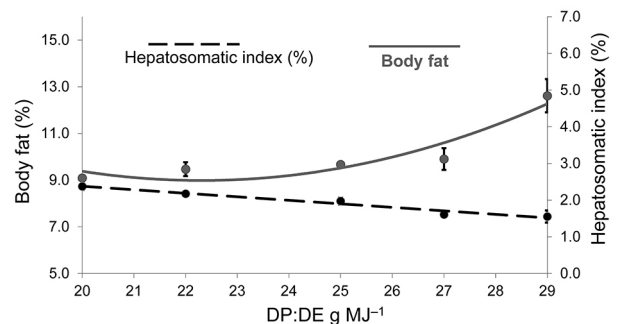
These findings indicate that diet cost, weight gain, and FCR significantly influenced the other economic indices. The best FCR was estimated for fish fed a diet with a DP:DE ratio of 25.38 g MJ<sup>-1</sup> (36.08 % DP and 14.03 MJ kg<sup>-1</sup> DE), whereas the lowest ACF was for a diet with a DP:DE ratio of 24.39 g MJ<sup>-1</sup> (33.83 % DP and 13.65 MJ kg<sup>-1</sup>) (Figure 2).

Moisture content, ash, crude protein, and the VSI were not altered by variation in the DP:DE ratio of the diets (Table 3), whereas body fat and HSI were significantly affected. An increase in fat content and a decrease in the HSI of jundiá was observed in response to the increasing DP:DE ratio in the diets (Figure 3).

Total ammonia nitrogen concentration, monitored during the last week of the feeding trial, increased linearly with an increase in protein concentration associated with the increase in DP:DE ratio (Figure 4). The highest value (0.49 mg TAN kg<sup>-1</sup>) was obtained when fish were fed the 29 g MJ<sup>-1</sup> DP:DE diet. The mean excretion of ammonia was estimated at 0.32 and 0.35 mg TAN kg<sup>-1</sup> body weight for fish fed diets containing DP:DE ratios of 23.72 and 24.39 g MJ<sup>-1</sup>, respectively.



**Figure 2** – Average feed cost ( $y = 0.06x^2 - 2.88x + 38.05$ ;  $R^2 = 0.77$ ;  $p < 0.0001$ ) and feed conversion ratio ( $y = 0.01x^2 - 0.65x + 9.36$ ;  $R^2 = 0.76$ ;  $p < 0.0001$ ) in jundiá (*Rhamdia quelen*) juveniles fed diets containing different digestible protein: digestible energy (DP:DE) ratios for 75 days.



**Figure 3** – Body fat ( $y = 0.07x^2 - 3.27x + 45.47$ ;  $R^2 = 0.73$ ;  $p < 0.05$ ) and hepatosomatic index ( $y = -0.1x + 4.3$ ;  $R^2 = 0.81$ ;  $p < 0.0001$ ) in jundiá (*Rhamdia quelen*) juveniles fed diets containing different digestible protein: digestible energy (DP:DE) ratios for 75 days.

## Discussion

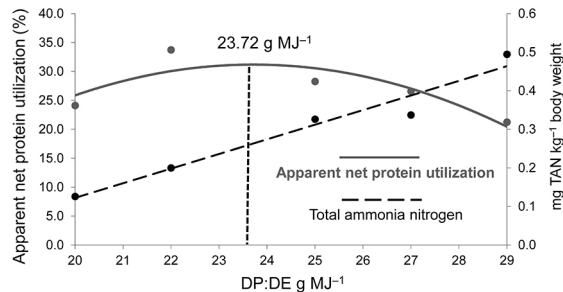
Most variables evaluated in this study reflect that jundiá responded to variations in the DP:DE ratio of



**Table 3** – Body composition (wet matter basis) and viscerosomatic index of jundiá (*Rhamdia quelen*) juveniles<sup>1</sup> fed diets containing different digestible protein: digestible energy (DP:DE) ratios for 75 days.

Variable <sup>2</sup> (%)	Digestible protein (DP, g kg <sup>-1</sup> ): Digestible energy (DE, MJ kg <sup>-1</sup> ) DP:DE ratio (g MJ <sup>-1</sup> )					p value
	20	22	25	27	29	
Moisture	70.97 ± 0.16	70.49 ± 0.12	69.76 ± 0.36	70.23 ± 0.36	69.18 ± 2.02	0.9886
Crude protein	14.40 ± 0.16	15.28 ± 0.03	14.60 ± 0.23	15.18 ± 0.20	15.03 ± 0.30	0.3162
Ash	5.97 ± 0.05	5.49 ± 0.05	6.31 ± 0.56	5.42 ± 0.03	4.80 ± 0.03	0.0629
Viscerosomatic index	8.55 ± 0.12	8.84 ± 0.19	8.43 ± 0.08	8.84 ± 0.24	8.78 ± 0.36	0.8701

<sup>1</sup>Initial weight: 31.54 ± 4.92 g. <sup>2</sup>Mean (± standard error) of three replicates. The polynomial regression analyses were not significant ( $p > 0.05$ ).



**Figure 4** – Apparent net protein utilization ( $y = -0.38x^2 + 18.24x - 185.17$ ;  $R^2 = 0.59$ ;  $p < 0.0001$ ) and the concentration of total ammonia nitrogen (TAN) in the water ( $y = -0.04x - 0.63$ ;  $R^2 = 0.86$ ;  $p < 0.0001$ ) of tanks stocked with jundiá (*Rhamdia quelen*) juveniles fed diets containing different digestible protein: digestible energy (DP:DE) ratios for 75 days.

the experiment diets. The best fish performance results were obtained for diets with DP:DE ratios between 22 and 27 g MJ<sup>-1</sup>, corresponding to a range of 29 to 39 % DP and 13.39 to 14.64 MJ kg<sup>-1</sup> DE, respectively.

Fish fed diets containing the extreme DP:DE ratios (20 and 29 g MJ<sup>-1</sup>) showed lower weight gain and ANPU than those which were fed the other diets. Fish that were fed the diet with 29 g MJ<sup>-1</sup> DP:DE exhibited the highest excretion of ammonia and the highest accumulation of body fat in comparison to fish that were fed the other diets. These findings suggest that the protein level of this diet is above that required by this species and is unbalanced in relation to the energy content. Under these circumstances, it is likely that amino acids are catabolized to meet energy needs, with a consequent reduction in growth rate, and surplus protein is stored as body fat which, in addition, could also be excreted in the form of nitrogen (Mock et al., 2019; NRC, 2011; Steffens, 1989).

Studies where jundiá was fed diets containing increasing protein levels and decreasing nitrogen-free extract (NFE, an indicator of the digestible carbohydrate content in the diet) have reported increased activity of digestive and liver enzymes related to amino acid and carbohydrate metabolism, as well as increased concentrations of free amino acids, ammonia, and urea in the plasma and liver (Melo et al., 2006; Melo et al., 2012; Melo et al., 2016). Such metabolic responses could

be related to the use of dietary protein as an energy source because of the excess of this nutrient, and the DP:DE ratio imbalance, both of which could explain the results observed in this study for fish fed the 29 g MJ<sup>-1</sup> DP:DE ratio diet. This diet had the highest protein and energy levels of those evaluated and contained only a small amount of corn (13.4 %), resulting in an NFE of only 7.49 %. This relatively low availability of a non-protein energy source, coupled with the high protein concentration of this diet, may have favored the use of protein as an energy supply to the detriment of protein synthesis, resulting in a relatively small increase in growth.

The diet with the lowest DP:DE ratio (20 g MJ<sup>-1</sup>), which contained the highest corn inclusion (58.9 %), affected fish growth negatively. Fish fed this diet showed the lowest values for all the performance variables evaluated. Studies report that jundiá is not a typical omnivore and has a low tolerance to dietary carbohydrates (Gominho-Rosa et al., 2015; Moro et al., 2010; Oliveira Filho and Fracalossi, 2006; Rodrigues et al., 2012). The NFE (32.73 %) in the diet with a 20 g MJ<sup>-1</sup> DP:DE ratio is close to the tolerance limit reported for this species where the inclusion of 30 % cornstarch in the diet (about 37 % NFE) promoted the accumulation of free fatty acids and triacylglycerols in the liver of these fish (mean weight 24 g), promoting high HSI values and hepatic steatosis. It has also been reported that diets rich in NFE favor the accumulation of hepatic glycogen in jundiá (Moro et al., 2010). Although these metabolic intermediates were not evaluated in the present study, fish fed the 20 g MJ<sup>-1</sup> DP:DE ratio diet exhibited the highest HSI values, suggesting that the effects reported in these previous studies may also have occurred in jundiá fed this diet.

The likely negative effects of excess non-protein energy seemed to impair the consumption and weight gain of fish fed the 20 g MJ<sup>-1</sup> DP:DE ratio diet. It is known that voluntary feed intake is essentially regulated by digestible energy in relation to the amount of protein in the diet (NRC, 2011) and this was observed in the present study. The diet with the lowest DP:DE ratio resulted in a considerably low DDI than the other diets. This, coupled with low protein retention, suggests that there may have been an interruption in feed intake even before the fish had consumed sufficient protein to meet the minimum requirement for this species. Similar

results were reported for small-sized jundiá (mean weight 1.52 g to 10.96 g) by Meyer and Fracalossi (2004), when fed diets rich in non-protein energy (excess carbohydrates).

The nutrient requirements of a species are determined by the minimum concentrations needed in the diet to provide maximum growth (Jobling, 2016; Lovell, 1998). In general, studies consider the optimal dietary P:E ratio as that which provides maximum weight gain, since this variable is usually the most suitable for estimating nutritional requirements in fish (Cowey, 1992). In the present study, the best DP:DE ratio estimate, based on weight gain, corresponded to 26.62 g MJ<sup>-1</sup> (38.64 % DP and 14.86 MJ kg<sup>-1</sup> DE). However, weight gain does not always accurately reflect how consumed nutrients and energy are used for growth. This is likely to be the case for the 27 and 29 g MJ<sup>-1</sup> DP:DE diets which, in comparison to the other diets, resulted in higher weight gain in the fish but sub-optimal ANPU and body fat values, suggesting less efficiency in the use of the feed consumed. To avoid any erroneous interpretation of the optimal DP:DE ratio, variables that express weight gain based on feed intake, such as the FCR should be used (Glencross et al., 2007; Jobling, 2001).

In the present study, the best FCR was estimated for a diet with a DP:DE ratio of 25.38 g MJ<sup>-1</sup> (36.08 % DP and 14.03 MJ kg<sup>-1</sup> DE). However, the FCR is calculated based on feed consumption which is difficult to measure accurately in the aquatic environment. In addition, the FCR is based on total feed intake which hampers the specific estimation of how energy and/or some of the dietary nutrients have been utilized by the fish. This is illustrated by the results obtained for the 25 and 27 g MJ<sup>-1</sup> DP:DE ratio diets, where the fish presented different feed intakes, but similar FCRs and significantly different ANPU and body fat deposition. One way to circumvent this is to estimate the best dietary DP:DE ratio from variables that reconcile weight gain and changes in fish body composition such as ANPU (Lupatsch, 2009).

The results showed that the best ANPU in jundiá juveniles would potentially be achieved with a DP:DE ratio of 23.72 g MJ<sup>-1</sup>, representing a diet of 32.23 % DP and 13.46 MJ kg<sup>-1</sup> DE. The experimental diets with protein and energy levels close to this ratio (22 and 25 g MJ<sup>-1</sup>) did not produce the greatest fish weight gain; however, they yielded better ANPU and FCR values, as well as lower accumulation of body fat. Lower body fat accumulation is significant because it directly influences the quality and shelf life of fish. Reduced fat content is related to lower rates of lipid oxidation, a major cause of fish decay (Davis et al., 1999; Sant'Ana et al., 2011). In addition, it was noted that better ANPU promoted by a favorable dietary DP:DE ratio resulted in the excretion of intermediate levels of TAN, as has been demonstrated in other studies (Debnath et al., 2012; Engin and Carter, 2001; Green and Hardy, 2008; McGoogan and Gatlin, 1999, 2000; Ozorio et al., 2001). Ammonia is the main

nitrogen product of protein metabolism in fish having a deleterious effect on water quality. The inclusion of adequate DE favors greater protein utilization and, consequently, lower nitrogen excretion (Mock et al., 2019).

Along with the aforementioned aspects, a definition of the best DP:DE ratio for jundiá juveniles should also consider the minimum cost of production by relating growth indices to economic variables (Lupatsch, 2009). Since the cost of feed accounts for a large proportion of the operational costs of an aquaculture enterprise, it is necessary to ensure, through the maintenance of an optimal DP:DE ratio, that dietary protein, which is relatively costlier, is utilized for growth, while lower cost carbohydrates and lipids are metabolized for energy supply (Lovell, 1998; Mock et al. 2019; Silva et al., 2020).

In the present study, it was estimated that the lowest AFC would be achieved with a DP:DE ratio diet of 24.39 g MJ<sup>-1</sup> (33.83 % DP and 13.65 MJ kg<sup>-1</sup>). Cost indices, calculated for each experimental diet, were influenced by weight gain, FCR, and diet costs *per se*. The 20 g MJ<sup>-1</sup> DP:DE ratio diet yielded the highest ACF and CI and lowest EEI values. Although this diet had a lower protein content, the inclusion of synthetic amino acids resulted in a higher cost compared to the diet with a 22 g MJ<sup>-1</sup> DP:DE.

Despite providing high weight gain, the diet with 29 g MJ<sup>-1</sup> DP:DE did not show economic potential, mainly because of its high protein content and worse FCR. In contrast, the diets with DP:DE ratios of 22 and 25 g MJ<sup>-1</sup> showed the best indices of economic efficiency. Although yielding the second lowest weight gain compared to other experimental diets, the low cost of the 22 g MJ<sup>-1</sup> DP:DE diet provided the best ACF, EEI, and CI values. The diet with 25 g MJ<sup>-1</sup> DP:DE, the ratio closest to those estimated for optimal ANPU and lower ACF, showed the best FCR and an intermediate ACF compared to that of the other treatment diets.

For successful farming of jundiá, it is necessary to determine the optimal dietary DP:DE ratios for other life stages since the present study focused only on the initial grow-out phase (30 to 80 g average weight). This need is related to dietary requirement changes during different developmental stages as well as to the reduction in utilization capacity of some nutrients as the fish grow (NRC, 2011).

## Conclusions

The estimated dietary DP:DE ratios for optimal ANPU and economic performance were 23.72 g MJ<sup>-1</sup> (32.23 % DP and 13.46 MJ kg<sup>-1</sup> DE) and 24.39 g MJ<sup>-1</sup> (33.83 % DP and 13.65 MJ kg<sup>-1</sup>), respectively. However, for an economically viable and environmentally friendly culture, it is necessary to balance the variables. Therefore, 25 g MJ<sup>-1</sup> DP:DE (34.05 % DP and 13.81 MJ kg<sup>-1</sup>) is proposed as the optimal DP:DE ratio for jundiá juveniles in the initial grow-out stage (30 to 80 g average weight).

## Acknowledgments

The authors acknowledge financial support from the Foundation for Research and Innovation Support of the Santa Catarina State (FAPESC, SC #2012000298), as well as the fellowships granted by the Coordination of Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) for the first and third authors, respectively. We also thank the Agricultural Research and Rural Extension (EPAGRI) for donating the fish used in the study, and the companies Evonik, *In vivo*, Tectron, Nicoluzzi, and IMCOPA for the donation of some of the ingredients used in the manufacture of the experimental diets.

## Authors' Contributions

**Conceptualization:** Freitas, L.E.L.; Fracalossi, D.M.  
**Data acquisition:** Freitas, L.E.L. **Data analysis:** Freitas, L.E.L.; Silva, T.S.C. **Design of methodology:** Freitas, L.E.L.; Fracalossi, D.M. **Writing and editing:** Freitas, L.E.L.; Fracalossi, D.M.; Silva, T.S.C.

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