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Digestible Protein Requirement of Pirarucu Juveniles (*Arapaima gigas*) Reared in Outdoor Aquaculture

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Abstract

Pirarucu (*Arapaima gigas*) is a fast-growing, carnivorous species reared commercially in Amazonian countries as Brazil. Lack of a nutritionally balanced and affordable diet is a major constraint in pirarucu aquaculture, and our investigation sought to estimate the dietary protein requirement for this species. Four diets were formulated to contain increasing concentrations of digestible protein (32, 35, 38, and 41%) and fed to triplicate groups of juvenile pirarucu of around 2 kg for 18 weeks. As result, pirarucu showed no differences in feed intake, survival, or fillet yield. However, regression analysis revealed that weight gain (WG) values showed a general increasing trend with increasing dietary digestible protein (DP) level up to 36.7%. In addition, feed conversion rates were also improved with increase in dietary DP up to 36.4%. Fish fed diets containing 32 and 41% had poor feed conversion rates. Thel protein retention rate (PRR) and protein efficiency index (PER) decreased as levels of DP increased. The optimal dietary protein content for juvenile pirarucu between 1.98 and 4 kg of BW is about 36% of digestible protein on diet.

Keywords: dietary formulation, feed efficiency, nutritional fish, optimal growth

1. Introduction

The pirarucu (*Arapaima gigas*) is considered to be among the largest freshwater fish in the world, reaching up to 3 m in length and 200 kg in body weight (BW) (Pereira-Filho & Roubach, 2005; Castello, 2008). Wild pirarucu are piscivorous, but can be conditioned to accept extruded feed in aquaculture conditions (Crescêncio et al., 2005; Imbiriba, 2001). As they can grow up to 10 kg in body weight (BW) in the first year of cultivation, and can be reared at high stocking densities (Cavero, Roubach, Itassú, Gandra, & Crescêncio, 2003; Brandão, Gomes, & Chagas, 2006), it has potential to become an economically valuable aquaculture species. In order for this to be fully realized, it is necessary to develop an optimized cultivation diet based on low-cost, nutritionally balanced formulations, in addition to research for reproduction control and low cost of fingerlings (Gatlin, 1995).

Few studies have been conducted concerning the nutritional requirements of this species. Those which have done so mostly observed only young fish of small size—for example, those of approximately 50-120 g body weight—over a relatively short time period (Santos, Pereira Filho, Sobreira, Ituassú, & Fonseca, 2010; Ituassú et al., 2005). Since pirarucu is difficult to work with experimentally due to its large size and relative cost, there are few laboratories with appropriate facilities dedicated to the study of larger fish. As nutritional requirements may vary with size, dietary protein source, and environmental conditions, previous results should be complemented with information regarding larger sized fish nutrition.

Protein is one of the main feed components in aquaculture and also one of the most expensive, largely determining the price of feed (Yang et al., 2016). Protein demand is the first nutritional parameter to be estimated for new species in production because it provides the basis for the formulation of practical rations, particularly when there is no additional nutritional information available (NRC, 2011; Kim & Lee, 2009). When dietary protein is provided in excess of its requirement, only a portion is used to synthesize new proteins, while most of the excess amino acids are catabolized and converted into energy (Wilson, 2002). Hence, it is important to optimize protein provision at all phases of growth, in order to keep costs as low as possible (Kumar et al., 2010; Cornelio et al., 2014). The use of digestible protein (DP) in studies of protein requirement allows for efficient aquafeed formulation (Gonçalves & Carneiro, 2003), resulting in improved utilization of nutrients, increased productivity and profitability of the product.

The farming of pirarucu is under development in Brazil, and the practical diet formulation is currently based on existing diets of other fish species. In the present study, conducted an experiment to determine the protein requirement for juvenile pirarucu, by measuring growth performance, feed efficiency, and body composition.

2. Materials and Methods

2.1 Experimental Design

A total of 624 juveniles pirarucu were obtained from a commercial hatchery (Aguavale Piscicultura, Ituberá, Bahia, Brazil) and maintained at Experimental Farm of Almada, in Ilhéus, Bahia (18°43′51″S latitude, 44°53′33″W longitude). Prior to experimentation, juveniles were kept in holding tanks for 21 days to acclimate to experimental conditions. During this time, they were fed a commercial diet (36% crude protein). After the period of acclimation had elapsed, the juveniles (initial body weight 1.98±0.50 kg) were placed randomly into 12 tanks (400 m^2 each), 156 fish per treatment, at a stocking density of 1 fish per 7.7 m^2. This yielded three replicates of each of the four different levels of dietary protein provision. The experiment was conducted outdoors, with photoperiod determined by natural lighting, simulating a practical production system. Fish were hand-fed with experimental diets three times per day, at 8:00 AM, 1:00 PM, and 5:00 PM, for 126 days. During the experimental period, water temperature was 26.6±1.2 °C and pH was 5.84±0.8 (YSI Professional Plus multiparameter digital, Yellow Springs, USA).

2.2 Experimental Diets

Four diets were formulated to contain increasing levels of protein, ranging from 32, 35, 38, and 41% digestible protein (DP), approximately 36, 39, 42, 44% crude protein (CP) (Tables 1 and 2). Each diet was designed to be isoenergetic and isolipidic, based on the digestibility studies developed in our laboratory (Cipriano et al., 2015, 2016). Ingredients of the diets were mixed and extruded mechanically with a 6 mm diameter die (Ferraz Máquinas e Engenharia LTDA, São Paulo, Brazil). Pellets were dried in an oven at 60 °C for 48 h. Dried feed was stored in plastic bags and refrigerated at 5 °C until use.

Table 1. Formulation and composition of four diets (% natural matter) for juvenile *Arapaima gigas*

Ingredient	Digestible protein content (%)			
	32	35	38	41
Soybean meal (45%)[1]	19.00	27.40	32.70	34.00
Corn gluten meal (60%)[1]	16.00	15.00	13.85	21.00
Fish meal (55%)[1]	14.00	14.50	15.00	14.00
Meat and bones meal (45%)[1]	7.10	7.10	-	-
Poultry offal meal[1]	5.80	7.10	15.10	16.50
Corn meal[1]	7.00	7.30	7.65	7.60
Wheat bran[1]	25.50	16.20	11.10	3.00
Soybean oil[1]	4.71	4.51	3.71	3.01
Salt	0.28	0.28	0.28	0.28
Mineral and vitamin premix*	0.50	0.50	0.50	0.50
Antifungal	0.08	0.08	0.08	0.08
Antioxidant (BHT)[2]	0.01	0.01	0.01	0.01
Vit. C mon 35[1]	0.02	0.02	0.02	0.02
Real proximate composition				
Moisture (%)	10.22	10.43	9.95	9.82
Crude protein (%)	35.72	39.25	42.08	44.31
Digestible protein*(%)	32.00	35.00	38.00	41.00
Gross energy (MJ kg^{-1})	20.45	19.26	19.02	19.29
Digestible energy (MJ kg^{-1})*	13.28	13.02	13.38	13.43
Ether extract (%)	9.13	8.90	8.14	8.55
Acid detergent fibre (%)	18.64	24.58	17.65	15.39
Neutral detergent fibre (%)	34.08	37.57	34.07	30.19

Note. *Protein and digestible energy based on the percentage of digestibility of ingredient for pirarucu juvenile (*Arapaima gigas*) of Cipriano et al. (2015, 2016).

[1] Pratigi Alimentos, Castro Alves, BA, BR.

Composition/kg of the 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. pyridoxine = 8.000 mg; Biotin = 2000 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; Cu = 15.000 mg; Co = 2000 mg; If = 400 mg;

[2] BHT = Butylated hydroxytoluene.

Table 2. Amino acid analysis of diets (dry matter)

Amino acids		Digestible protein content (%)			
		32	35	38	41
Essentials	Isoleucin	9.35	8.70	8.95	9.50
	Leucin	5.55	5.50	4.85	5.20
	Lysine	5.90	5.55	5.6	5.25
	Metionin	2.10	1.90	1.80	2.30
	Phenylalanine	6.75	6.25	6.55	7.60
	Treonin	4.75	4,00	3.90	3.95
	Valine	4.80	5.65	5.55	5.20
	Arginine	7.20	7.40	7.20	6.95
	Histidine	2.75	2.60	2.65	2.55
Non - Essentials	Alanine	5.30	4.85	5.05	5.05
	Aspartate	8.15	9.10	8.85	9,00
	Cisteín	1.30	2.70	2.70	2.55
	Glutamine	18.5	19.1	18.15	18.5
	Glycine	3.25	4.40	4.55	4.35
	Proline	7.00	6.55	7.05	6.75
	Serine	5.90	5.85	6.25	5.90
	Tyrosine	5.05	4.35	4.20	3.95
	Taurine	0.25	0.45	0.45	0.50

2.3 Sample Collection

At the end of the trial period, all fish were sacrificed using a pneumatic captive bolt gun (TEC 10 PP; Cachoeirinha, Brazil). Two individuals per tank were sampled for fillet analysis, which was conducted at the Fish Processing Unit and Health Inspection Service of the Bahia state government by members of staff, without the intervention of the researchers.

2.4 Growth Parameters

At the end of the trial, following variables per tank were evaluated:

Weight gain (BW) = final BW (kg) – initial BW (kg).

Feed intake (FI) = ∑[(daily feed intake per tank (g))/(number of fish)]

Feed conversion rate (FCR) = live weight gain (g)/dry feed intake (g).

Survival = 100 × (final number of fish/initial number of fish).

Fillet yield (FY): FY = 100 × (final BW/fillet weight).

Protein retention rate (PRR) = 100 × (final BW × final body protein mass) – (initial BW × initial body protein mass)/protein intake.

Protein efficiency ratio (PER) = weight gain/protein intake.

2.5 Chemical Analysis

The composition of experimental diets and fillets were analyzed according to AOAC (2005). Diet moisture was determined by drying the samples for 24 h at 105 °C. Crude protein content was estimated using the micro-Kjeldahl method (N × 6.25). Crude fat content was estimated using diethyl ether extraction, ash by heating at 550 °C for 24 h and nitrogen free extract (NFE) as the remainder. The gross energy of diets and fillets were calculated using bomb calorimetry (IKA Instrument Co., Staufen, BKG, DE).

2.6 Statistical Analysis

The association between the protein levels of the experimental diets with weight gain and feed conversion rate was established using regression analyses to estimate the optimal DP level of the diets for pirarucu juveniles. The statistical program R Development Core Team (2008) was used for all statistical analyses.

3. Results

Since pirarucu have bimodal respiration (Lefevre et al., 2016), dissolved oxygen is not a key requirement for cultivation; however, this parameter was routinely measured (3.29±1.6 mg L^{-1}). All parameters evaluated were within tolerance ranges according to Del Risco et al. (2008). No mortalities occurred during the experiment. Feed intake (p = 0.0897), fillet yield (p = 0.6187) and final weight (p = 0.6104) were not affected by dietary protein levels (Table 3). However, weight gain (WG) (p = 0.0237), feed conversion rate (p = 0.0012), protein retention rate (p = 0.0025) and protein efficiency ratio (p = 0.001) did differ significantly (Table 3).

Table 3. Growth parameters of pirarucu juveniles fed with experimental diets

	Digestible protein content (%)				P-value
	32	35	38	41	
Initial weight (kg)	2.00±0.08	2.01±0.09	1.90±0.10	2.01±0.06	-
Final weight (kg)	3.96±0.38	4.28±0.27	4.27±0.42	4.02±0.36	0.6104
Weight gain(kg)*	1.96±0.06	2.27±0.11	2.37±0.14	2.01±0.08	0.0237
Feed intake (kg)	4.78±0.71	4.52±0,80	5.30±0.57	5.55±0.73	0.0897
Feed conversion rate*	2.48±0.18	2.03±0.03	2.24±0.22	2.46±0.12	0.0012
Protein retention rate*	24.88±0.41	23.41±0.29	20.33±0.28	18.12±0.36	0.0025
Protein efficiency ratio*	1.20±0.01	1.22±0.02	1.00±0.06	0.97±0.12	0.0001
Fillet yield (%)	37.72±0.45	37.57±0.23	38.13±0.34	38.46±0.40	0.6187

Note. *Weight gain ($y = -0.0187x^2 + 1.3613x - 22.87$); Feed conversion rate ($y = 0.0310x^2 - 2.2344x + 42.44$); Protein retention rate ($y = -0.030x + 2.197$); Protein efficiency ratio ($y = -0.778x + 50.09$).

Weight gain increased up to the optimum level of 36.4% of digestible protein in the diet, then decreased with increasing protein level in the diet (Figure 1). Feed conversion rate (FCR) also improved with increasing DP level (Figure 2).

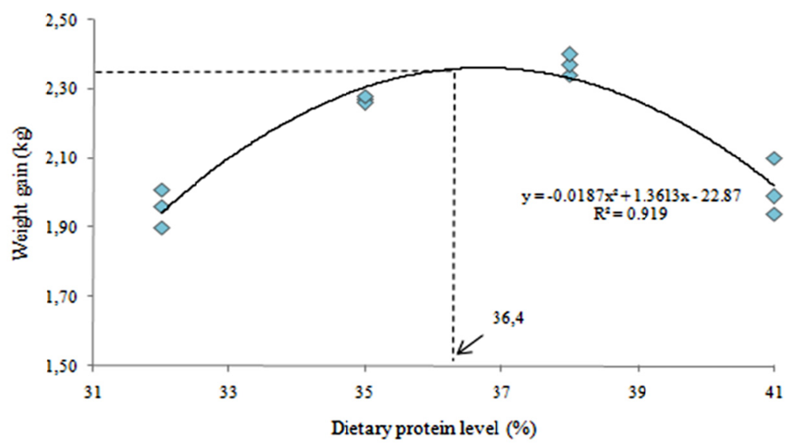

Figure 1. Polynomial regression of weight gain for pirarucu juveniles fed with experimental diets

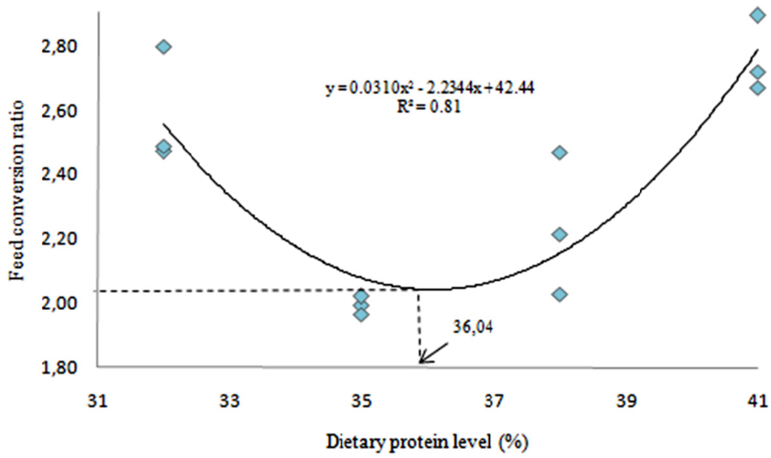

Figure 2. Polynomial regression of the feed conversion ratio and digestible protein levels for pirarucu juveniles fed with experimental diets.

Quadratic analysis showed that the optimum feed conversion rate was 2.13, achieved with 36% of digestible protein in the diet. In analyzed fillets, no significant differences were observed across the diets in ash content, moisture, protein, ether extract, or gross energy (Table 4).

Table 4. Proximate composition of pirarucu filets fed with experimental diets (based on natural matter)

Variable	Digestible protein content (%)				P-value
	32	35	38	41	
Ashes (%)	0.98±0.09	0.87±0.10	0.97±0.08	0.96±0.11	0.3969
Humidity (%)	76.61±0.68	77.92±0.58	76.38±0.66	77.54±0.61	0.7348
Crude protein (%)	20.0±0.71	18.75±0.62	20.16±1.10	19.16±0.66	0.8792
Ether extract (%)	3.67±0.33	3.31±0.24	3.68±0.31	3.49±0.17	0.8421
Gross energy (MJ/kg)	5.31±0.24	5.13±0.21	5.44±0.17	5.13±0.19	0.6202

4. Discussion

Although final weight and survival were not different across treatments, some performance data of juvenile pirarucu showed a favorable trend with increasing dietary protein level up to a point, followed by subsequent decrease. We observed a positive relationship between feed conversion rate (FCR) and a diet with ~36% of DP. Similarly, fish weight gain further improved under a diet with 35 to 38% of DP. These results are different to studies with juvenile pirarucu (~120 g), which showed differences in specific growth rates and weight gain related to the level of protein in the diet, but not in feed conversion rate (Ituassú et al., 2005). However, Del Risco et al. (2008) observed no significant differences in FCR between pirarucu fed at 40% and 45% CP, in juveniles of 85-470 g BW. Mattos et al. (2016), studying the regulation of protein intake and diet selection, concluded that pirarucu of ~650 g BW preferred a protein intake at 45% CP. Similar studies performed in other tropical freshwater carnivorous species, showed a protein requirement of 45% DP for barred sorubim (*Pseudoplatystoma reticulatum*) of 16 g BW and 38% CP for spotted catfish (*Pseudoplatystoma* sp.) of 360 g BW (Cornelio et al., 2014; Honorato et al., 2015). According to Ituassú et al. (2005), the bioavailability of food in different studies may have influenced findings. In our study, more recently available information on digestible dietary ingredients (Cipriano et al., 2015, 2016) guided the choice of ingredients for the diet formulation, which may have improved dietary protein utilization. It is clear that differences in the digestibility of protein and various energy sources used in diet preparation can have significant effects on the estimation of protein requirements. However, we also suggest that the difference in results is due to the difference in size of fish. Some previous protein requirement experiments with pirarucu have been performed using fingerlings (Ituassú et al., 2005; Del Risco et al., 2008), animals with low initial weight and rapid growth responses over a short time, whereas the present study utilized much larger individuals. Our study also differs in our use of extruded feed rather than pellet feed, which may have increased the bioavailability of the nutrients.

Varying levels of protein in the diet did not affect feed intake, but did affect feed conversion rate (FCR). Fish fed a diet with ~36% DP by polynomial regression analysis showed optimum FCR (2.03), suggesting that this protein level is suitable for maximal protein synthesis. The FCR found in the present study was similar to Ituassú et al. (2005), who found an FCR of 2.3 in 120 g fish. However, those results differed from Del Risco et al. (2008), who found an FCR of 1.07 in fish of 86-470 g fed CP at 40%. These variable findings could be due to energy use in protein deposition. Additionally, it is known that the energy-to-protein ratio plays an important role in fish nutrition, which can affect growth performance and feed efficiency. According to Yang et al. (2016) and NRC (2011), expressing protein requirement based on the protein-to-energy ratio is more rational than the dietary crude "protein requirement" expression. Using this expression, pirarucu in our study fed a digestible energy/digestible protein ratio (E/P) of 35.8 kJ kg^{-1} showed optimum performance in weight gain and feed conversion rate. This suggests that this ratio was suitable for maximal protein synthesis.

Skinless fillet yield (FY) had no significant differences among treatments. This result is consistent with a study of a carnivorous freshwater specie (*Pseudoplatystoma* sp.) reared under similar conditions of commercial aquaculture (Honorato et al., 2015). However, higher yields of 47-50% were recorded by Fogaça, Oliveira, Carvalho, and Santos (2011) evaluating pirarucu juveniles of 7-16 kg BW. In assessing the skinless FY of cultivated pirarucu, Oliveira, Jesus, Batista, and Lessi (2014) reported a mean value of 41% for fish weighing between 6-9 kg. This variation may be due to the age of the fish.

PER and PRR decreased significantly with increased dietary protein content. As previously discussed, excess dietary protein beyond requirement is directed towards energy production (Yang et al., 2016). The trends in protein use observed in this study are similar to several previously studies for other carnivorous fish (Abbas, Siddiqui, & Jamil, 2011; K. Kim, Lim, Kang, K. Kim, & Son, 2012; Mohseni, Pourkazemi, Hosseni, Hassani, & Bai, 2013; Coutinho et al., 2014). In some cases, fish body protein content tends to increase with increasing protein level in the diet (Zhang, Gong, Y. Yuan, Chu, & H. Yuan, 2009; Zhang et al., 2010). However, in the current study, the protein content in the pirarucu youth fillet was not significantly affected by the dietary DP levels. These results concurred with those observed in the body protein content of other fish species (Abbas et al., 2011; Guo et al., 2012; Deng et al., 2014). Similarly, ash content, moisture, crude energy, and fillet lipids were not affected by changes in dietary protein levels. This is also consistent with other studies in some fish species (Monentcham, Pouomogne, & Kestemont, 2010; Akpinar, Sevgili, Ozgen, Demir, & Emre, 2012). Dry matter and CP values were similar to those found in pirarucu juvenile fillets of 7-16 kg by Fogaça et al. (2011). These results lead us to propose studies on liver metabolism in pirarucu. Liver plays an important function in maintaining body composition in fish, but there is some variation of information in the literature on fish liver function subjected to dietary protein changes (Kim & Lee, 2009; Yang et al., 2016).

5. Conclusion

The optimal dietary protein requirement for juvenile pirarucu between 1.98 and 4 kg of BW is estimated to be around 36% digestible protein. This information will be useful in developing accurately balanced diets for cultivating this species.

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