

Optimum dietary crude protein requirement for juvenile cachama *Piaractus brachypomus*

Exigência ótima de proteína bruta para juvenis de pirapitinga, *Piaractus brachypomus*

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ABSTRACT

A growth experiment was conducted to determine the optimal dietary protein requirement for juvenile cachama, *Piaractus brachypomus*. Six semi-purified isoenergetic experimental diets using casein and gelatin as protein sources were formulated to contain graded levels of protein (16, 20, 24, 28, 32 and 36%). Fish initially averaging 15.52 ± 0.33 g (mean \pm SD) were randomly distributed into the tanks (500L) as groups of 20 fish and fed to apparent satiation twice daily for 60 days. The results showed that feed consumption, feed conversion ratio, fish weight gain, specific growth rate, protein efficiency ratio and protein productive value were affected by dietary protein level ($P < 0.05$). No significant differences were observed in proximal composition of carcass ($P < 0.05$) in response to dietary protein. Analysis of dietary protein level \times WG with a second order polynomial regression suggested a requirement of 31.6% CP for optimum growth.

Key words: tropical fish, protein requirements, growth performance, feed utilization, whole-body composition.

RESUMO

O presente experimento foi realizado para determinar o requerimento ótimo de proteína em dietas para juvenis de pirapitinga, *Piaractus brachypomus*. Foram formuladas seis dietas experimentais semi-purificadas, isoenergéticas e com diferentes níveis de proteína (16, 20, 24, 28, 32 e 36% de PB) usando caseína e gelatina como fontes de proteína. Os peixes com peso inicial $15,52 \pm 0,33$ g (média \pm SD) foram distribuídos aleatoriamente em grupos de 20 animais por tanque; foram alimentados durante 60 dias, duas vezes/dia até aparente saciedade. Os resultados mostraram que o

consumo de alimento, taxa de conversão de alimento, ganho de peso, taxa de crescimento específico, taxa de eficiência de proteína e valores produtivos da proteína e da energia foram significativamente afetados pelos níveis de proteína das dietas ($P < 0,05$). Não foram observadas diferenças significativas na composição proximal da carcaça ($P < 0,05$) entre tratamentos. A análise do ganho de peso em função dos níveis de proteína da dieta, realizada utilizando regressão polinomial de segundo grau, deu um requerimento de 31,6% de proteína para ótimo crescimento de juvenis de pirapitinga.

Palavras-chave: peixe tropical, níveis de proteína, desempenho de crescimento, utilização de alimento, composição corporal.

INTRODUCTION

A protein is the main organic component of the fish tissues and at the same time, the most abundant and costly in commercial feeds. In addition to being used for growth and repair of tissues, protein is also used extensively for providing energy in routine metabolism by fish (GUILLAUME et al., 2004). It is therefore, an essential nutrient for both maintenance and growth (DE SILVA & ANDERSON, 1995). Determining the minimum protein level in the diet which allows maximum growth for each species is one of the first challenges nutrition research which enable the development of highly efficient and low cost commercial feeds. In general, fish require relatively high levels of

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protein in their diet compared to other terrestrial warm-blooded animals (NRC, 1993). Demands among the different group species are not very different and depend upon factors such as: feeding habits, water temperature (cold or tropical), environment (marine or freshwater) and stage of physiological development. According to reports by NRC (1993), for maximum growth the majority of fish species on commercial farms in the world require diets with protein level that vary between 31 and 56%. Most of these values were estimated from dose-response curves, yielding the minimum amount of dietary protein which resulted in maximum growth (WILSON, 2002). Information also shows that such protein needs decrease as fish weight increases. Protein ingested by fish is used in maintenance and growth, but when they are overfed only one part of this protein is effectively used for synthesis of new proteins and the remaining is metabolized to produce energy (WILSON, 2002), with an unavoidable increase of nitrogen excretion to the aquatic system (OISHI et al., 2010; WEBB & GATLIN III, 2003). Consequently, when fish are fed with optimal dietary protein levels they retain a proportionally higher amount of protein in the body (growth), decreasing production costs and pollution (THOMAN et al., 1999).

Neotropical and omnivorous freshwater species such as *Piaractus* and *Colossoma* (Characidae, Serrasalminae) (ARAUJO-LIMA, 1997), are very important for commercial aquaculture industry in South America and other Central American countries (aproxim. 90.000MT year⁻¹, IBAMA, 2009; MADR, 2009). However, there is little information on dietary protein requirements for growth in these species. Thus, it is important that studies on its nutrition should be carried out to develop cost-effective and nutritionally balanced feed formulation for these fish. Consequently, the present study was designed to investigate the dietary protein requirement and evaluate the effects of dietary protein level on growth performance for juveniles cachama, *Piaractus brachypomus*.

MATERIALS AND METHODS

Six isoenergetic diets with 16, 20, 24, 28, 32, and 36% of CP were formulated. Protein levels were adjusted as in VÁSQUEZ-TORRES et al. (2002). Dextrin and oils (50% oil corn + 50% oil fish) proportions were adjusted to maintain diets isoenergetic with approximately 3080kcal DE kg⁻¹. Concentration of other ingredients was the same for all diets (Table 1). Three

Table 1 - Ingredients and proximate composition of experimental diets.

Ingredients (%)	-----Dietary protein levels (%)-----					
	16	20	24	28	32	36
Casein ¹	17.90	22.40	26.90	31.40	35.90	40.30
Gelatin ²	2.20	2.70	3.20	3.80	4.30	4.90
Dextrin	37.90	34.50	31.10	27.60	24.20	20.80
Fish oil + Corn oil	13.0	11.4	9.80	8.20	6.60	5.00
Others ingredients ³	29.0	29.0	29.0	29.0	29.0	29.0
	-----Proximate analysis (% of dry matter basis)-----					
Dry matter (%)	62.60	57.30	59.50	57.90	56.90	57.80
Crude protein	14.80	20.80	24.30	28.20	32.60	37.40
Crude lipids	12.40	10.70	9.10	8.90	5.70	5.40
Ash	3.90	4.30	4.30	4.90	4.70	4.60
Gross Energy (kcal kg ⁻¹) ⁴	5527	5452	5511	5482	5421	5431
Estimated DE (kcal kg ⁻¹) ⁵	3116	3100	3085	3071	3056	3041

¹Casein: DM 93.0%, CP 86.42%, Lipid 2.29%, Ash 3.66%, EAA (g kg⁻¹ CP): Met 29.1, Lys 80.6, Thr 41.4, Arg 34.3, I-leu 50.2, Leu 93.4, Val 65.4, His 31.0, Phe 50.2.

²Gelatin: DM 91.0%, CP 94.02%; EAA (g kg⁻¹ CP): Met 9.2, Lys 38.1, Thr 18.7, Arg 82.5, I-leu 15.2, Leu 30.2, Val 23.7, His 7.2, Phe 20.7.

³Others ingredients: Vitamin mixture Rovimix 0.1% Lab. Roche S.A.: Vit. A 8x10⁶UI; Vit. D₃ 1.8x10⁶UI, Vit. E 66.66g, Vit. B₁ 6.66g, Vit. B₂ 13.33g, Vit. B₆ 6.66g, Panthotenic acid 33.33g, Biotin 533.3mg, Folic acid 2.66g, Ascorbic acid 400.0g, Nicotinic acid 100.0g, Vit. B₁₂ 20.0mg, Vit. K₃ 6.66g, filer csp. 1.0kg, Vitamin C (StayC-35) 0.03% Lab. Roche S.A.

Mineral mixture: microminerais 0.05%: Mg 10, Zn 160, Fe 40, Cu 10, I 5.0, Se 0.5, Co 0.1, filer csp. 1.0kg; macro-minerals: 4.02%: Ca(H₂PO₄) 136g; Ca Lactate 348.5g; 2MgSO₄.7 H₂O, 132g; KH₂PO₄ 240g; NaCl 45g; AlCl₃ 0.15g, CMC 98.35g.

Cellulose 18.0%; CMC 6.8%.

⁴Gross Energy: measured through a ballistic bomb calorimeter (PARR 121 EA. USA)

⁵Calculated using DE (kcal kg⁻¹) values for catfish (NRC. 1993): Casein: 4400, gelatin: 4700, dextrin: 2920, oils 8600.

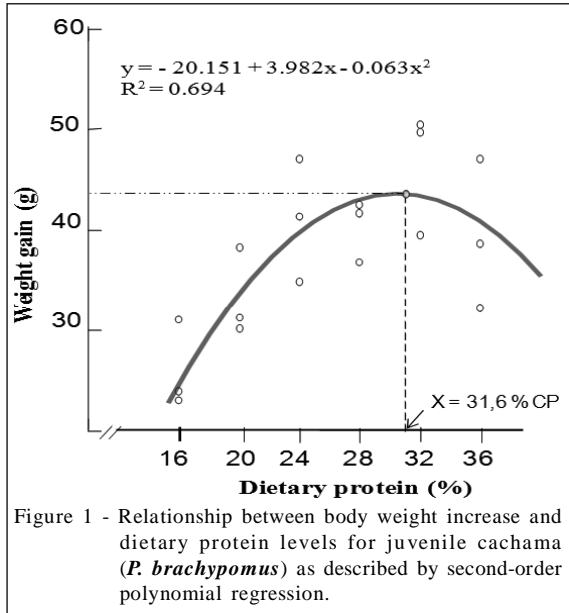
hundred and sixty fish *P. brachypomus* juveniles obtained by induced spawning at the Instituto de Acuicultura de la Universidad de los Llanos were acclimatized to the experimental conditions for two weeks prior to the experiment. Fish initially averaging 15.52 ± 0.3 g were distributed into the 500L tanks in groups of 20 fishes. The six experimental diets were randomly assigned to the tanks (six treatments in triplicate). Juveniles were fed twice daily (at 8:00 and 15:00 hours) until apparent satiety for 60 days, registering daily food intake for each experimental unit. Each tank was adjusted to maintain a continuous flow of water at a rate of 2 L min^{-1} and constant aeration to maintain dissolved oxygen near 60% saturation. Water was continuously recycled through a unit that included a mechanical filter to remove suspended particles and a series of four bio-filters for nitrification (removal of ammonia and nitrite). Water quality parameters were monitored three times a week between 10:00 and 11:00h. During the experiment period, temperature was $26 \pm 1.2^\circ\text{C}$, pH 7.3 ± 0.2 , hardness $40\text{-}80 \text{ mg L}^{-1} \text{ CaCO}_3$, ammonia nitrogen was lower than 0.02 mg L^{-1} and dissolved oxygen was not less than 4.7 mg L^{-1} . At the end of the growth trial, fish were starved for 24h and then weighed. A sample of 20 fish from the initial group and 10 of each replicate at the end of the experiment were sacrificed for carcass proximate analysis. Crude protein, crude lipid, moisture and ash in diets and fish whole body composition were performed in triplicate by the standard methods. Water quality parameters were measured using a multiparameter probe YSI 556 (YSI Incorporated, Yellow Springs, OH USA). At the end of the experiment, after a 24h fasting period, all fish were weighted individually. Criteria to evaluate dietary effects were: Weight Gain (WG) = $W_f \text{ (g)} - W_i \text{ (g)}$; Specific Growth Rate (SGR) = $(\ln W_f - \ln W_i) \times 100/t$; Dry Feed Intake $\text{g DM kg}^{-1} \text{ days}^{-1}$ (DFI) = $100 \times \text{FI} / [(W_i + W_f) / 2 \times t]$; Feed Conversion Ratio (FCR) = $\text{FI (g)} / \text{WG}$; Protein Efficiency Ratio (PER) = WG / CPI ; Protein Productive Value (PPV) = $100(\text{CP}_f - \text{CP}_i) / \text{CPI}$; Energy Productive Value (EPV) = $100(\text{CE}_f - \text{CE}_i) / \text{CEI}$ Where W_f its final body weight, W_i is initial body weight, t is experimental duration in days, FI is dry feed intake (g), CPI is crude protein intake (g), CP_i and CP_f are estimates of crude protein content (g) of the biomass at the start and end of the experiment, CE_i and CE_f are estimates of energy content of the biomass at the start and end of the experiment and CEI is the crude energy intake.

Results are expressed as mean \pm SD. All data were subjected to one-way ANOVA followed by the Tukey's test to determine significant differences among treatment groups and $P < 0.05$ was considered to be statistically significant. Protein requirements were

estimated from body weight gain using the second-order polynomial regression analysis model. From the regression, the optimal level supporting the maximum growth response was calculated. All statistical analyses were performed using Systat for Windows (v10.2, SPSS Inc., USA).

RESULTS AND DISCUSSION

Data on growth show that there was a gradual increment in WG (46.5 ± 5.0 g) as dietary protein levels increased to 32% CP. Fish fed diet beyond 36% CP could not produce additional growth. There is some evidence that at very high feeding levels, protein deposition tends to level off (plateau) (BUREAU et al., 2002). Fish fed diets with lower CP levels showed reduced weight gain and efficiency of feed utilization. Dietary protein is the most important factor affecting growth performance of fish and feed cost. Generally, increasing protein level in diets can lead to improved fish production, especially for carnivorous fish. The demand of fish protein for growth is different according to species, feeding habits, physiological and development state, conditions of culture, sources of protein, dietary energy levels and the protein: energy ratio (ROBINSON & LI, 1999; LUPATSCHEK et al., 2001). However, it has been determined that the average protein requirements in diets for growth of the main fishes species cultivated in the world may oscillate between 31 and 55% (NRC 1993) equivalents to 300-700 parts kg^{-1} of GE content on diet in the protein form. Differences on WG in relation to the protein content in the diet, similar to those observed in this study, have been described for different species of fishes omnivorous such as jundiá *Rhamdia quelen* (MEYER & FRACALOSSO, 2004), catfish *Clarias gariepinus* (ALI & JANNEY, 2005) and Tilapia *O. niloticus* (ABDEL-TAWWAB et al., 2010). In this study the maximum WG in cachama juveniles was observed with 32% of dietary protein level. The influence of increased dietary protein levels on the growth response was examined by fitting dose-response data to a polynomial curve (Figure 1). The relationship between WG and dietary protein level was described by the equation $Y = -20.15 + 3.98X - 0.063X^2$ ($R^2 = 0.694$). The optimal dietary protein level was determined to be 31.59% for maximum weight gain. This value is consistent with the results of GUTIERREZ et al. (1996) who determined that 29.8% of protein was necessary in practical growth diets for this species. Values obtained for fishes of the same family and similar feeding habitat, 26% CP for pacu (FERNANDES et al., 2000) or 27% (BICUDO et al., 2010) and for tambaqui, or 30% (OISHI et al., 2010), were comparatively similar



to those observed in this study. These values are also comparable with those of other omnivorous species such as catfish *I. punctatus* (ROBINSON & LI, 1999), piracanjuba (SÁ & FRACALOSSO, 2002), jundiá (MEYER & FRACALOSSO, 2004; SALHI et al., 2004) and tilapia (ABDEL-TAWWAB & AHMAD, 2009)

The highest SGR PER and best FCR, were also observed in fish fed diet containing 32% CP (Table 2). SGR varied significantly between 1.6 in fish fed with the diet with the lowest protein content up to 2.2 in those fed with the diet with 32% CP. Values of SGR obtained in this experiment for cachama juveniles fed with dietary protein levels above 20% (1.9 to 2.2), were higher than those observed for tambaqui (0.8-0.9) (OISHI et al., 2010), for pacu (0.75-0.81) (ABIMORAD & CARNEIRO, 2007) and similar to those observed for

this same species by FERNANDES et al. (2000); BICUDO et al. (2010). The increase of dietary protein to 36%, above the optimum level determined in the present study, caused a significant decrease on WG and SGR in a similar way to that described for bagrid catfish *Pseudobagrus fulvidraco* (LEE & LEE, 2005), jundiá (MEYER & FRACALOSSO, 2004) and bonitongue *Heterotis niloticus* (MONENTCHAN et al., 2010). According to DE SILVA & ANDERSON (1995), this pattern in fish can be caused by an imbalance in the relationship protein/energy of diets when non proteic energetic sources diminish with a concomitant increase of protein contents. Such disproportion can shift the metabolic partition of protein towards the production of energy instead of protein synthesis. Since this is a frequently observed response in different fish species, it is suggested that in this experiment the diet with 36% CP, which was unbalanced in protein/energy ratio (37.4 CP/12.7kj DE kg⁻¹) due to protein excess, was one of the causes for growth depression of fish fed with it.

Feed intake was higher in fish fed with the lowest dietary protein level and gradually diminished in fish fed with 32% (Table 2). Low food consumption and high WG obtained in fish fed with 32% CP produced a FCR of 1.1±0.03, significantly better than that observed in fish fed with dietary protein levels above and below this level (P<0.05); differences in consumption due to dietary variations in CP levels similar to register in this experiment, were observed in tambaqui (VAN DER MEER et al., 1995) and jundiá (SALHI et al., 2004). On the other hand, in pacu (FERNANDES et al., 2000) and catfish *I. punctatus* (ROBINSON & LI, 1999), no significant influence of dietary protein levels on the consumption of dry matter were stated. The fishes, as well as terrestrial animals, eat to satisfy nutrients and energy demands. It is also

Table 2 - Growth response and feed utilization of juvenile Cachama (*P. brachyomus*) fed with diets containing graded levels of protein; Specific Growth Rate (SGR), Protein Efficiency Rate (PER), Dry Feed Intake (DFI), Feed Conversion Ratio (FCR). Protein Productive Value (PPV%), Energy Productive Value (EPV%)¹.

	-----Protein levels (%)-----						
	16	20	24	28	32	36	P
WG (g)	26.0±3.56 ^d	33.2±3.58 ^c	41.1±4.92 ^b	40.3±2.46 ^b	46.5±5.01 ^a	39.3±6.05 ^b	0.009
SGR	1.63±0.15 ^d	1.89±0.07 ^c	2.11±0.12 ^{ab}	2.11±0.06 ^{ab}	2.25±0.15 ^a	2.07±0.19 ^b	0.006
PER	4.7±0.24 ^a	3.8±0.41 ^b	3.5±0.17 ^{bc}	3.2±0.04 ^{cd}	2.9±0.10 ^d	2.4±0.10 ^e	0.000
DFI	1.51±0.02 ^a	1.40±0.05 ^{ab}	1.40±0.04 ^{ab}	1.32±0.01 ^c	1.32±0.06 ^{bc}	1.34±0.04 ^{bc}	0.005
FCR	1.5±0.11 ^a	1.3±0.08 ^b	1.2±0.07 ^{bc}	1.2±0.03 ^{bc}	1.1±0.03 ^c	1.2±0.03 ^{bc}	0.000
PPV(%)	80.2±1.4 ^a	69.3±2.8 ^b	65.4±5.5 ^b	53.5±1.7 ^c	47.2±1.6 ^{cd}	38.9±2.7 ^e	0.000
EPV(%)	30.5±5.5	35.8±3.0	35.3±4.8	34.7±0.6	33.5±2.2	33.0±0.8	0.653

¹ Data are means of triplicate ±SD. Means in the same column sharing the different superscript letter are significantly different determined by Tukey test (P<0.05). Initial weight was 15.52±0.82g.

believed that fish adjust food consumption to satisfy the metabolic energy requirements, however once minimum requirement are satisfied, the daily consumption may be related to dietary quality such as protein content (DE SILVA & ANDERSON, 1995). In such cases, fish can adjust ingestion of food as a function of DE contents of the diet. In contrast, a diet containing excess energy can reduce feed consumption and thus lower the intake of the necessary amount of protein and other essential nutrient for maximum growth (NRC, 1993). In different species, it has been observed that when the diet is low in energy or in an essential nutrient for growth such as proteins, the animal compensates such deficiency by increasing its consumption (GUILLAUME et al., 2004). According to SANTINHA et al. (1999), when a fish is fed to satiety, weight gain will depend not only on the energy and nutrient content of the diet, also in its ability to regulate food intake. Consequently, these theories could explain the higher feed ingestion by *P. brachypomus* fed diets isoenergetics with low protein. In agreement with the results of the present experiment (Table 2), GUTIERREZ et al. (1996) observed a decrease in the FCR value from 3.9 to 2.4 when *P. brachypomus* were fed with increasing dietary protein levels from 27 to 30%. A similar pattern was observed by FERNANDES et al. (2000) in pacu when dietary protein increased from 22 to 30% and also by SÁ & FRACALLOSSI (2002) in *B. orbignyanus* fed with 24 to 36% CP. Values of FCR obtained in this study, between 1.3 and 1.1 for fish fed with dietary protein levels from 20 to 36%, besides being in an average acceptable range for fishes (DE SILVA & ANDERSON, 1995) and may be considered excellent when compared with those observed in experiments of close species as pacu (BECHARA et al., 2005) and tambaqui (OISHI et al., 2010). These values are also superior to those observed in other teleosts as *R. quelen* (SALHI et al., 2004), tilapia (*O. niloticus*) (ABDEL-TAWWAB et al., 2010) and catfish

(ROBINSON & LI, 1999). PER was negatively correlated with dietary protein levels and consequently the efficiency of the use of dietary protein for biomass gain was lower in fish fed with high protein levels. PER varied within a maximum value of 4.7 in fish fed with the lowest dietary protein level to 2.4 in those fed with the diet with the highest protein content PPV values, which showed that the amount of retained protein by fish had a similar behavior to that observed with PER and PPV were 2.9 and 47.2 respectively in fish fed with 32% CP, dietary protein level which led to maximum WG (Table 2). A negative relationship between dietary protein levels and PER and PPV was observed in pacu (BACHARA et al., 2005) and piracanjuba *B. orbignyanus* (SÁ & FRACALLOSSI, 2002). Several studies demonstrated that high CP levels in the diet lead to lower PER and PPV values (MATHIS et al., 2003; LEE & LEE, 2005). Apparently, this response is associated with the lack of digestible energy coming from non protein sources in the diets with the highest levels of CP inclusion. In order to keep the dietary energy constant, in this experiment lipid was used to regulate dietary energy, so lipid content in the diets decrease with increasing dietary protein level. Thus, in this study, the reason for the negative relationship between levels of dietary protein and PER, was that a greater amount of protein in the diets was used as an energy source. When fishes are fed with these diets, protein is then partially used as source of energy diminishing its possibilities of use for deposition in the tissues (FERNANDES et al., 2000; BICUDO et al., 2010). PPV in body significantly increased with increasing dietary protein levels from 16 to 20%, but remained similar thereafter ($P < 0.05$). At the end of the experiment the content of moisture, crude protein, lipid and gross energy in whole body were not significantly affected ($P > 0.05$) (Table 3). Similar results have been described by several researchers in studies with different species in the same experimental conditions,

Table 3 - Whole body proximate composition (% on fresh/weight basis), gross energy and protein and energy efficiencies of juvenile cachama (*P. brachypomus*) fed diets containing different protein levels¹.

	-----Protein levels (%)-----						P
	16	20	24	28	32	36	
Moisture	67.8±0.51	68.0±0.20	68.2±2.79	69.8±0.90	70.9±1.13	70.6±0.63	0.051
Crude protein	17.0±1.65	19.9±0.41	17.7±0.67	16.3±0.31	16.2±0.37	16.1±0.74	0.545
Ether Extract	8.65±2.47	11.57±0.88	10.19±2.21	10.10±0.76	9.73±0.93	9.72±0.74	0.724
Ash	3.22±0.59	3.39±0.48	3.21±0.45	3.27±0.37	3.24±0.23	3.27±0.09	0.998
GE (kcal kg ⁻¹)	6337±707	6248±211	6274±181	6183±201	6026±386	6019±121	0.836

¹Data are means of triplicate ±SD. Means in the same column sharing different superscript letter are significantly different determined by Tukey test ($P < 0.05$).

WEBSTER et al. (2000) with catfish, SÁ & FRACALOSSO (2002) with *B. orbignyanus*, MATHIS et al. (2003) with *Perca fluviatilis*, MEYER & FRACALOSSO (2004) with *R. quelen* and ABDEL-TAWWAB & AHMAD (2010) with tilapia *O. niloticus*.

CONCLUSION

It may be concluded that for *P. brachypomus* juveniles a dietary protein level of 31.6% CP is the optimum level for maximum growth and Feed Conversion Ratio. An increase on dietary protein level beyond 36% seems to generate decrease on WG. The PER and PPV were negatively related with dietary protein levels.

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