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# Dietary protein requirements for tambaqui *Colossoma macropomum* (Cuvier, 1818) juvenile[1]

## Exigência de proteína dietética para juvenis de tambaqui *Colossoma macropomum* (Cuvier, 1818)

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**ABSTRACT -** The freshwater fish, tambaqui, *Colossoma macropomum*, is a native species of Brazilian fauna that has the potential to become a widespread aquaculture species, howerer, studies regarding the nutrient requirements remain scarce, often producing conflicting results. Hence, the currently available information is insufficient to formulate the specific diet of this species, for the different growth stages. This study aimed to evaluate the effects of dietary crude protein levels on the growth performance, body composition and ammonia excretion rates in tambaqui of 2–20 g body weight. Juvenile (average weight, 1.70 ± 0.05 g) were fed four times a day over a 60 days period using diets containing 300; 350; 400 and 450 g kg$^{-1}$ crude protein in a completely randomized experimental design. The tambaqui juvenile fed diet containing 300 g kg$^{-1}$ crude protein exhibited higher feed intake, specific growth rate, protein efficiency ratio, and protein productive value. This finding shows an improvement in protein utilization efficiency when fish are fed the lowest level of this nutrient in the diet; however, more viscero-somatic fat deposition and body lipid content was observed in these fish. The total ammonia excretion rates were lower in tambaqui fed diet containing 300 g kg$^{-1}$ crude protein than those fed diets containing 450 g kg$^{-1}$ crude protein. From the present investigation, it can be conclude that 300 g kg $^{-1}$ crude protein should be fed to tambaqui juvenile of 2–20 g body weight.

**Key words:** Crude protein. Growth performance. Body composition. Total ammonia nitrogen excretion. Protein utilization efficiency.

**RESUMO -** O tambaqui, *Colossoma macropomum*, é uma das espécies nativas da fauna brasileira com grande potencial de expansão, todavia, os estudos de exigências nutricionais são escassos e apresentam resultados conflitantes, a ponto de não termos informações suficientes para formular dietas específicas para a espécie, nas diferentes fases de crescimento. Objetivou-se com esta pesquisa avaliar a influência de níveis de proteína bruta sobre o desempenho produtivo, composição de carcaça e taxa de excreção de amônia em tambaqui na categoria de peso de 2 a 20 g. Os juvenis (peso médio 1,7 ± 0,05 g) foram alimentados quatro vezes ao dia, durante um período de 60 dias, com dietas contendo 300; 350; 400 e 450 g kg$^{-1}$ de proteína bruta. O experimento foi executado em delineamento inteiramente casualizado com quatro níveis de proteína e cinco repetições. Os juvenis de tambaqui alimentados com a dieta contendo 300 g kg$^{-1}$ de proteína bruta apresentaram maior consumo de ração, taxa de crescimento específico, taxa de eficiência proteica e valor produtivo da proteína, demonstrando que os animais alimentados com o menor nível de proteína bruta apresentaram melhor eficiência de utilização deste nutriente. Por outro lado, observou-se maior deposição de gordura viscero-somático e lipídio corporal nestes animais. As taxas de excreção de amônia foram menores nos tambaquis alimentados com a ração contendo 300 g kg$^{-1}$ proteína bruta do que naqueles que receberam ração contendo 450 g kg$^{-1}$. Recomenda-se o nível de 300g kg$^{-1}$ de proteína bruta para tambaqui de 2 a 20 g de peso vivo.

**Palavras-chave:** Proteína bruta. Desempenho produtivo. Composição corporal. Excreção de amônia. Eficiência de utilização da proteína.

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## INTRODUCTION

Tambaqui, *Colossoma macropomum*, a native fish species of the Amazon basin, is member of the family Serrasalmidae that include 80 species and 15 genera, and its distribution is restricted to neotropical areas in the main rivers of South America (ORTÍ *et al*., 2008). This species has the potential to become a widespread aquaculture species due to it already being established in the market, good growth rates in fish-culture farms, presence of artificial propagation technology, and an established production chain (NUNES *et al*., 2006).

The diet of wild adult tambaqui is primarily herbivorous; however, during the juvenile and sub-adult phases higher quantities of protein are consumed, especially animal protein. Hence, this species is considered an omnivore (ARAUJO-LIMA; GOULDING, 1997). Since tambaqui reared in captivity are juveniles, the nutritional requirements for protein and energy must be determined (ARAUJO-LIMA; GOMES, 2013) to obtain high growth rates under captive conditions.

In general, studies regarding the nutrient requirements of tambaqui remain scarce, often producing conflicting results. Hence, the currently available information is insufficient to formulate the specific diet of this species, in which the appropriate levels of protein, amino acids, vitamins, minerals, fatty acids, among others are delineated for the different growth stages (ONO; OLIVEIRA, 2008). In addition, knowledge on the nutritional requirements facilitates the generation of economically viable feed formulas, which increase species performance, while minimizing nutrient excretion rates into the environment.

Protein is an essential macronutrient in the diet of fishes, providing essential and non-essential amino acids for body protein synthesis (MANBRINI; GUILLAUME, 2004). However, protein is also the most expensive food component; hence, studies on nutritional requirements have been prioritized (ROTTA, 2002). Furthermore, Van Der Meer, Zamora and Verdegem (1997) found that protein is the most important macronutrient that determines food intake in tambaqui.

Protein and nitrogen intake contained in the diet directly influence the ammonia excretion rates in teleosts (KAUSHIK; COWEY, 1990). Protein quality (amino acid composition of the diet) is the determinant factor that influences the production of nitrogenous waste, because excess amino acids in the diet result in catabolism, with subsequent ammonia excretion (ARZEL *et al*., 1995).

Thus, the current study aimed to evaluate the effects of dietary crude protein levels on the growth performance, body composition, and ammonia excretion rates in tambaqui of 2–20 g body weight.

## MATERIAL AND METHODS

### Diet

Four isocaloric diets were formulated, each containing 300; 350; 400 and 450 g kg$^{-1}$ crude protein (Table 1). The protein sources were fish meal and soybean meal. To minimize the differences in nutrient utilization among treatments, a baseline level of approximately 150 g kg$^{-1}$ fish meal was included in all experimental diets. After mixing the ingredients, the diets were dry pelleted (Chavante®), broken into small pieces, and sieved into appropriate pellet size. All diets were stored at -20 ºC until use.

### Growth Performance and Body Composition Experiment

For the growth performance experiment, tambaqui juvenile, with an average initial weight of 1.70 ± 0.05 g and body standard length of 3.6 ± 0.1 cm, were randomly distributed into 0.25 m$^3$ experimental cages (30 fish per cage) with five replicate cages for each treatment. The cages were made of a 0.6 mm plastic mesh, and placed in an 500 m$^2$ earthen pond. The distance between cages was approximately 0.50 m.

The experiment diets were provided during the entire breeding period with a 15-10% body weight ration, four times a day (08:00; 11:00; 14:00 and 17:00 hours) over a 60 days period. Fish were weighed fortnightly, and the daily ration was adjusted accordingly.

The water temperature and dissolved oxygen levels (measuring using an oximeter) were measured daily at 09:00 hours. Fortnightly, water samples were collected (09:00 hours) to determine the pH (by using a digital pH meter), and total alkalinity, hardness, and carbon dioxide levels by using the method described by Sá (2012). Water transparency was determined using a Secchi disk, with the aim of maintaining a low concentration of phytoplankton in the earthen pond.

At the end of the experimental period, the tambaqui were subject to 24 hours fasting period to empty the digestive tract. The total number of remaining fish in each cage was counted, and their final body weights were measured. Three fish from each cage were randomly killed by administering an overdose of benzocaine (100 mg L$^{-1}$), and the separate weights of the whole body, liver, eviscerated carcass (without viscera), and viscero-somatic fat were obtained. These values were used to calculate the hepatosomatic index, carcass yield, and viscero-somatic fat index.

At the beginning of the experiment, 250 fish from the original population were killed to determine the initial

**Table 1** - Feed and chemical composition of experimental diets

| Ingredients (g kg$^{-1}$) | Crude protein levels (g kg$^{-1}$) | | | |
|---|---|---|---|---|
| | 300 | 350 | 400 | 450 |
| Fish meal | 150.0 | 150.0 | 150.0 | 169.8 |
| Soybean meal | 396.9 | 529.0 | 662.6 | 778.0 |
| Corn meal | 420.4 | 296.0 | 163.5 | 2.9 |
| Soybean oil | 11.6 | 5.0 | 5.0 | 4.0 |
| Vitamin-mineral premix* | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin C | 0.1 | 0.1 | 0.1 | 0.1 |
| Vitamin E | 0.4 | 0.4 | 0.4 | 0.4 |
| Choline chloride | 0.5 | 0.5 | 0.5 | 0.5 |
| Dicalcium phosphate | 5.0 | 4.7 | 4.5 | 2.1 |
| Limestone | 8.5 | 7.7 | 6.8 | 4.4 |
| Selenium | 0.4 | 0.4 | 0.4 | 0.4 |
| BHT | 0.1 | 0.1 | 0.1 | 0.1 |
| Inert (sand) | 1.1 | 1.1 | 1.1 | 32.3 |
| Chemical composition (Analyzed) | | | | |
| Crude protein (g kg$^{-1}$) | 303.4 | 359.9 | 401.5 | 460.5 |
| Lipid (g kg$^{-1}$) | 37.1 | 34.4 | 24.9 | 28.5 |
| Dry matter (g kg$^{-1}$) | 903.0 | 902.8 | 918.9 | 921.7 |
| Mineral matter (g kg$^{-1}$) | 66.0 | 68.9 | 79.6 | 116.7 |
| Gross energy (kcal kg$^{-1}$) | 4144.27 | 4249.78 | 4286.11 | 4070.60 |
| Amino acid profiles (Calculated) | | | | |
| Lysine | 1.92 | 2.31 | 2.69 | 3.10 |
| Methionine | 0.46 | 0.51 | 0.56 | 0.63 |
| Methionine + Cystine | 0.82 | 0.93 | 1.04 | 1.16 |
| Threonine | 1.09 | 1.28 | 1.47 | 1.66 |
| Arginine | 2.01 | 2.40 | 2.80 | 3.20 |

BHT, butylated hydroxytoluene; * Vitamin-mineral premix: guaranteed levels per kg of product. Vit. A, 6000 UI; Vit. D, 6000 UI; Vit. K, 6.30 mg; Vit. B1, 11.76 mg; Vit.B2, 15.36 mg; Vit. B6, 12.74 mg; Vit. B12, 40 mcg; Folic acid, 1.92 mg; Pantotenic acid, 39.20 mg; Choline, 800 mg; Niacine, 400 mg; Biotine, 0.2 mg; Antioxidant, 300 mg; Iron, 257.15 mg; Zinc, 300 mg; Manganese, 133.45 mg; Copper, 19.60 mg; Iodine, 9.40 mg

whole body composition. At the end of the experiment, five fish per cage were killed to determine the final whole body composition. The eviscerated carcass composition was only determined in samples collected from three fish per cage at the end of the experiment. Specimens for body analysis were frozen and stored in a freezer (-20 ºC) until chemical analysis.

The analysis of diet and fish samples for moisture, crude protein, lipids, and mineral matter contents followed standard AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1995) methods. The gross energy content of the diets was determined using a calorimetric bomb (Parr 6100).

The following formulas were used to calculate the performance characteristics and nutrient retention efficiency: Weight gain = final weight - initial weight; Feed conversion ratio = feed intake (g)/weight gain (g); Specific growth rate = 100 × (In final weight - In initial weight)/day; Survival rate = 100 × (final number of fish/ initial number of fish); Carcass yield = 100 × (carcass weight without viscera/body weight); Hepatosomatic index = 100 × (liver weight/body weight); Viscero-somatic fat index = 100 × (visceral fat/body weight); Protein efficiency rate = weight gain (g)/protein intake (g); Protein productive value = 100 × (% final body protein × final body weight) - (% initial body protein

× initial body weight)/protein intake (g); Protein growth rate = [100 × (ln final protein content - ln initial protein content)]/day.

**Total Ammonia Nitrogen Excretion Trial**

In the total ammonia nitrogen excretion ($NH_3$ + $NH_4$) trial, tambaqui (average weight, 8.7 g) were placed in individual 2.5 L plastic tanks. The fish were fed the experimental diets four times a day (08:00; 11:00; 14:00 and 17:00 hours) for a three days period, to allow them to acclimate to the experimental conditions. The fish group used as the positive control was starved for 72 hours before the measurement day to measure their endogenous ammonia excretion rates.

On the measurement day, water samples were collected from the 2.5-L aquariums without fish, to determine the initial background concentrations (time 0). Each experimental fish, excluding the fish used for positive control, was fed the respective experimental diets between 08:00 and 09:00 hours to satiation (until the intake and regurgitation of pellets was no longer observed). After the completion of feeding trial, the treated fish and control fish were carefully transferred from the tank and placed individually in the aquariums. Then, water samples for total ammonia nitrogen determination were collected from each aquarium at 2; 4; 8; 12 and 24 hours after feeding. The water samples were immediately stored at -20 ºC until analysis. This procedure was repeated thrice, with each day being considered as a replicate. The water temperature in both the plastic tanks and aquariums was maintained at about 27 ºC, with continuous aeration. A spectrophotometer (Micronal B582) was used to measure the total ammonia nitrogen levels, following the methodology described by Koroleff (1976). Ammonia excretion rates were expressed in mg N $kg^{-1}$ $h^{-1}$.

**Statistical Analysis**

Data were analyzed by one-way ANOVA and, when significant differences were indicated at the 0.05 levels, the treatment effects were analyzed using polynomial regression models for the growth performance and body composition. Total ammonia nitrogen excretion rates were compared using by Tukey's test. Os dados foram analisados pelo procedimento GLM utilizando-se o programa computacional Software "*Statistical Analysis System*" Version 9.3 (SAS INSTITUTE INC, 2012).

## RESULTS AND DISCUSSION

During the experimental period, the pH ranged from 7.1 to 8.05; dissolved oxygen 4.1 to 5.3 mg $L^{-1}$; alkalinity 22.75 to 28.00 mg $CaCO_3$ $L^{-1}$; hardness 37 to 42.5 mg $CaCO_3$ $L^{-1}$; and carbon dioxide 3.5 to 5.5 mg $L^{-1}$. The physical and chemical parameters of water were maintained within the tolerance range for most teleost species used in aquaculture according Sá (2012). The temperature ranged 24 to 25.5 ºC, and was only slightly below the ideal range for tambaqui on days 30 to 45 of the experiment, with the ideal range being 25 to 34 ºC according Araujo-Lima and Gomes (2013). Water transparency remained high (range, 68 to 84 cm) throughout the experimental period, with the aim to reduce the concentration of natural food so it did not interfere with the nutrient utilization of fish diets.

The protein levels in the diet did not interfered with final body weight, weight gain, feed conversion ratio, hepato-somatic index, carcass yield or survival (Table 2). However, the food intake, specific growth rate and viscero-somatic fat index of tambaqui were influenced by the protein levels in a linear decreasing manner. This result showed that the values of these variables decreased with increasing crude protein level in the diet.

The tambaqui fed diet containing 300 g $kg^{-1}$ crude protein consumed higher amount of feed, resulting in higher intake of digestible energy, which may have provided a greater viscero-somatic fat deposition in these fish. This result is similar to those shown by Van der Meer, Zamora and Verdegem (1997) who determined that tambaqui (1.12 g) fed diet containing 300 g $kg^{-1}$ crude protein showed higher feed intake and protein utilization efficiency than those fed diet containing 400 g $kg^{-1}$. However, the fish became fat, with high body lipid and intraperitoneal fat levels. Similar results were obtained by Gutiérrez *et al.* (2010), who concluded that feeding tambaqui (6.73 g) with diets containing 250 or 270 g $kg^{-1}$ of crude protein and supplemented with methionine, lysine and methionine + cystine, will guarantee its successful growing.

For tambatinga hybrid (*Colossoma macropomum x Piaractus brachypomus*) with initial weight of 14.76 g, Uzcátegui-Varela *et al.* (2014) recommend crude protein level greater than 260 g $kg^{-1}$ (using casein as a protein source) to ensure optimum growth. Similar results were obtained by Araripe *et al.* (2011), who recommended a 280 g $kg^{-1}$ crude protein level with amino acid supplementation (methionine, lysine and threonine) to tambatinga fingerlings (6.52 g).

In pacu, *Piaractus mesopotamicus*, which is an omnivorous fish native to Brazil, belonging to the same family as tambaqui, Abimorad and Carneiro (2007) and Abimorad, Carneiro and Urbinati (2007), found that the minimum crude protein requirement for pacu juvenile is 250 g $kg^{-1}$. Furthermore, Bicudo, Sado and Cyrino (2010) recommended that a 270 g $kg^{-1}$ of dietary protein should be used. The results of the current study are similar to those

**Table 2** - Growth performance of tambaqui *Colossoma macropomum* fed with diets containing different crude protein levels

| Variables | Crude protein levels (g kg$^{-1}$) | | | | P Value | CV (%) |
|---|---|---|---|---|---|---|
| | 300 | 350 | 400 | 450 | | |
| Final weight (g) | 18.97 ± 1.78 | 18.27 ± 1.71 | 18.55 ± 1.14 | 16.33 ± 0.78 | 0.076$^{ns}$ | 8.71 |
| WG (g fish$^{-1}$) | 17.28 ± 1.75 | 16.55 ± 1.72 | 16.76 ± 1.16 | 14.65 ± 0.68 | 0.074$^{ns}$ | 9.55 |
| Feed intake (g fish$^{-1}$)[1] | 32.68 ± 3.08 | 32.31 ± 2.38 | 32.07 ± 1.02 | 28.32 ± 1.99 | 0.047 | 7.96 |
| FCR | 1.89 ± 0.08 | 1.95 ± 0.13 | 1.92 ± 0.09 | 1.93 ± 0.08 | 0.821$^{ns}$ | 5.76 |
| SGR (% day$^{-1}$)[2] | 4.06 ± 0,14 | 3.93 ± 0,17 | 3.89 ± 0,13 | 3.79 ± 0,09 | 0.047 | 3.68 |
| HSI (%) | 2.13 ± 0.17 | 2.12 ± 0.24 | 1.87 ± 0.14 | 2.02 ± 0.13 | 0.143$^{ns}$ | 9.20 |
| Carcass yield (%) | 88.79 ± 0.34 | 89.72 ± 1.30 | 89.62 ± 0.59 | 88.98 ± 1.13 | 0.413$^{ns}$ | 1.16 |
| VSFI (%)[3] | 2.18 ± 0.30 | 1.63 ± 0.16 | 0.89 ± 0.18 | 0.61 ± 0.22 | 0.001 | 18.5 |
| Survival rate (%) | 98.00 ± 2.98 | 100.00 ± 0.00 | 98.67 ± 1.83 | 98.00 ± 2.98 | 0.491$^{ns}$ | 2.33 |

Results represent mean ± SD (n = 5); CV = variation coefficient; ns = not significant; WG = Weight gain; FCR = feed conversion rate; SGR = specific growth rate; HIS = hepato-somatic index; VSFI = viscero-somatic fat index; [1] Linear effect ($\hat{y}$ = 41.34-0.026x, $r^2$ = 0.72); [2] Linear effect ($\hat{y}$ = 4.555-0.0017x, $r^2$ = 0.96); [3] Linear effect ($\hat{y}$ = 5.465-0.011x, $r^2$ = 0.97)

reported for other omnivorous Brazilian fish species, such as the pirapitinga *Piaractus brachypomus* (316 g kg$^{-1}$) by Vásquez-Torres, Pereira-Filho and Arias-Castellanos (2011).

It was found that the protein efficiency ratio and the protein productive value were affected by the crude protein levels in a linear decreasing manner (Table 3). However, the protein growth rate was not influenced by the dietary protein levels. The highest protein efficiency ratio and protein productive value were obtained from the diet containing 300 g kg$^{-1}$ crude protein, while the lowest values were obtained from the diet containing 450 g kg$^{-1}$ crude protein. These results show that protein utilization was better in fish that were fed with lower levels of crude protein.

Protein growth rate is represented by the percentage of protein deposited on a daily basis, which in the present study ranged from 4.05 to 4.29% day$^{-1}$. As dietary protein levels had no significant effect on protein growth rates, our data reinforces the affirmation of Manbrini and Guillaume (2004), who explained that protein deposition (protein synthesis) is limited by the potential of the animal, which depends on genetic and physiological factors. Therefore, there will be an upper limit to the daily protein deposition, independent of the intake.

The amount of protein and mineral matter in the entire whole body and eviscerated carcass was not affected by the dietary protein levels; however, water and lipid contents of whole body and eviscerated carcass were linearly influenced by the dietary protein levels (Table 4). The water content increased with increasing dietary protein levels, whereas lipid values declined with increasing dietary protein levels. This finding confirmed that fish with higher body lipid content contained less water. Fish fed diets containing 300 g kg$^{-1}$ crude protein had higher body lipid content, corroborating the viscero-somatic fat index values. Increasing crude protein levels with decreasing body lipid content in fish have been shown in other studies on tambaqui (VAN DER MEER; ZAMORA; VERDEGEM, 1997) and pacu (BICUDO; SADO; CYRINO, 2010).

According to Bureau *et al.* (2000), the inverse relationship between lipid and water content in the body of fish occurs because water is substituted by the lipid

**Table 3** - Protein utilization efficiency of tambaqui *Colossoma macropomum* fed diets containing different crude protein levels

| Variables | Crude protein levels (g kg$^{-1}$) | | | | P Value | CV (%) |
|---|---|---|---|---|---|---|
| | 300 | 350 | 400 | 450 | | |
| PER[1] | 1.74 ± 0.08 | 1.42 ± 0.11 | 1.26 ± 0.07 | 1.12 ± 0.05 | 0.001 | 5.82 |
| PPV (%)[2] | 24.94 ± 2.35 | 22.65 ± 1.95 | 19.69 ± 1.34 | 17.11 ± 1.33 | 0.001 | 8.45 |
| PGR (% day$^{-1}$) | 4.22 ± 0.34 | 4.29 ± 0.37 | 4.27 ± 0.27 | 4.05 ± 0.38 | 0.667$^{ns}$ | 8.21 |

Results represent mean ± SD (n = 5); CV = variation coefficient; ns = not significant; PER = protein efficiency rate; PPV = protein productive value; PGR = protein growth rate; [1] Linear effect ($\hat{y}$ = 2.906-0.0041x, $r^2$ = 0.96); [2] Linear effect ($\hat{y}$ = 40.935-0.0529x, $r^2$ = 0.99)

**Table 4** - Proximate composition of whole body, and eviscerated carcass of tambaqui *Colossoma macropomum* fed diets containing different crude protein levels

| Variables | Crude protein levels (g kg$^{-1}$) | | | | P Value | CV (%) |
|---|---|---|---|---|---|---|
| | 300 | 350 | 400 | 450 | | |
| Whole body | | | | | | |
| Water (%)[1] | 75.28 ± 0.49 | 76.42 ± 0.66 | 77.59 ± 0.62 | 78.79 ± 0.70 | 0.001 | 0.81 |
| Protein (%) | 13.99 ± 0.86 | 15.18 ± 0.49 | 14.77 ± 0.60 | 14.62 ± 0.49 | 0.067$^{ns}$ | 4.29 |
| Lipid (%)[2] | 6.21 ± 0.45 | 5.15 ± 0.25 | 3.80 ± 0.35 | 3.12 ± 0.39 | 0.001 | 8.11 |
| Mineral matter (%) | 3.01 ± 0.22 | 2.82 ± 0.03 | 3.21 ± 0.15 | 3.01 ± 0.14 | 0.070$^{ns}$ | 4.92 |
| Eviscerated carcass | | | | | | |
| Water (%)[3] | 75.99 ± 1.08 | 76.72 ± 0.62 | 78.23 ± 1.29 | 78.76 ± 0.89 | 0.001 | 1.29 |
| Protein (%) | 14.92 ± 0.60 | 15.42 ± 0.51 | 14.67 ± 0.92 | 15.26 ± 0.69 | 0.353$^{ns}$ | 4.63 |
| Lipid (%)[4] | 4.73 ± 0.27 | 4.02 ± 0.14 | 3.14 ± 0.21 | 2.58 ± 0.22 | 0.001 | 5.98 |
| Mineral matter (%) | 3.07 ± 0.24 | 3.09 ± 0.24 | 3.10 ± 0.22 | 3.11 ± 0.05 | 0.993$^{ns}$ | 6.62 |

Results represent mean ± SD (n = 5); CV = variation coefficient; ns = not significant; [1] Linear effect ($\hat{y}$ = 68.245+0.0234x, $r^2$ = 0.99); [2] Linear effect ($\hat{y}$ = 12.535-0.0212x, $r^2$ = 0.98); [3] Linear effect ($\hat{y}$ = 70.06+0.0196x, $r^2$ = 0.97); [4] Linear effect ($\hat{y}$ = 9.115-0.0147x, $r^2$ = 0.99)

deposited in tissues. The inverse relationship between lipid and water content in the muscle of fish has also been recorded by several authors (ABIMORAD; CARNEIRO; URBINATI, 2007; BICUDO; SADO; CYRINO, 2010; CAULA; OLIVEIRA; MAIA, 2008; GUINAZI *et al*., 2006).

The fat deposition in fish is closely related to the amino acids balance and the energy/ protein ratio of the diet (BOTARO *et al*., 2007). Further, it is possible that the energy/protein ratio of the experimental diets may have influenced the body fat deposition. According to Manbrini and Guillaume (2004), a low energy/protein ratio may reduce fish growth rates, due to the increased metabolic demand for nitrogen excretion. Alternatively, excess energy in the diet may cause excessive body lipid and intraperitoneal fat deposition in fish, as well as reduce feed intake levels and inhibit the use of other nutrients.

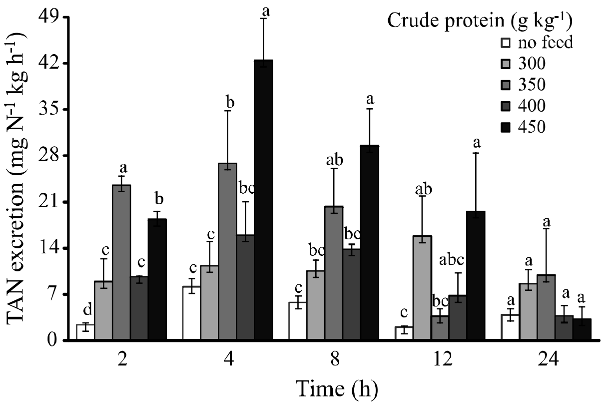
In this study, the energy/protein ratios of the diets containing 300; 350; 400 and 450 g kg$^{-1}$ crude protein were 13.66; 11.81; 10.67 and 8.84 kcal g$^{-1}$, respectively. This data showed that the diet of 300 g kg$^{-1}$ crude protein presented the highest energy/protein ratio, whereas the diet of 450 g kg$^{-1}$ presented the lowest. This outcome explains the greater body lipid and intraperitoneal fat deposition in fish fed with low protein level diet. Thus, further studies are required to determine the appropriate energy/protein ratio, which would help to promote the production of fish with low body lipid and intraperitoneal fat levels in the weight category of 2–20 g.

Total ammonia excretion rates were influenced by the dietary crude protein levels (Figure 1). In general, fish fed diet containing 300 g kg$^{-1}$ crude protein excreted less ammonia than those fed diet containing 450 g kg$^{-1}$ crude protein. The peak of ammonia excretion by tambaqui fed diets containing 350; 400 and 450 g kg$^{-1}$ crude protein was observed at 4 hours after feeding. In tambaqui that were fed diet containing 300 g kg$^{-1}$ crude protein, the peak of the ammonia excretion was observed at 12 hours after feeding. The peak rates were maintained for a short period, and then the rates slowly declined and were not significantly different to the rates of unfed fish (endogenous excretion) after 24 hours, which ranged from 3.23 to 9.0 mg N kg$^{-1}$ h$^{-1}$.

These results are in agreement with those in previous studies stating that ammonia excretion rates in teleosts are directly related to protein intake and dietary nitrogen content (KAUSHIK; COWEY, 1990). In previous studies of tambaqui (BRANDÃO *et al*., 2009), pacu (ABIMORAD *et al*., 2009) and red drum *Sciaenops ocellatus (WU; GATLIN III*, 2014) it was found that diets with high crude protein levels resulted in high total ammonia content in water. For rainbow trout *Oncorhynchus mykiss*, Green and Hardy (2008) confirmed that a diet with optimum dietary amino acid pattern and lowest digestive protein: digestive energy ratio produced the lowest total nitrogen and ammonia excretion.

The amino acid profile of protein, especially the dietary essential and nonessential amino acids ratio, may also influence the ammonia excretion rates. For instance, Carter and Houlihan (2001) explain that for each amino acid to be retained with maximum efficiency, all the essential amino acids need to be at an optimum ratio with respect to each other. Bonaldo *et al*. (2011) evaluated the replacement of fish meal protein with a complementary mixture of plant protein (wheat gluten, soybean meal, and

**Figure 1** - Total ammonia nitrogen (TAN) (mg N/kg/h) excretion rates of *Colossoma macropomum* juvenile in relation to dietary protein levels during the 24 h post-feeding period. Results represent mean ± SD (n = 3). Means not sharing the same letter are significantly different, p≤0.05




corn gluten meal) in isoproteic diets for juvenile turbot, *Psetta maxima*, and found that the ammonia excretion was higher in fish fed diet containing 66% of replacement of fish meal protein by mixture of plant protein, and lower in fish fed diet containing 25% of the replacement, in which the essential and nonessential amino acids ratios were 0.95 and 0.79, respectively.

For the amino acid profile of the experimental diets, the relationship between the limiting amino acids, lysine, methionine, methionine+cystine, threonine and arginine remained stable, whereas an excess of protein in the diets increased the rate of ammonia excretion.

Soybean meal includes such anti-nutritional factors as phytic acid, tannin, saponins, and the oligosaccharides, raffinose and stachyose, which are less affected in processing raw material. This incomplete inactivation of the anti-nutritional factors may limit the use of soybean meal in feed (BUTOLO, 2002; HOULIHAN; BOUJARD; JOBLING, 2001;). Nevertheless, in experiments with juveniles of pacu (FERNANDES; CARNEIRO; SAKOMURA, 2000), piavuçu *Leporinus macrocephalus* (FARIA; HAYASHI; SOARES, 2001) and Nile tilapia *Oreochromis niloticus* (NGUYEN; DAVIS; SAOUD, 2009), it was found that soybean meal can completely replace fishmeal in diets.

The best growth performance, protein utilization efficiency and total ammonia excretion rates were obtained from the diet containing low crude protein level. This diet contributed to a low production cost and environmental impact, since protein levels in the diet were lower, thus reducing the nitrogenous compounds excretion due to low levels of protein catabolism.

## CONCLUSIONS

The results obtained in the current study indicate that 300 g $kg^{-1}$ crude protein should be fed to tambaqui juvenile of 2–20 g body weight under aquaculture conditions.

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## REFERENCES

ABIMORAD, E. G. *et al*. Dietary supplementation of lysine and/or methionine on performance, nitrogen retention and excretion in pacu Piaractus mesopotamicus reared in cages. **Aquaculture**, v. 295, n. 3/4, p. 266–270, 2009.

ABIMORAD, E. G.; CARNEIRO, D. J. Digestibility and performance of pacu (*Piaractus mesopotamicus*) juveniles - fed diets containing different protein, lipid and carbohydrate levels. **Aquaculture Nutrition**, v. 13, n. 1, p. 1-9, 2007.

ABIMORAD, E. G.; CARNEIRO, D. J.; URBINATI, E. C. Growth and metabolism of pacu (*Piaractus mesopotamicus* Holmberg 1887) juveniles fed diets containing different protein, lipid and carbohydrate levels. **Aquaculture Research**, v. 38, n. 1, p. 36-44, 2007.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis of AOAC International**. 16th ed. Arlington: AOAC International, 1995.

ARARIPE, M. N. B. A. *et al*. Redução da proteína bruta com suplementação de aminoácidos em rações para alevinos de tambatinga. **Revista Brasileira de Zootecnia**, v. 40, n. 9, p. 1845-1850, 2011.

ARAÚJO-LIMA, C. A. R. M.; GOMES, L. C. Tambaqui (*Colossoma macropomum*). *In*: BALDISSEROTTO, B.; GOMES, L. C. **Espécies nativas para piscicultura no Brasil**. 2. ed. Santa Maria: UFSM, 2013. cap.7, p. 175-204.

ARAÚJO-LIMA, C.; GOULDING, M. **So fruitful a fish**: ecology, conservation and aquaculture of the Amazon's tambaqui. New York: Columbia University Press, 1997. 157 p.

ARZEL, J. *et al*. The protein requirement of brown trout (*Salmo trutta*) fry. **Aquaculture**, v. 130, n. 1, p. 67-78, 1995.

BICUDO, A. J. A.; SADO, R. Y.; CYRINO, J. E. P. Growth performance and body composition of pacu *Piaractus mesopotamicus* (Holmberg 1887) in response to dietary protein and energy levels. **Aquaculture Nutrition**, v. 16, n. 2, p. 213-222, 2010.

BONALDO, A. *et al*. Increasing dietary plant proteins affects growth performance and ammonia excretion but not digestibility and gut histology in turbot (*Psetta maxima*) juveniles. **Aquaculture**, v. 318, n. 1, p. 101-108, 2011.

BOTARO, D. *et al*. Redução da proteína da dieta com base no conceito de proteína ideal para tilápias-do-nilo (*Oreochromis niloticus*) criadas em tanques-rede. **Revista Brasileira de Zootecnia**, v. 36, n. 3, p. 517-525, 2007.

BRANDÃO, L. V. *et al*. Suplementação de metionina e/ou lisina em rações para juvenis de tambaqui (*Colossoma macropomum* Cuvier, 1818). **Acta Amazônica**, v. 39, n. 3, p. 675-680, 2009.

BUREAU, D. P. *et al*. Pattern and cost of growth and nutrient deposition in fish and shrimp: potencial implications and applications. *In*: **Avances en nutrición acuícola**: SIMPOSIUM INTERNACIONAL DE NUTRICIÓN ACUÍCOLA, 5., 2000, Mérida. **Abstract…** Mérida, 2000. p. 119-122.

BUTOLO, J. E. **Qualidade de ingredientes na alimentação animal**. Campinas: J. E. Butolo, 2002. 430 p.

CARTER, C. G.; HOULIHAN, D. E. Protein synthesis. *In*: WRIGHT, P. A.; ANDERSON, P. M. **Fish physiology**: nitrogen excretion. Amsterdam: Academic Press, 2001. cap. 2, p. 31-75.

CAULA, F. C. B.; OLIVEIRA, M. P.; MAIA, E. L. Teor de colesterol e composição centesimal de algumas espécies de peixes do estado do Ceará. **Ciência e Tecnologia de Alimentos**, v. 28, n. 4, p. 959-963, 2008.

FARIA, A. C. E. A.; HAYASHI, C.; SOARES, C. M. Substituição parcial e total da farinha de peixe pelo farelo de soja em dietas para alevinos de piavuçu, *Leporinus macrocephalus* (Garavello & Britski, 1988). **Acta Scientiarum**, v. 23, n. 4, p. 835-840, 2001.

FERNANDES, J. B. K.; CARNEIRO, D. J.; SAKOMURA, N. K. Fontes e níveis de proteína bruta em dietas para alevinos de pacu (*Piaractus mesopotamicus*). **Revista Brasileira de Zootecnia**, v. 29, n. 3, p. 646-653, 2000.

GREEN, J. A.; HARDY, R. W. The effects of dietary protein: energy ratio and amino acid pattern on nitrogen utilization and excretion of rainbow trout *Oncorhynchus mykiss* (Walbaum). **Journal of Fish Biology**, v. 73, n. 3, p. 663-682, 2008.

GUINAZI, M. *et al*. Composição química de peixes de água doce frescos e estocados sob congelamento. **Acta Scientiarum Technology**, v. 28, n. 2, p. 119-124, 2006.

GUTIÉRREZ, F. W. *et al*. Utilización de la proteína dietaría por alevinos de la gamitana, *Colossoma macropomum*, alimentados con dietas isocalóricas. **Revista Peruana de Biologia**, v. 17, n. 2, p. 219-223, 2010.

HOULIHAN, D.; BOUJARD, T.; JOBLING, M. **Food intake in fish**. Oxford: Blackwell Science, 2001. 418 p.

KAUSHIK, S. J.; COWEY, C. B. Dietary factors affecting nitrogen excretion by fish. *In*: COWEY, C. B.; CHO, C. Y. **Nutritional strategies and aquaculture waste**: Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. Ontario: University of Guelf, 1990. p. 3-19.

KOROLEFF, F. Determination of nutrients. *In*: GRASS-HOFFK, K. **Methods of seawater Analysis**. Germany: Verlag Chemie Weinhein, 1976. p. 117-187.

MANBRINI, M.; GUILLAUME, J. Nutrición proteica. *In*: GUILLAUME, J. *et al*. **Nutrición y alimentación de peces y crustáceos**. Madrid: Mundi-Prensa, 2004. cap. 6, p. 113-145.

NGUYEN, T. N.; DAVIS, A.; SAOUD, I. P. Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, *Oreochromis spp*. **Journal of the World Aquaculture Society**, v. 40, n. 1, p. 113-121, 2009.

NUNES, E. S. S. *et al*. Enzimas digestivas exógenas na alimentação do tambaqui. **Pesquisa Agropecuária Brasileira**, v. 41, n. 1, p. 139-143, 2006.

ONO, E. A.; OLIVEIRA, S. R. Rações para a aquicultura brasileira: os problemas e as possíveis soluções. **Panorama da Aquicultura**, v. 18, n. 106, p. 20-30, 2008.

ORTÍ, G. *et al*. Phylogeny of the Serrasalmidae (Characiformes) based on mitochondrial DNA sequences. **Genetics and Molecular Biology**, v. 31, n. 1, p. 343-351, 2008.

ROTTA, M. A. **Utilização da energia e da proteína pelos peixes**. Corumbá: Embrapa Pantanal, 2002. 24 p.

SÁ, M. V. C. **Limnocultura**: limnologia para aquicultura. Fortaleza: Edições UFC, 2012. 218 p.

SAS INSTITUTE INC. SAS/STAT ® Software, Version 9.3, Cary: SAS Institute Inc, 2012.

UZCÁTEGUI-VARELA, J. P. *et al*. Evaluación de dietas con diferente contenido proteico sobre el desempeño productivo de alevines del híbrido cachamay (*Colossoma macropomum* ♂ *x Piaractus brachypomus* ♀) en condiciones de cautiverio. **Revista Científica de la Facultad de Ciencias Veteriarias**, v. 24, n. 5, p. 458- 465, 2014.

VAN DER MEER, M. B.; ZAMORA, J. E.; VERDEGEM, M. C. J. Effect of dietary lipid level on protein utilization and the size and proximate composition of body compartments of *Colossoma macropomum* (Cuvier). **Aquaculture Research**, v. 28, n. 6, p. 405-417, 1997.

VÁSQUEZ-TORRES, W.; PEREIRA-FILHO, M.; ARIAS-CASTELLANOS, J. A. Optimum dietary crude protein requirement for juvenile cachama *Piaractus brachypomus*. **Ciência Rural**, v. 41, n. 2, p. 2183-2189, 2011.

WU, X.; GATLIN III, D. M. Effects of altering dietary protein content in morning and evening feedings on growth and ammonia excretion of red drum (*Sciaenops ocellatus*). **Aquaculture**, v. 434, p. 33-37, 2014.