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Lysine requirement for tambaqui juveniles

Exigência de lisina para juvenis de tambaqui

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Abstract

The aim of this study was to determine the requirement for lysine in the diet of juvenile tambaqui (*Colossoma macropomum*). In total, 750 juvenile fish (0.34 ± 0.02g) were distributed in tanks arranged in a randomized block design with six treatments, five replications in two blocks, and 25 fish per unit, for 50 d. Six levels of digestible lysine were tested (1.30, 1.48, 1.66, 1.84, 2.02, and 2.20%), in formulated diets based on the ideal protein concept. The performance, feed efficiency, daily deposition of protein and body fat, and nitrogen retention efficiency of the fish were evaluated. The consumption of rations and protein, specific growth rate, body composition, and deposition of body fat were not influenced by the lysine levels tested. The consumption of digestible lysine increased linearly and the efficiency of digestible lysine for weight gain decreased linearly with the increase in lysine levels. The levels of digestible lysine that optimized weight gain and body protein deposition were estimated at 1.73 and 1.78%, respectively. The feed conversion and nitrogen retention efficiency were most improved at 1.66% and 1.84%, respectively. The recommended level of dietary lysine for providing better weight gain and body protein deposition in juvenile tambaqui is 1.78%, equivalent to 2.00% total lysine.
Key words: Body composition. *Colossoma macropomum*. Initial growth phase. Protein nutrition.

Resumo

Objetivou-se determinar a exigência de lisina nas rações para juvenis de tambaqui (*Colossoma macropomum*). 750 peixes (0,34 ± 0,02g) foram distribuídos em tanques organizados em delineamento de blocos casualizados, com seis tratamentos, cinco repetições em dois blocos e vinte e cinco peixes por parcela, durante cinquenta dias. Foram testados seis níveis de lisina digestível (1,30; 1,48; 1,60; 1,84; 2,02; e 2,20%), em dietas formuladas com base no conceito de proteína ideal. Avaliaram-se o desempenho, eficiência alimentar, deposições diárias de proteína e gordura corporais e a eficiência de retenção de nitrogênio. Os consumos de ração e de proteína, taxa de crescimento específico, composição corporal e deposição de gordura corporal não foram influenciados pelos níveis de lisina testados. O consumo de lisina digestível aumentou linearmente e a eficiência de lisina digestível para o ganho de peso reduziu de forma linear com a elevação dos níveis de lisina. O nível de lisina digestível da

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ração que otimizou o ganho de peso e deposição de proteína corporal foi estimado em 1,73 e 1,78%, respectivamente. A conversão alimentar e eficiência de retenção de nitrogênio melhoraram no nível de 1,66% e 1,84%, respectivamente. A recomendação do nível de lisina digestível em rações para juvenis de tambaqui é de 1,78%, equivalente a 2,00% de lisina total por proporcionar melhor ganho de peso e deposição de proteína corporal.
Palavras-chave: *Colossoma macropomum*. Composição corporal. Fase inicial. Nutrição proteica.

Introduction

The tambaqui (*Colossoma macropum*) is a species native to the Amazon region that has potential for zootechnical farming due to its productive and organoleptic characteristics (ARAUJO-LIMA and GOMES, 2005). On the other hand, there is a lack of information on its nutritional requirements, especially in relation to the normally limiting amino acids in feeding practices (DAIRIKI; SILVA, 2011; OLIVEIRA et al., 2013).

Fish, similarly to birds and mammals, do not have a dietary requirement for protein, but instead require a quantitative balance of essential and non-essential amino acids. Accordingly, a ration formulated based on crude protein may not meet the nutritional requirements for all amino acids. As a strategy to meet the amino acid requirements of the species, the ideal protein concept has been used in previous studies to formulate feed (TAKISHITA et al., 2009).

The ideal protein corresponds to an ideal amino acid balance, without excesses or deficits, that meets the requirements for maintenance and growth. The amino acid used as a reference to the profile of the ideal protein is lysine (BAKER; HAN, 1994; PARSONS; BAKER, 1994; FURUYA et al., 2005; BOMFIM et al., 2008).

Lysine is a reference amino acid used in the determination of the ideal protein standard because it is the most limiting in some protein sources alternative to fish meal, mainly in cereals (such as gluten), being found in higher concentrations in proteins of animal origin. In addition, the laboratory analysis of its levels in ingredients, rations, and tissues is more accessible, besides being an amino acid primarily directed to body protein deposition (BAKER; HAN, 1994; KIM; LALL, 2000; RODEHUTSCORD et al., 2000; FURUYA et al., 2004; DAIRIKI et al., 2007; ABIMORAD et al., 2008; BRANDÃO et al., 2009; OVIE; EZE, 2013).

Given the lack of information regarding the requirement of lysine in tambaqui feed, this study was carried out to determine the requirement of lysine in the rations for juveniles of this species.

Material and Methods

The experiment was conducted in accordance with the ethical standards for animal research, after approval by the Ethics Committee on Animal Use of the Federal University of Maranhão (Protocol: 23115004063/2012 - 95), and was carried out over 50 d at Laboratory of Food and Nutrition of Aquatic Organisms in the center of Agricultural and Environmental Sciences of the Federal University of Maranhão (UFMA), Chapadinha, Maranhão.

The experiment used 750 tambaqui, with an initial weight of 0.34 ± 0.02g, in a randomized block design, consisting of six treatments with five replications in two blocks, and 25 fish per experimental unit.

The treatments consisted of six experimental isoproteic, isonenergetic, isocalcium, and isophosphorus rations with different levels of digestible lysine (1.30, 1.48, 1.66, 1.84, 2.02, and 2.20%), formulated using the supplementation with amino acids technique based on the ideal protein concept. In the rations, the methionine plus cystine:lysine ratio, as well as the other amino acids:lysine ratios, were kept at least five percentage points above the values recommended by Souza (2014) for tambaqui, and estimated from the values of requirements for Nile tilapia *(Oreochromis niloticus*) proposed by the NRC (2011), respectively, in order to avoid the occurrence of another limiting amino acid for each level of supplementation of digestible lysine evaluated (Table 1).

Table 1. Percentage and chemical composition of the experimental rations (natural matter).

Ingredients (%)	Level of digestible lysine (%)					
	1.30	1.48	1.66	1.84	2.02	2.20
Soy bran	51.511	51.511	51.511	51.511	51.511	51.511
Corn	34.830	34.830	34.830	34.830	34.830	34.830
Corn starch	0.000	0.291	0.599	0.974	1.406	2.177
Soybean oil	3.885	3.797	3.697	3.552	3.384	3.246
Lysine HCl (78.4%)	0.000	0.230	0.460	0.690	0.920	1.150
DL-Methionine (99.0%)	0.194	0.322	0.450	0.579	0.707	0.835
L-Threonine (98.5%)	0.155	0.308	0.461	0.613	0.766	0.919
L-Tryptophan (98.0%)	0.000	0.044	0.090	0.136	0.182	0.228
L-Isoleucine (99.0%)	0.000	0.000	0.023	0.143	0.263	0.383
L-Valine (96.5%)	0.000	0.000	0.000	0.000	0.070	0.179
L-Arginine (99.0%)	0.000	0.000	0.000	0.000	0.004	0.162
L-Glutamic Acid (98.5%)	5.046	4.288	3.499	2.592	1.577	0.000
Dicalcium phosphate	3.310	3.310	3.310	3.310	3.310	3.310
Vitamin and mineral premix[5]	0.500	0.500	0.500	0.500	0.500	0.500
Vitamin C[4]	0.050	0.050	0.050	0.050	0.050	0.050
Salt	0.500	0.500	0.500	0.500	0.500	0.500
(Antioxidant BHT)	0.020	0.020	0.020	0.020	0.020	0.020
Calculated composition[1]						
Crude protein (%)	29.14	29.14	29.14	29.14	29.14	29.14
Digestible protein (%)[3]	26.78	26.78	26.78	26.78	26.78	26.78
Digestible energy (kcal kg^{-1})[3]	3000.01	3000.00	3000.00	3000.00	3000.00	3000.01
Ether extract (%)	6.01	5.92	5.82	5.68	5.51	5.37
Crude fiber (%)	3.33	3.33	3.33	3.33	3.33	3.33
Total Ca (%)	0.95	0.95	0.95	0.95	0.95	0.95
P available (%)[2]	0.70	0.70	0.70	0.70	0.70	0.70
Total lysine (%)[2]	1.517	1.698	1.878	2.058	2.239	2.419
Digestible lysine (%)[2]	1.300	1.480	1.660	1.840	2.020	2.200
Digestible methionine. + cysteine (%)[2]	0.910	1.036	1.162	1.288	1.414	1.540
Digestible threonine (%)[2]	1.066	1.214	1.361	1.509	1.656	1.804
Digestible tryptophan (%)[2]	0.345	0.389	0.434	0.479	0.525	0.570
Digestible isoleucine (%)[2]	1.073	1.073	1.096	1.214	1.333	1.452
Digestible arginine (%)[2]	1.754	1.754	1.754	1.754	1.757	1.914
Digestible valine (%)	1.143	1.143	1.143	1.143	1.212	1.320
Digestible lysine/energy (g $Mcal^{-1}$)	0.433	0.493	0.553	0.613	0.673	0.733

[1] Based on the values proposed by Rostagno et al. (2011);
[2] Based on the digestibility coefficients of industrial aminoacids proposed by Rostagno et al. (2011) and for amino acids and phosphorus availability for corn and soybean meal proposed by Furuya (2010);
[3] Based on the digestibility coefficients proposed by Furuya (2010) for Nile tilapia;
[4] Vitamin C: Calcium L-ascorbic acid 2-monophosphate, 42% of active ingredient;
[5] Vitamin and mineral supplement (5 kg t^{-1}), with guaranteed levels per kilogram of product: Total vitamins, 1,200,000 IU; vit. D_3, 200,000 IU; vit. E, 1,200 mg; vit. K_3, 2,400 mg; vit. B_1, 4,800 mg; vit. B_2, 4,800 mg; vit. B_6, 4,800 mg; vit. B_{12}, 4,800 mg; folic acid, 1,200 mg; Ca pantothenate, 12,000 mg; vit. C, 48,000 mg; biotin, 48 mg; choline chloride, 108 g; niacin, 24,000 mg; Fe, 50,000 mg; Cu, 3,000 mg; Mn, 20,000 mg; Zn, 30,000 mg; I, 100 mg; Co, 10 mg; Se, 100 mg.

The fingerlings were kept in 30 polyethylene boxes, 12 of 1,000 L and 18 of 500 L, with individual water supply, drainage, and aeration systems.

The water supply for the aquariums was derived from an artesian well. The water temperature was measured daily, at 7h30 and 17h30, with the help of a mercury bulb thermometer, graduated from 0 to 50 °C. The monitoring of pH, dissolved oxygen, and ammonia in the water was performed every 7 d with a a pH-meter, pulse oximeter, and commercial colorimetric kit to test for toxic ammonia, respectively.

The maximum and minimum temperatures remained at 26.1 ± 0.64 °C and 24.2 ± 0.76 °C, respectively; the concentration of dissolved oxygen in the water was 7.63 ± 0.70 ppm, while pH was 5.8 ± 0.39 and total ammonia was ≤ 1.00 ppm. The water quality parameters remained within the recommended standards for the breeding of the species (GOMES et al., 2010; MENDONÇA et al., 2012).

The ingredients of the experimental rations were mixed, moistened in water heated to about 50 °C, and the mixture was pelleted using a meat grinder with 5 mm sieve (C.A.F. Meat grinder; model 98 STI). Then, the rations were dried in open air, crushed, and sieved, to attain pellets of around 3 mm in diameter.

The rations were offered daily in six meals (08h00, 10h00, 12h00, 14h00, 16h00, and 18h00). At every meal, the rations were offered in small quantities, with successive passes to apparent satiation, thus avoiding over- or underdelivering of feed. Cleaning of the boxes was performed daily by siphoning after measuring water temperature.

At the beginning of the experiment, 50 fish were stunned and euthanized in water with ice, and frozen for later determination of initial body composition. In the end, all fish in each box, after 24 hours of fasting, were stunned, euthanized, weighed, and frozen for determination of final body composition.

After thawing, the whole fish were pre-dried in an oven with forced air circulation, pre-degreased in glass containers using petroleum ether as solvent, milled in a ball mill and placed in containers for laboratory analysis. The samples were analyzed for body composition (moisture, crude protein, and lipids) according to the procedures described by Silva and Queiroz (2005). The laboratory analyses were performed in the Laboratory of Animal Nutrition at the Federal University of Maranhão (UFMA).

The following parameters were evaluated: survival rate (SR), feed consumption (FC), consumption of crude protein (CCP), consumption of digestible lysine (CDL), weight gain (WG), specific growth rate (SGR), feed conversion (FC), protein efficiency rate (PER), efficiency of digestible lysine for weight gain (ELW), chemical body composition (moisture, protein, and fat), rates of daily body protein (BPD) and fat deposition (BFD), and retention efficiency of nitrogen (NRE), according to the equations:

- $\text{SR (\%)} = \dfrac{\text{final number of fish} \times 100}{\text{initial number of fish}}$
- FC (g) = feed consumed during the experimental period
- $\text{CCP (g)} = \dfrac{[\text{consumption of feed (g)} \times \text{level of crude proteins in feed (\%)}]}{100}$
- $\text{CDL (mg)} = \dfrac{[\text{consumption of feed (mg)} \times \text{level of digestible lysine in feed (\%)}]}{100}$

- $WG\ (g) = \text{final mean weight (g) - initial mean weight (g)}$
- $SGR\left(\%\ day^{-1}\right) = \dfrac{\{[\text{natural logarithm of final weight (g) - natural logarithm of initial weight (g)}] \times 100\}}{\text{experimental period (days)}}$
- $FC\left(g\ g^{-1}\right) = \dfrac{\text{consumption of feed (g)}}{\text{Weight gain (g)}}$
- $PER\left(g\ g^{-1}\right) = \dfrac{\text{weight gain (g)}}{\text{consumption of crude protein (g)}}$
- $BPD\left(mg\ day^{-1}\right) = \dfrac{\{[(\text{final body protein, \%} \times \text{final weight, mg}) - (\text{initial body protein, \%} \times \text{initial weight, mg})] / 100\}}{\text{Experimental period (days)}}$
- $BFD\left(mg\ day^{-1}\right) = \dfrac{\{[(\text{final body fat, \%} \times \text{final weight, mg}) - (\text{initial body fat, \%} \times \text{initial weight, mg})] / 100\}}{\text{Experimental period (days)}}$
- $NRE(\%) = \dfrac{[\text{final body N (\%)} \times \text{final weight (g)}] - [\text{initial body N (\%)} \times \text{initial weight (g)}]}{(\text{feed consumption, g} \times \text{level of N in ration, \%})/100}$

The variables were subjected to analysis of variance with 5% significance. Variables that displayed significant effects of digestible lysine by variance analysis were subjected to linear and quadratic regressions. The discontinuous linear response plateau (LRP) model was also evaluated. The best fit model considered the value of significance (*P*) and the R^2 (SQ the model/SQ treatment). In addition, variables were submitted to the model proposed by Baker et al. (2002) to estimate the intermediary requirement, which were obtained with the quadratic-plus-LRP models individually.

In variables where there was no significant fit ($P < 0.10$) to the regression models, the Student-Newman-Keuls (SNK) test was used to compare the means of each treatment. Statistical analyses were performed using SAS (2002) 9.0 software.

Results and Discussion

The rate of survival, feed consumption of crude protein, and specific growth rate were not influenced ($P > 0.05$) by the level of digestible lysine in the ration (Table 2). Given that the diets contained different levels of digestible lysine, the consumption of digestible lysine increased linearly ($P < 0.05$) with the increase in dietary lysine levels (Tables 2 and 3). These results indicate that the significant effects observed in other variables were caused by the difference in the consumption of digestible lysine, since it was the most limiting essential amino acid in all experimental rations. The lack of variation in feed intake may be associated with the fact that the diets were isoenergetic, as the energy level of the diet can influence fish intake (NRC, 2011).

Table 2. The survival rate (SR), feed consumption (FC), consumption of crude protein (CCP), consumption of digestible lysine (CDL), weight gain (WG), specific growth rate (SGR), feed conversion (FC), protein efficiency rate (PER), and efficiency of digestible lysine for weight gain (ELW) of juvenile tambaqui, and a summary of the analysis of variance, depending on the level of digestible lysine in rations.

Level of digestible lysine (%)	Variable								
	SR (%)	FC (g)	CCP (g)	CDL (mg)	WG (g)	SGR (% day^{-1})	FC (g g^{-1})	TEP (g g^{-1})	ELW (g g^{-1})
1.30	95.20	5.45	1.55	70.81	3.73	5.17	1.39^{a}	2.42^{b}	57.99
1.48	96.80	6.09	1.73	90.12	4.52	5.28	1.38^{a}	2.60^{ab}	50.23
1.66	94.40	5.81	1.66	96.38	5.33	5.56	1.14^{b}	3.34^{a}	57.35
1.84	94.40	6.25	1.78	115.04	5.35	5.48	1.21^{ab}	2.99^{ab}	46.37
2.02	98.40	5.80	1.43	117.17	4.59	5.30	1.25^{ab}	2.95^{ab}	39.34
2.20	98.40	6.33	1.80	139.32	5.24	5.51	1.21^{ab}	2.91^{ab}	37.76
$P > F$[1]	0.9999	0.2820	0.0601	0.0001	0.0113	0.2094	0.0049	0.0323	0.0001
CV (%)	4.87	10.77	12.35	10.49	15.28	5.20	8.21	11.93	14.46

CV: Coefficient of variation (%);
$P > F$: Significance of "F" test of analysis of variance;
Means in the column followed by the same letters do not differ by the SNK test (P > 0.05).

Table 3. Adjusted regression equations, P values, coefficients of determination and values of the requirement for the variables, consumption of digestible lysine (CDL), weight gain (WG), and efficiency of digestible lysine for weight gain (ELW) of juvenile tambaqui as a function of levels of digestible lysine in rations.

Variable	Model	Equation	$P > F$	R^2	Requirement (%)
CDL (mg)	Linear	CLD = - 18.0742 + 70.2184Lys	0.0001	0.97	-----
WG (g)	Linear	GP = 2.6313 + 1.2352Lys	0.0946	0.42	-----
WG (g)	Quadratic	GP = - 8.8618 + 14.7885Lys - 3.8724Lys^2	0.0918	0.71	1.91
WG (g)	LRP	GP = 5.1280 - 4.3889(1.6190 - Lys)	0.0630	0.81	1.62
WG (g)	Quadratic + LRP	5.1280 = - 8.8618 + 14.7885Lys - 3.8724Lys^2	-	-	1.73
ELW (g g^{-1})	Linear	ELG = 88.3967 - 22.9857Lys	0.0044	0.80	-----

$P > F$: Significance of "F" analysis of variance test.

The weight gain displayed a quadratic effect (P<0.10) as a function of digestible lysine in feed rations, estimating the requirement at 1.910%. However, the LRP model was the best fit ($P < 0.07$) to the data, estimated at 1.619% digestible lysine, from which there was a plateau (Tables 2 and 3).

It should be emphasized that the quadratic model can provide an overestimation in requirement, because the model assumes bilateral symmetry of the response to increasing nutrient, since the quadratic model describes the drop in gain in the same intensity as the loss in gain. Furthermore, the quadratic function is very sensitive to the difference between the levels studied, tending to estimate the optimal values within the range of levels (EUCLYDES; ROSTAGNO, 2002). Although it has a good statistical fit, the LRP model, in turn, does not consider the physiological aspects of the animal and the law of diminishing returns; therefore, in many cases, it underestimates the optimal level, since it does not consider the increases that could justify additional improvements in performance

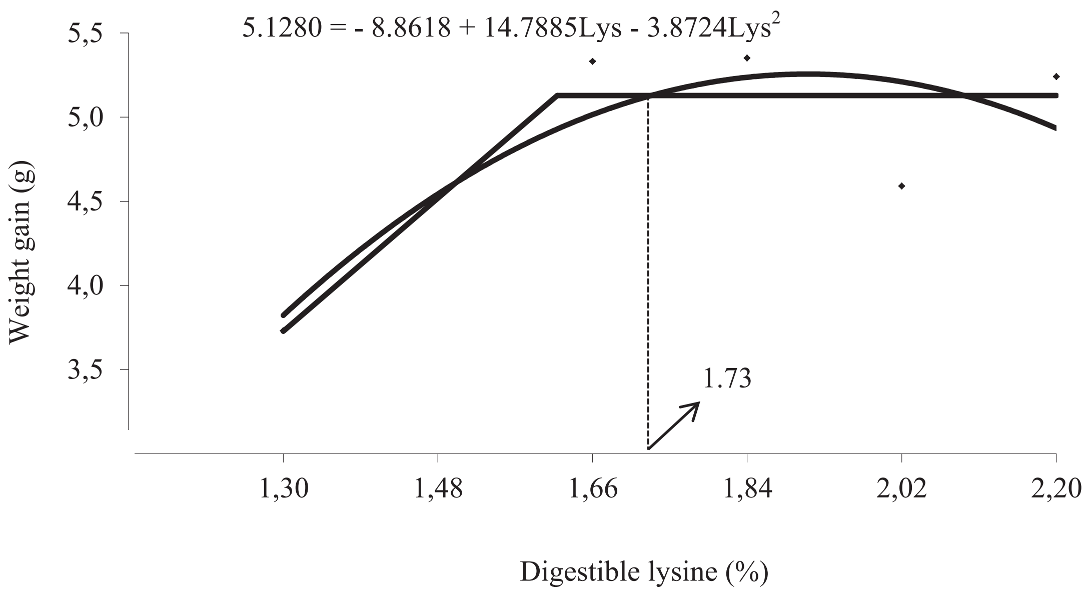
(PACK, 1996; PACK et al., 2003; SAKOMURA; ROSTAGNO, 2007; SIQUEIRA et al., 2009).

Thus, it is possible to estimate the level of lysine in an intermediate form obtained with the LRP plus quadratic models individually, as a way to circumvent the criticisms of these models. According to Baker et al. (2002), the value of the first intersection is an excellent way of representing the requirements, because it is an objective value. The level of digestible lysine in the diet estimated to optimize weight gain, corresponding to the first intersection of the quadratic equation with the plateau estimated by the LRP model, was 1.73% (Figure 1).

The results for the weight gain of fish fed with diets containing less than 1.73% of digestible lysine demonstrate that the use of levels below the requirement restricts animal growth, probably due to the reduction in the formation of lean tissue. This is because lysine is an essential amino acid found in higher concentrations in the carcass than others, and is used almost exclusively for protein synthesis (FURUYA et al., 2005; BOMFIM et al., 2010; NRC, 2011; BOMFIM, 2013).

Lysine requirements for Nile tilapia show different results than those estimated in this study to optimize weight gain (1.73%). It was higher than those determined by Furuya et al. (2004), for fish in the 5.34 to 77.26 g stage, which estimated the lysine level at 1.42%; by Furuya et al. (2013) for fish in the 87 to 226 g stage, fed diets balanced for arginine:lysine ratio, which estimated the requirement to be 1.31% digestible lysine; and by Furuya et al. (2006) with juveniles (5.72 to 35.05 g), which determined the requirement to be 1.56% digestible lysine. On the other hand, the result of the present study was similar to that determined by Bomfim et al. (2010), working with fingerlings (1.12 g), which estimated 1.70% digestible lysine as the minimum level to obtain the optimum weight gain; and was lower than that found for Nile tilapia fingerlings (0.98 g) by Takishita et al. (2009), who estimated the requirement to be 2.17% digestible lysine, both using the ideal protein concept in the formulation of experimental feed.

Figure 1. A graphical representation of weight gain for tambaqui juveniles as a function of digestible lysine levels in rations.



For pacu (*Piaractus mesopotamicus*), an omnivorous species with morphometric and ecological characteristics similar to tambaqui, fed diets with different levels of lysine using the ideal protein concept at the 8.66 to 49.18 g stage, the dietary requirement to optimize weight gain was 1.64% digestible lysine (ABIMORAD et al., 2010), similar to that estimated in this study using the LRP model.

Besides the statistical criterion for the interpretation of the results (linear, quadratic, exponential, LRP, models, or means test), the level of energy used in the experimental feed can also influence the value of estimated requirement for a same species, since it directly affects the consumption of feed and, consequently, in the amount of amino acids consumed per unit of gain and its use for protein deposition. In addition, a deficiency in any one of the essential amino acids in the experimental feed may limit the synthesis of protein at higher lysine levels and, consequently, underestimate the lysine requirement obtained (SAKOMURA; ROSTAGNO, 2007; SIQUEIRA et al., 2009; BOMFIM et al., 2010).

The improvement in weight gain, together with the similar consumption of feed, resulted in an improved feed conversion ($P < 0.05$) as a function of an increase in digestible lysine in the feed. However, there was no fit ($P > 0.10$) for any of the regression models evaluated. Based on the SNK test, a level of 1.66% digestible lysine presented better results compared to lower levels, without variation compared to higher levels, and is therefore recommended for this variable (Table 2). It should be emphasized that according to Sampaio (2007), the quantitative nature of the treatments employed suggests the study of models, however, this procedure alone does not replace the comparison of means.

The improvement in feed conversion, especially in terms of similar energy and feed consumption, indicates that there was a higher efficiency of protein utilization for the formation of lean tissue; this includes a higher percentage of water in its composition (BOMFIM et al., 2010).

In a study on juvenile Nile tilapia, Furuya et al. (2006), observed an improvement in feed conversion of fish fed with a diet until the estimated level of 1.44% of digestible lysine, a lower result than the value observed in this study. Abimorad et al. (2010), in an experiment with pacus (8.66 to 49.18g), identified 1.75% digestible lysine for better feed conversion, which is a higher value than that found in the present study. On the other hand, in studies conducted by Bomfim et al. (2010), and Takishita et al. (2009), with Nile tilapia fingerlings, the increase in digestible lysine in feed provided a linear improvement in feed conversion.

The protein efficiency rate displayed a variation ($P < 0.05$) according to the levels of digestible lysine. However, there was no fit ($P > 0.10$) for any of the regression models evaluated. Based on the SNK test, 1.66% of digestible lysine showed better results compared to the lowest level tested, without variation in relation to other levels (Table 2). The efficiency of digestible lysine for weight gain deteriorated linearly ($P < 0.01$) with an increase in digestible lysine in feed (Tables 2 and 3).

The protein efficiency rate is influenced by the protein:energy ratio of the feed, as the energy level directly influences feed consumption and, consequently, the availability of dietary protein to meet the nutritional requirements for maintenance and body protein deposition (MATHIS et al., 2003; OISHI et al., 2010). In this case, as the rations were isoproteic and isoenergetic (same protein:energy ratio), this gain is probably due to the improvement of the biological value of the protein (amino acids) balance provided by raising the level of digestible lysine (digestible lysine:digestible energy ratio), since there was no other essential amino acid that limited the potential use of each level of lysine evaluated. This suggests that an improvement in the rate of body protein deposition and nitrogen retention efficiency was observed in this study (BOISEN, 2003; TAKISHITA et al., 2009; BOMFIM et al., 2010).

Gonçalves et al. (2009), highlighted that high levels of digestible lysine supplementation in diets with low protein levels do not provide the same response, highlighting the importance of lysine in balance with other amino acids. Furthermore, the adequate balancing of amino acids in the diet, as well as the use of moderate levels of synthetic amino acids and short feeding intervals can maintain a more stable plasma concentration of amino acids. This optimizes the capacity/velocity of protein synthesis by specialized tissues, increasing the efficiency of nitrogen retention and significantly reducing the excretion of nitrogen into the environment (TENGJAROENKUL et al., 2000; BOMFIM et al., 2008).

The body composition (moisture, protein, and fat) of fish and the deposition of body fat did not vary (P > 0.05) with the variation in digestible lysine of the feed (Table 4).

The increase in digestible lysine in feed provided a quadratic effect on the body protein deposition, ($P < 0.10$), increasing this variable until the estimated level of 1.948%. Despite the quadratic increase, the LRP model was the best fit ($P < 0.09$) to the data, estimating that there was a plateau at the 1.683% digestible lysine level (Tables 4 and 5). Using the first intersection of the quadratic equation with the plateau obtained by the LRP equation, the level of digestible lysine of ration estimated for this variable was 1.78% (Table 5, Figure 2). For the efficiency of nitrogen retention, the LRP model provided a better fit ($P < 0.10$) to the data, estimated that there was a plateau at the 1.58% digestible lysine level (Tables 4 and 5).

Table 4. Contents of body moisture (MC), fat (FC), and protein (PC); deposition of body protein (DBP) and body fat (DBF); and nitrogen retention efficiency (NRE) of juvenile tambaqui, and a summary of the analysis of variance, as a function of the digestible lysine levels in rations.

Level of digestible lysine (%)	Variable					
	MC (%)	FC (%)	PC (%)	DGC (mg day^{-1})	DBP (mg day^{-1})	NRE (%)
Initial	75.89	4.47	14.87	-	-	-
1.30	78.83	7.69	10.70	6.02	7.62	24.94
1.48	76.62	8.06	11.58	7.63	10.12	29.31
1.66	78.10	8.80	10.22	9.95	10.54	32.47
1.84	79.22	5.95	11.36	6.71	12.01	33.53
2.02	79.07	6.62	10.81	6.53	9.67	29.49
2.20	78.31	7.19	11.20	7.73	11.51	32.02
$P > F$	0.0539	0.0887	0.1165	0.0534	0.0138	0.0324
CV (%)	1.70	20.86	7.12	26.20	17.51	13.31

CV: Coefficient of variation;
$P > F$: Significance of "F" test of analysis of variance.

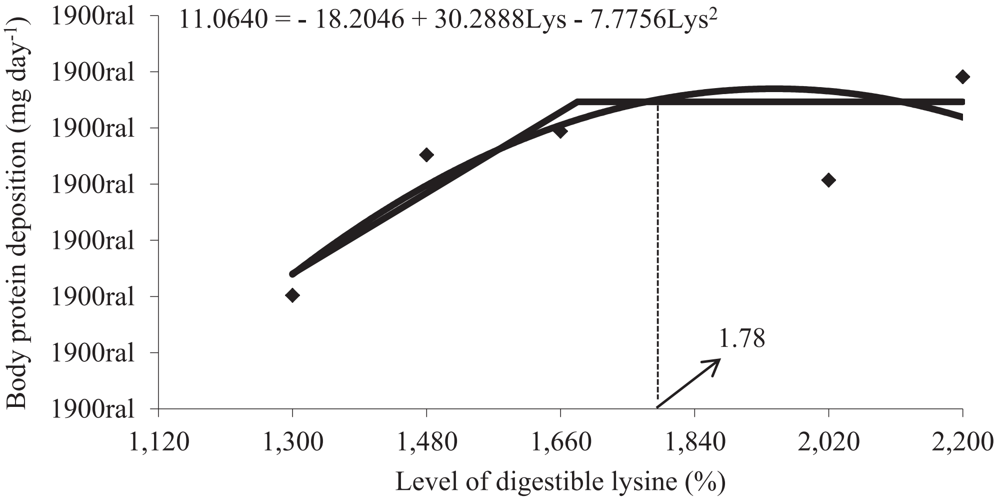
Table 5. Adjusted regression equations, coefficients of determination, and values of the requirement for the body protein deposition (DBP) and nitrogen retention efficiency (NRE) variables of juvenile tambaqui as a function of digestible lysine levels in rations.

Variable	Model	Equation	*P>F*	R^2	Requirement (%)
DBP (mg day^{-1})	Linear	DPC = 4.87333 + 3.0743Lys	0.0707	0.45	-----
DBP (mg day^{-1})	Quadratic	DPC = - 18.2046 + 30.2888Lys - 7.7756Lys^2	0.0928	0.65	1.95
DBP (mg day^{-1})	LRP	DPC = 11.0640 - 7.9999(1.6827 - Lys)	0.0821	0.68	1.68
DBP (mg day^{-1})	Quadratic + LRP	11.0640 = - 18.2046 + 30.2888Lys - 7.7756Lys^2	-	-	1.78
NRE (%)	LRP	ERN = 31.8775 - 24.278(1.58 - Lys)	0.0774	0.81	1.58

P > F: Significance of F analysis of variance test.

Based on the results obtained to optimize the weight gain and protein deposition, the recommended level of digestible lysine in the diet of tambaqui fingerlings is 1.78%, equivalent to 2.00% total lysine. These percentages are higher than the 1.60% of digestible lysine recommended for Nile tilapia by the NRC (2011) and the recommended level in the Brazilian table for the nutrition of tilapias (1.53% of digestible lysine). However, it approaches the value of 1.70% digestible lysine recommended by Bomfim et al. (2010) and is lower than the 2.12% calculated by Takishita et al. (2009) in diets for Nile tilapia fingerlings, in which they also used the ideal protein concept to formulate the experimental rations.

Figure 2. Graphical representation of body protein deposition for tambaqui juveniles, depending on the levels of digestible lysine in rations.



Conclusion

The recommended level of digestible lysine in the diet of tambaqui fingerlings is 1.78%, equivalent to 2.00% total lysine, for providing increased weight gain and body protein deposition.

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