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Threonine to lysine ratio in diets of tambaqui juveniles (*Colossoma macropomum*)[1]

Relação da treonina com a lisina em dietas para juvenis de tambaqui (*Colossoma macropomum*)

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Abstract

The objective of this study was to determine the optimal digestible threonine to lysine ratios in diets of tambaqui (*Colossoma macropomum*) juveniles. Five-hundred fish with a mean ± SE initial weight of 2.16 ± 0.03 g were used in a completely randomized design, consisting of four treatments, five replicates per treatment, and 25 fish per experimental unit. The treatments consisted of four isoenergetic, isophosphoric, isocalcic, and isolisinic (1.45%) diets, consisting of a basal diet supplemented with four L-threonine levels (1.013, 1.085, 1.158, and 1.230%), resulting in different threonine to lysine ratios (70, 75, 80, and 85%). Fish were maintained in twenty 500-L aquaria with independent water supply, drainage, and aeration systems, and were fed to apparent satiation six times a day for 45 days. Performance, feed efficiency, daily protein and fat deposition, body moisture content, and nitrogen retention efficiency of fish were evaluated. The digestible threonine intake increased linearly, and the efficiency of threonine for weight gain decreased quadratically, with increasing digestible threonine to lysine ratios. Weight gain, specific growth rate, feed:gain ratio, protein efficiency for weight gain, and nitrogen retention efficiency of fish increased in a quadratic manner with increasing digestible threonine to lysine ratios up to the levels of 75.96, 76.06, 76.36, 76.47, and 74.02%, respectively. It was concluded that the digestible threonine to lysine ratio for use in diets of juvenile tambaqui to achieve optimal performance and nitrogen retention efficiency is 76 and 74%, respectively, which corresponds to a digestible threonine level of 1.102 and 1.073%.
Key words: Digestible amino acids. Early life stage. Protein nutrition. Ideal protein.

Resumo

Objetivou-se determinar a relação da treonina com a lisina digestível em dietas para juvenis de tambaqui (*Colossoma macropomum*). Utilizaram-se 500 peixes com peso inicial de 2,16 ± 0,03 g distribuídos em delineamento inteiramente casualizado com quatro tratamentos, cinco repetições por

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tratamento e vinte e cinco peixes por unidade experimental. Os tratamentos consistiram de quatro dietas experimentais isoenergéticas, isofosfóricas, isocálcicas e isolisínicas digestíveis (1,45%), sendo uma basal suplementada com três níveis de L-treonina (1,013%; 1,085%; 1,158% e 1,230%), resultando em diferentes relações da treonina com a lisina digestível (70%, 75%, 80% e 85%). Os peixes foram mantidos em 20 aquários de polietileno com capacidade de 500 L, equipados com sistemas individuais de abastecimento de água, drenagem e aeração. As dietas foram ofertadas até a saciedade aparente, em seis refeições diárias durante 45 dias. Avaliaram-se variáveis de desempenho, eficiência alimentar, deposições diárias de proteína e gordura corporais e a eficiência de retenção de nitrogênio dos peixes. O consumo de treonina digestível aumentou linearmente e a eficiência de treonina para o ganho de peso reduziu de forma quadrática, em função da elevação da relação treonina com a lisina digestível. O ganho de peso, a taxa de crescimento específico, a conversão alimentar, a eficiência de proteína para o ganho de peso e a eficiência dos peixes na retenção do nitrogênio, aumentaram de forma quadrática com a elevação da relação treonina com a lisina digestível até os níveis de 75,96%, 76,06%, 76,36%, 76,47% e 74,02%, respectivamente. A deposição de gordura corporal reduziu linearmente com a elevação da relação da treonina com a lisina digestível. Concluiu-se que a relação da treonina com a lisina digestível em dietas para juvenis de tambaqui para otimizar o desempenho e a eficiência de retenção de nitrogênio é de 76 e 74%, que correspondem aos níveis de treonina digestível de 1,102 e 1,073%, respectivamente.
Palavras-chave: Aminoácidos digestíveis. Fase inicial. Nutrição proteica. Proteína ideal.

Introduction

The tambaqui (*Colossoma macropomum* Cuvier, 1818) is a naturally occurring species in the Amazon and Orinoco river basins (GOMES et al., 2010; RODRIGUES, 2014). It is a robust species with rheophilic habits that presents resistance to reduced levels of oxygen dissolved in the water, enduring values below 1 mg L^{-1} of oxygen. Tambaqui are also omnivorous, allowing for the use of food of both animal and vegetable origin in their diet (ARAÚJO-LIMA; GOULDING, 1998; LOPERA-BARRETO et al., 2011; RODRIGUES, 2014; RIBEIRO et al., 2016; SILVA et al., 2003).

Currently, tambaqui is the most intensively cultivated native species in the country of Brazil, with a registered annual production of more than 136 thousand tons (IBGE, 2016). Despite their productivity and economic importance, there is still a lack of information about the nutritional requirements of tambaqui, as highlighted in recent reviews by Dairiki and Silva (2011) and Oliveira et al. (2013).

Among the nutrients required by this species, the determination of optimal dietary levels of crude protein has been prioritized, especially during the hatchery stage (HERNANDES et al., 1995; SILVA et al., 2006; VAN DER MEER et al., 1995; VIDAL JÚNIOR et al., 1998) due to the importance of nutrients for their development, growth, and maintenance, as well as the great variability in the recommendations proposed by studies thus far. This variability may be related to differences in energy levels of experimental diets, water temperature, and feeding frequency used in previous studies, which influence fish ration consumption rates and, consequently, the estimated ideal nutrient levels in the diet (BOMFIM et al., 2010; COTAN et al., 2006; LUZZANA et al., 1998).

As fish do not have a specific metabolic requirement for protein, but rather need the correct, balanced dietary supply of essential and non-essential amino acids, a formulated ration based on crude protein may not meet their requirements for all amino acids. Because of this, the determination of their requirements for each amino acid, especially those that are usually limiting in practical diets, is necessary (BOMFIM, 2013).

Currently, the determination of amino acid requirements based on the ideal protein concept has been prioritized. In the ideal protein concept, the amino acid composition of proteins supplied in the diet would be identical to the requirements of the animals for these for their maintenance and growth, where all essential amino acids would be expressed in ideal proportions or relative to a reference amino acid, such as lysine. The principle behind the concept is that, although the quantitative requirements of amino acids can be influenced by several factors, the proportions between them remain relatively constant (MITCHELL, 1962). To determine the dietary ratio of a certain essential amino acid (lysine) needed in the diets of fish by Boisen (2003), levels of lysine were set at values considered sub-optimal to ensure that it was the second most limiting amino acid in the basal ration, and the other essential amino acids or amino acid:lysine ratios were set at amounts higher than the required values reported in the literature. In this way, the response to the increment in the levels of the evaluated amino acid could be maximized, as a result of lysine levels being a limiting factor (BOMFIM et al., 2008; BOMFIM, 2013; TAKISHITA, 2012; VIANA, 2012).

Threonine is the third most limiting amino acid in diets used for non-ruminants made from ingredients of vegetable origin, preceded by lysine and methionine (AHMED et al., 2004; ROSA et al., 2001). Besides its use for protein synthesis, this amino acid is involved in other physiological functions, such as digestion and immunity. It is abundant in immunoglobulin and mucin molecules, and is considered the first amino acid that is demanded for the production of these biomolecules (VIANA, 2012).

The dietary requirements of threonine for omnivorous and herbivorous fish species vary significantly in both diet percentages (%) and % crude protein (CP) (NRC, 2011). For channel catfish (*Ictalurus punctatus*), the threonine requirement is 0.5% of diet (2.2% of CP), for common carp (*Cyprinus carpio*) it is 1.5% (3.9% of CP), for the Indian carp (*Cirrhinus mrigala*) it is 1.8% (4.8% of CP), and for Nile tilapia (*Oreochromis niloticus*) it is 1.1% (3.8% of CP).

Because of its importance for body maintenance processes, the ratio of threonine to lysine may increase with the age and weight of the animal as a consequence of increases in the ratio of the amino acids used for maintenance in comparison to those used for protein deposition and growth, which decreases as the animal matures. Additionally, studies published by the NRC (2011) demonstrated that the requirement of threonine by fish is higher than that recommended for poultry and swine (ROSTAGNO et al., 2017), probably because significant amounts of mucus are produced for the coating of fish skin, which is induced under culture conditions such as population stress, transport, or exposure to heavy metals and large concentrations of ammonia.

The objective of this study was to determine the required ratio of threonine to lysine (based on digestible amino acid ratios) in diets used for juvenile tambaqui (*Colossoma macropomum*).

Materials and Methods

The experiment performed in this study was conducted following to the ethical norms for animal research, after being approved by the Animal Ethics Committee of the Federal University of Maranhão (Protocol Nº: 23115004063/2012-95). It was carried out in the Research Sector in Fish Nutrition of the Center for Agrarian and Environmental Sciences of the Federal University of Maranhão (UFMA), in Chapadinha, Maranhão, Brazil, and lasted 45 days.

Five-hundred tambaqui juveniles (*Colossoma macropomum*) with a mean ± standard error (SE) initial weight of 2.16 ± 0.03 g were used in a completely randomized design, composed of four treatments, with five replicates per treatment, and 25 fish per experimental unit.

The treatments consisted of four isonutritive and isoenergetic diets, based on corn and soybean meal supplemented with four different levels of threonine

(1.013, 1.085, 1.158, and 1.230%), resulting in four different digestible threonine:lysine ratios: 70, 75, 80, and 85%, respectively. To ensure that the diets maintained equal digestible protein values, the addition of L-threonine (an essential amino acid) was proportional to the decrease in the glutamic acid content (a non-essential amino acid) of the diets. The level of digestible lysine used in the experimental diets was 1.45%, a level considered sub-optimal in relation to the recommended minimum level in diets for tambaqui juveniles of 1.73% estimated by Silva (2016), according to the methodology proposed by Boisen (2003). The ratios of methionine + cystine:lysine and tryptophan:digestible lysine, as well as of the other essential amino acids with lysine, were maintained at least three points above the values estimated by Souza (2014), Takishita (2012) and Furuya et al. (2010), respectively (Table 1).

Table 1. Percent (%) chemical composition of different experimental diet rations (organic matter) used in this study.

Ingredients (%)	Threonine:digestible lysine ratio (%)			
	70	75	80	85
Soybean meal	58.071	58.071	58.071	58.071
Corn	32.155	32.155	32.155	32.155
Corn starch	0.000	0.029	0.057	0.086
Soybean oil	4.739	4.739	4.739	4.739
DL-Methionine	0.171	0.171	0.171	0.171
L-Threonine	0.000	0.075	0.150	0.225
Glutamic Acid	0.516	0.413	0.310	0.206
Dicalcium phosphate	3.277	3.277	3.277	3.277
Vitamin and Mineral Premix [5]	0.500	0.500	0.500	0.500
Vitamin C [4]	0.050	0.050	0.050	0.050
Salt	0.500	0.500	0.500	0.500
Antioxidant (BHT)	0.020	0.020	0.020	0.020
Calculated composition[1]				
Crude protein (%)*	29.94	29.94	29.94	29.94
Digestible Protein (%) [3]	27.33	27.33	27.33	27.33
Digestible Energy (kcal kg^{-1})	3000	3000	3000	3000
Ethereal Extract (%)	6.87	6.87	6.87	6.87
Crude fiber (%)	3.63	3.63	3.63	3.63
Total Ca (%)	0.95	0.95	0.95	0.95
Available P (%) [2]	0.70	0.70	0.70	0.70
Total Lysine (%)	1.694	1.694	1.694	1.694
Digestive Lysine (%) [2]	1.450	1.450	1.450	1.450
Digestible Met+Cyst (%) [2]	0.957	0.957	0.957	0.957
Total Threonine (%)2	1.137	1.210	1.284	1.358
Digestive Threonine (%) [2]	1.013	1.085	1.158	1.230
Digestible Tryptophan (%) [2]	0.364	0.364	0.364	0.364

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continuation

Digestive Lysine ratio/ED (g $Mcal^{-1}$)	0.565	0.565	0.565	0.565
Met+Cyst /Dig. Lysine ratio	66	66	66	66
Threonine/Digestive Lysine ratio	70	75	80	85
Tryptophan/Digestive Lysine ratio	25	25	25	25

* Based on laboratory analysis of the ingredients;
[1] Based on the values proposed by Rostagno et al. (2017);
[2] Based on the digestibility coefficients for industrial amino acids proposed by Rostagno et al. (2017) and for the amino acids and phosphorus availability of corn and soybean meal proposed by Furuya et al. (2010);
[3] Based on the digestibility coefficients proposed by Furuya et al. (2010) for Nile tilapia;
[4] Vitamin C: calcium salt ascorbic acid 2-monophosphate, 42% active principle components;
[5] Vitamin supplement and commercial mineral (5 kg/t), with guaranteed levels per kilogram of the product: Vit. A, 1.200.000 UI; Vit. D3, 200.000 UI; Vit. E, 1.200 mg; Vit. K3, 2.400 mg; Vit. B1, 4.800 mg; Vit.B2, 4.800 mg; Vit.B6, 4.800 mg; Vit.B12, 4.800 mg; Vit.C, 48 g; 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.

Fish were kept in 20 expanded polyethylene tanks with a volume of 500 L each, which were each provided with their own systems for water supply, drainage, and aeration. The water used to supply the aquaria came from an artesian well, and its temperature was measured daily at 7:30AM and at 5:30PM with a mercury bulb thermometer, graduated from 0 to 50°C. The pH, dissolved oxygen content, and total ammonia in the water was measured every seven days with a pH meter, an oximeter, and a commercial colorimetric kit of the brand Labcon Test®, respectively.

Water quality parameters were kept within the standard ranges recommended for raising this species (GOMES et al., 2010; MENDONÇA et al., 2012). The maximum and minimum water temperatures averaged 26.2 ± 1.04°C and 24.7 ± 0.68°C, respectively. The concentration of dissolved oxygen in the water was around 7.43 ± 0.76 ppm, pH averaged 5.9 ± 0.33, and total ammonia was always ≤ 1.00 ppm.

The ingredients of the experimental diets were mixed, moistened with heated water at 50°C, and pelletized in an adapted meat grinder for processing. The diet material was then dried in a forced air circulation oven at 50°C and ground with a manual mechanical grain grinder, sieved, and graded according to the diameter of the pellet, which varied from 2 to 4 mm.

The diets were given to fish six times a day at 08:00AM, 10:00AM, 02:00PM, 04:00PM, and 06:00PM. The diets were given in small quantities each time, little by little, until apparent satiation was reached, thus avoiding leftovers or under-delivery of food. The tanks were cleaned daily by siphoning, always after measuring the water temperature.

All fish were weighed at the beginning and the end of the experiment. At the beginning of the experiment, 50 fish were desensitized and euthanized by immersion in a solution containing overdose levels of benzocaine (300 mg L^{-1}). They were then frozen for further determination of the initial body composition. At the end, after a 24-hour fast, all fish in each box were desensitized, euthanized, weighed, and frozen for determination of the final body composition.

After freezing, the fish (carcasses and viscera) were dried in an oven with forced air circulation, pre-degreased, ground in a ball mill, and packaged in containers for laboratory analyses. The samples were analyzed for their body composition (moisture content, crude protein, and lipids) according to procedures described by Detmann et al. (2012). The laboratory analyses were performed at the

Laboratory of Animal Nutrition of the Federal University of Maranhão (UFMA).

The following variables were evaluated: weight gain (WG), specific growth rate (SGR), ration consumption (RC), digestible threonine consumption (DTC), feed conversion (FC), protein efficiency for weight gain (PEG), digestible threonine efficiency for weight gain (TEG), body protein deposition (BPD), body fat deposition (BFD), body chemical composition (moisture, protein, and body fat contents), and nitrogen retention efficiency (NRE). These variables were calculated according to the following equations:

- WG (g) = final mean weight - initial mean weight;
- RC (g) = ration consumed during the experiment;
- DTC (mg) = [ration consumption (mg) × digestible threonine ration content (%)] / 100;
- FC (g g^{-1}) = ration consumption / weight gain;
- SGR (% day^{-1}) = [(natural logarithm of the final weight (g) - natural logarithm of initial weight (g)) × 100] / experimental observation period (days);
- PEG (g g^{-1}) = weight gain / (ration consumption × crude protein content of the ration);
- TEG (g g^{-1}) = weight gain / (ration consumption × digestible threonine content of the ration);
- BPD (mg day^{-1}) = [(final body protein (mg)) - (initial body protein (mg)) / (experimental observation period);
- BFD (mg day^{-1}) = {[(final body fat) - (initial body fat)] × 100} / (experimental observation period).
- NRE (%) = {[final body N) - (initial body N)] × 100} / consumption of N

Statistical analyses were performed using the software SAEG 9.1 (RIBEIRO JUNIOR; MELO, 2007). The data were tested with analysis of variance (ANOVA) at a five percent significance level. For those variables that showed significant effects of threonine ratios in the ANOVAs, linear and quadratic regression analyses were then performed. It was also verified whether there needed to be an adjustment for the discontinuous model “Linear Response Plateau” (LRP). To choose the best-fitting model, their P (significance) and R^2 (Sum-of-squares (SS) of the model / SS of the treatment) values were compared, according to the procedures described by Ribeiro Junior and Melo (2008).

Results and Discussion

It was observed that the consumption of food did not change significantly (P > 0.05) with increased threonine:digestible lysine ratios in the diet (Table 2). Considering that the consumption did not vary among the treatments (P > 0.05) and that the nutritional composition of the diets differed only in their threonine levels, the consumption of threonine increased linearly (P < 0.01). Thus, the significant effects of the other variables evaluated were due to differences in the consumption of digestible threonine and, as a consequence, the differences in the ratio of threonine to digestible lysine.

Table 2. Initial weight (IW), ration consumption (RC), digestible threonine consumption (DTC), weight gain (WG), specific growth rate (SGR), apparent feed conversion (FC), protein efficiency for weight gain (PEG), and digestible threonine efficiency for weight gain (TEG) of tambaqui juveniles and summary of the analyses of variance comparing effects of diets with different threonine:digestible lysine ratios in the ration on these variables.

Threonine: digestible lysine ratio (%)	Variable							
	IW (g)	RC (g)	DTC (mg)	WG (g)	SGR (%/day)	FC (g/g)	PEG (g/g)	TEG (g/g)
70	2.17	14.33	145.12	14.56	4.54	0.99	3.48	100.30
75	2.17	13.83	150.07	14.85	4.57	0.93	3.67	98.86
80	2.16	13.74	159.08	15.47	4.67	0.94	3.67	95.48
85	2.15	13.68	168.23	13.11	4.35	1.05	3.28	77.83
Effect	NS	NS	L	Q	Q	Q	Q	Q
$P > F$[1]	0.999	0.999	< 0.001	0.031	0.047	< 0.001	< 0.001	< 0.001
CV[2] (%)	1.47	4.42	4.54	7.38	3.52	4.03	4.00	4.10

[1] Significance of the F-Test of Analysis of Variance;
[2] Coefficient of variation;
NS - Non-significant effect (P > 0.05);
L - Linear Effect;
Q - Quadratic effect.

The weight gain and specific growth rate increased (P < 0.05) in a quadratic manner as a result of the ratio of threonine to lysine in the diet up to the estimated ratios of 76.96% (1.116% digestible threonine) and 76.06% (1.106% threonine), respectively (Figure 1; Tables 2, 3 and 4). This increase in performance can be attributed to the better balance of the protein fraction of the diet with the increased level of threonine, which allowed better use of the amino acids, favoring the deposition of body protein and improved fish performance, which was similar to the observations by Bomfim et al. (2008) in Nile tilapia (*Oreochromis niloticus*).

Figure 1. Graphical representation of mean ± standard error (SE) weight gain (WG) of tambaqui juveniles as a result of the threonine:digestible lysine ratio (Rel) in their diet.

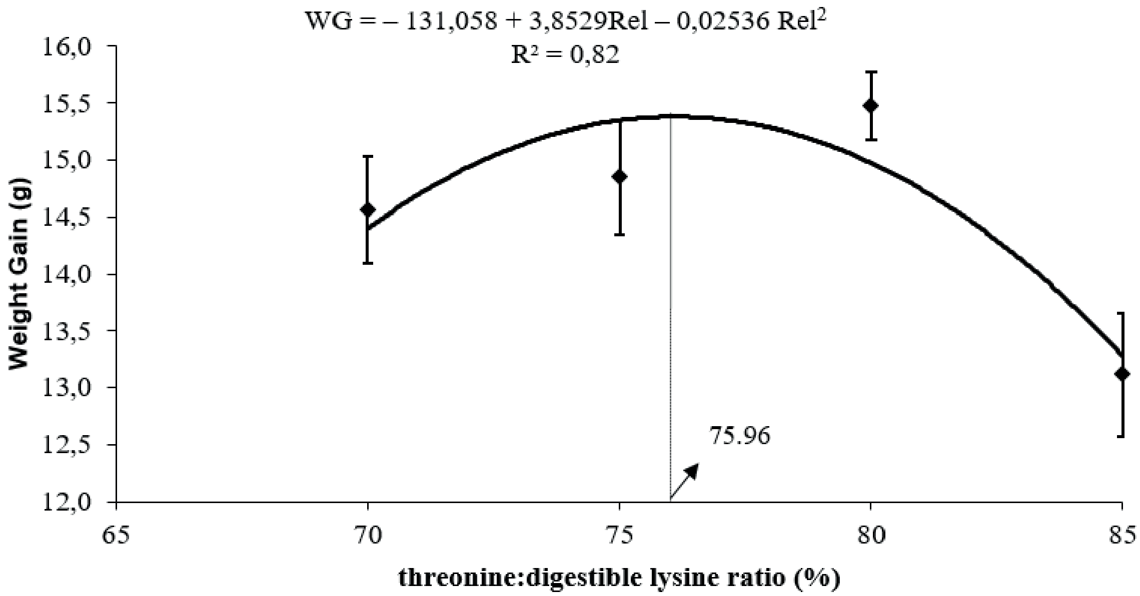


Table 3. Body moisture content (MC), body fat (BF), body protein (BP), body protein deposition (BPD), body fat deposition (BFD), and nitrogen retention efficiency (NRE) in tambaqui juveniles and summary of the analyses of variance comparing effects of diets with different threonine:digestible lysine ratios in the ration on these variables.

Threonine:digestible lysine ratio (%)	Variable					
	MC (%)	BF (%)	BP (%)	BFD (mg/day)	BPD (mg/day)	NRE (%)
Initial	76.07	5.77	11.93	-	-	-
70	76.31	6.03	13.26	19.64	44.40	47.11
75	76.12	5.69	12.96	18.70	43.15	48.11
80	76.44	5.60	12.67	18.32	41.81	46.85
85	75.61	5.61	13.02	16.23	38.48	43.27
Effect	NS	NS	NS	L	NS	Q
P > *F*[1]	0.311	0.999	0.999	0.043	0.179	0.019
CV (%) [2]	0.90	6.95	5.60	11.71	8.75	5.79

[1] Significance of the F-Test of Analysis of Variance;
[2] Coefficient of variation;
NS - Non-significant effect (P > 0.05);
L - Linear Effect;
Q - Quadratic effect.

Table 4. Adjusted regression equations, coefficients of determination (R^2), and requirement values for the variables digestible threonine consumption (DTC), weight gain (WG), specific growth rate (SGR), feed conversion (FC), protein efficiency for weight gain (PEG), digestible threonine for weight gain (TEG), body fat deposition (BFD), and nitrogen retention efficiency (NRE) in tambaqui juveniles as a result of the threonine:digestible lysine ratio (Rel) in their dietary ration.

Variable	Model	Equation	R^2	Requirement (%)
DTC (mg)	Linear	DTC = 34.1543 + 1.5673Rel	0.96	-----
WG (g)	Quadratic	WG = - 131.058 + 3.8529Rel - 0.02536 Rel^2	0.82	75.96
SGR (%/day)	Quadratic	SGR = - 15.0351 + 0.517476Rel - 0.003402Rel^2	0.81	76.06
FC (g/g)	Quadratic	FC = 10.2736 - 0.244913Rel + 0.00160374Rel^2	0.91	76.36
PEG (g/g)	Quadratic	PEG = - 30.6600 + 0.899074Rel - 0.0058784Rel^2	0.91	76.47
TEG (g/g)	Quadratic	TEG = - 740.521 + 23.0621Rel - 0.157975Rel^2	0.98	72.99
BFD (mg/day)	Linear	BFD = 34.6582 - 0.212061Rel	0.91	-----
NRE (%)	Quadratic	NRE = - 207.811 + 6.85079Rel - 0.0458451Rel^2	0.98	74.02

Improvement in performance with increased dietary levels of threonine were also observed by Takishita (2012) and Michelato et al. (2016) in Nile tilapia, and by Ahmed et al. (2004) in Indian carp (*Cirrhinus mrigala*), demonstrating that similar to what was observed in this study, the level of threonine in the basal diet was limiting to fish performance. However, when evaluating diets for tambaqui juveniles with digestible threonine levels in the ration ranging from 0.70 to 0.98%, Gonçalves Júnior (2015) did not observe a significant response in weight gain. However, a quadratic response was observed in the fish growth rate in the present study, with the best result obtained with the estimated level of 0.89% digestible threonine in the ration. The values of the estimated threonine:digestible lysine ratio based on the above-mentioned variables resemble the value of 74% (1.35% threonine) determined by Silva et al. (2006), are higher than the 69% (0.930% threonine) recommended by Bomfim et al. (2008), and lower than the 88% (1.358% threonine) observed by Takishita (2012), all in trials with Nile tilapia.

The improvement in fish weight gain associated with ration consumption and, consequently, with crude protein consumption implied a quadratic improvement ($P < 0.01$) in feed conversion with increasing threonine ratios up to the level of 76.36% (1.107% threonine), protein efficiency for weight gain ($P < 0.01$) to the level of 76.47% (1.109% threonine), and digestible threonine efficiency for weight gain ($P < 0.01$) to the level of 72.49% (1.051% threonine) (Tables 2 and 4). Lean tissue is composed of a greater percentage of water, approximately three parts of water to one part of protein, in relation to adipose tissue, which presents a high concentration of dry matter. Therefore, four times more food is needed to produce one kilogram of fat than what is needed to produce one kilogram of meat (HACKENHAAR, 2000). In this sense, the improvement observed for the food efficiency variables indicates that the better amino acid balance provided greater efficiency of the use of the protein in the diet ration for deposition of body protein and, consequently, increased the weight gain of the animals (BOMFIM et al., 2010).

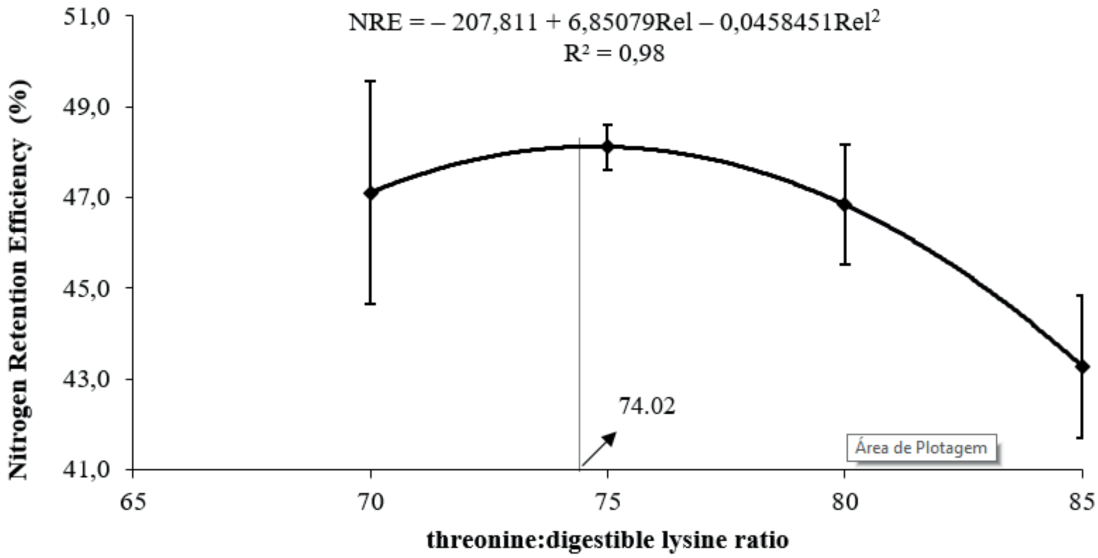
A similar effect was previously observed in juvenile carp (AHMED et al., 2004), Nile tilapia juveniles (TAKISHITA, 2012), and tambaqui juveniles (GONÇALVES JÚNIOR, 2015) in response to elevated dietary levels of threonine. However, Silva et al. (2006) observed a linear effect of increased threonine levels on feed conversion of Nile tilapia juveniles. Araripe et al. (2011) did not find significant differences in feed conversion in his trials with tambatinga juveniles, which is a hybrid of a female tambaqui (*Colossoma macropomum*) and a male pirapitinga (*Piaractus brachypomus*). Effects of threonine ratios on feed conversion were also not observed by Bomfim et al. (2008) in his studies with Nile tilapia.

The divergence between the results of different studies may be due to factors such as the stage of development of the fish, among others, as protein demand varies according to the life history stage of the animal. It is also known that the requirement for threonine can be altered in situations of health challenges, as threonine is the amino acid used in greater quantities in the synthesis of mucin and immunoglobulins (BOMFIM et al., 2008; VIANA, 2012). The reduction of threonine efficiency for weight gain above a given dietary level was similar to that observed in tambatinga by Araripe et al. (2011) and demonstrates that increases in threonine consumption above the required level does not increase its usage efficiency for any process.

The body composition (fat, moisture, and protein contents) and protein deposition were not influenced by the diet treatments ($P > 0.05$). However, elevation of the ratio of threonine to lysine reduced body fat deposition linearly ($P < 0.05$). This was probably due to the additional energy spent on the increased protein deposition, or to amino acid catabolism in the higher amino acid ratios, implying in both cases that there was less energy available for deposition of body fat at higher threonine ratios (BOMFIM et al., 2010).

Regarding the efficiency of fish nitrogen retention, increasing the ratio of threonine to lysine increased this variable in a quadratic way ($P < 0.05$) up to the estimated level of 74.02% (1.073% threonine), indicating that the amino acid balance up to the estimated level favored the efficiency of the use of dietary protein for body protein deposition. This corroborated the results observed for the performance and food efficiency variables (Figure 2, Tables 3 and 4).

Figure 2. Graphical representation of mean ± SE nitrogen retention efficiency (NRE) of tambaqui juveniles as a result of the threonine:digestible lysine ratio (Rel) in their diet.



The reduction observed in nitrogen retention efficiency above the level of 74.02% was similar to that observed by Araripe et al. (2011) and Takishita (2012), indicating that excessive levels of threonine may also negatively influence the efficiency of the use of the protein fraction of the diet (amino acids) for deposition of lean tissue. Consequently, the body deposition of protein and fish fat was reduced, as the amino acids that are not used for protein synthesis are catabolized, increasing the energy spent and consequently reducing the energy available for the deposition of body fat (BOMFIM et al., 2008, 2010; NELSON; COX, 2014). Although there were no statistically significant changes ($P > 0.05$) in body protein deposition, it was observed that the fish fed with a higher ratio of threonine to lysine (85%) had lower absolute values of body protein deposition when compared to those fed diets with lower ratios.

The determination of the ratio of threonine to lysine, rather than the requirement for threonine, minimizes the possibility of underestimating the value of this feed requirement if the fixed level of another essential amino acid in the experimental diets limits the use of the higher threonine levels (BOMFIM, 2013). The need for this precision in fish nutrition is because the amino acid studied (threonine) is important to fish health, as it is the first limiting amino acid for the production of mucin, which is synthesized in large quantities in the digestive tract and used mainly for skin covering and synthesis of immunoglobulins (BOMFIM et al., 2008; NRC, 2011).

The ratio of threonine to digestible lysine of 76% and the requirement of 1.73% of digestible lysine for tambaqui juveniles estimated in a previous study (SILVA, 2016) agrees well with the estimated optimal level of approximately 1.315% of digestible threonine in the present study. This ratio thus satisfies the dietary requirements of this species for this amino acid.

Conclusions

It was concluded that the ratio of threonine to digestible lysine to use in diets for tambaqui juveniles to optimize performance and nitrogen retention efficiency is 76% and 74%, respectively, which corresponds to digestible threonine levels of 1.102% and 1.073%.

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