Ciência e Agrotecnologia
SCIENCE AND AGROTECHNOLOGY

Ciência e Agrotecnologia, 45:e023520, 2021
http://dx.doi.org/10.1590/1413-7054202145023520

Animal Science and Veterinary
eISSN 1981-1829

Digestible threonine requirement in diets for tambatinga (♀*Colossoma macropomum* x ♂*Piaractus brachypomus*) fingerlings

Exigência de treonina digestível em rações para alevinos de tambatinga (*Colossoma macropomum* ♀ x *Piaractus brachypomus* ♂)

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Received in August 27, 2020 and approved in February 10, 2021

ABSTRACT

Cross breeding of native fish species is a technique to produce hybrids that can express higher weight gain and feed efficiency compared to the parental species. The digestible threonine requirement in diets for tambatinga fingerlings (*Colossoma macropomum* ♀ x *Piaractus brachypomus* ♂) was determined in this study. For this, 700 fingerlings with an average initial weight of 2.39 ±0.02 g and average final weight of 35.96 ±2.03 g were distributed in a completely randomized design consisting of six treatments (0.600, 0.800, 1.000, 1.200, 1.400, and 1.600% digestible threonine) and five replicates per treatment, with 20 fish per experimental unit. Diets were formulated by the "diet dilution" technique using the ideal protein concept. Performance, feed efficiency, body depositions of protein, ash and fat, and nitrogen retention efficiency of the fish were evaluated. The digestible threonine levels that optimized weight gain, specific growth rate, and feed conversion ratio were 1.40, 1.27 and 1.10%, respectively. Body deposition of fat was reduced in a quadratic manner ($p<0.01$), and the body depositions of protein and ash, together with the efficiency of nitrogen retention, were optimized by the digestible threonine level of 1.20; 1.33, and 0.82%, respectively. The recommended digestible threonine level in the diet for tambatinga fingerlings is 1.20 to 1.40% (0.40 and 0.47% Mcal DE^{-1}) to obtain higher body deposition of protein and weight gain, respectively.

Index terms: Fish nutrition; essential amino acid; deposition of body protein; zootechnical performance.

RESUMO

O cruzamento de espécies de peixes nativas é uma técnica para produzir híbridos que possam expressar maior ganho de peso e eficiência alimentar quando comparado às espécies parentais. A exigência de treonina digestível em dietas para alevinos de tambatinga (*Colossoma macropomum* ♀ x *Piaractus brachypomus* ♂) foi estudada. Utilizaram-se 700 alevinos com peso médio inicial de 2,39 ±0,02 g e peso final de 35,96 ± 2.03 g, distribuídos em delineamento inteiramente casualizado composto por seis tratamentos (0,600; 0,800; 1,000; 1,200; 1,400 e 1,600% de treonina digestível) e cinco repetições por tratamento, com vinte peixes por unidade experimental. As rações foram formuladas pela técnica da "diluição de dietas", utilizando o conceito de proteína ideal. Avaliaram-se o desempenho, a eficiência alimentar, as deposições de proteína, cinzas e gordura corporal e a eficiência de retenção de nitrogênio. O nível de treonina digestível que otimizou o ganho de peso, taxa de crescimento específico e conversão alimentar foi de 1,40; 1,27 e 1,10%, respectivamente. A deposição de gordura corporal reduziu de forma quadrática ($p<0,01$), e as deposições de proteína e cinzas corporais, juntamente com a eficiência de retenção de nitrogênio foram otimizadas com valores de 1,20; 1,33 e 0,82% de treonina digestível, respectivamente. A recomendação do nível de treonina digestível na dieta para alevinos de tambatinga é de 1,20 a 1,40% (0,40 e 0,47% Mcal de DE^{-1}), por proporcionar maior deposição corporal de proteína e ganho de peso, respectivamente.

Termos para indexação: Nutrição de peixes; aminoácido essencial; deposição de proteína corporal; desempenho zootécnico.

INTRODUCTION

Cross breeding of native fish species is a technique to produce hybrids that can express/achieve higher weight gain and feed efficiency compared to the parent species and is widely used in fish farming. The hybrid produced by crossing the female of *Colossoma macropomum* (tambaqui) with the male of *Piaractus brachypomus* (pirapitinga) is popularly known as tambatinga. This hybrid has shown superior zootechnical characteristics, such as rapid growth, rusticity, and tolerance to temperature variations and oxygen levels, compared to its parent species (Dias; Tavares-Dias; Marchiori, 2012; Hashimoto et al., 2012).

In fish farming, the use of diets with high levels of crude protein can increase the cost of production and make the activity unfeasible since protein is the most expensive nutrient in the formulation of feed (Alencar Araripe et al., 2011; Pianesso et al., 2015; Lee et al., 2020). Moreover, the use of diets with high levels of crude protein results in increased excretion of nitrogen compounds into the aquatic environment (Nunes et al., 2014). This practice does not always result in improved performance, since fish, like other animals, need a balanced amount of essential and non-essential amino acids to enhance their growth and health (Abimorad et al., 2010; Yue et al., 2014; Firmo et al., 2018). Among the essential amino acids for fish, threonine is the third limiter in practical fish feeds when ingredients of plant origin are used in the manufacture of feeds (Yue et al., 2014; Michelato et al., 2016).

The amino acid requirements of fish have been determined using dose-response studies by the amino acid-supplementation technique (Bomfim et al., 2008; National Research Council - NRC, 2011; Firmo et al., 2018). However, the use of this method has been criticized as the fixation of the levels of other amino acids in diets can limit the response of animals to the higher levels of the amino acid (Bomfim et al., 2010; Silva et al., 2018). Furthermore, a systematic change may occur in the amino acid balance in different dietary treatments, which may affect the growth response and feed intake (Ullah-Khan et al., 2019). Also, higher amounts of crystalline amino acids used in experimental diets may facilitate leaching in the aquatic environment, which can lead to inaccuracies in the obtained results (Lanna et al., 2016).

An alternative technique proposed by Fisher and Morris (1970) is the diet dilution technique. In this technique, the amino acid balance of the treatments remains constant since it is based on the use of a high-protein diet deficient in the evaluated amino acid, which is diluted in a protein-free isoenergetic diet, resulting in intermediate levels of the evaluated amino acid (NRC, 2011; Siqueira et al., 2013). It is based on the hypothesis that amino acid utilization is independent of the dietary protein level and avoids the problem of change in amino acid balance in successive dietary treatments (Ullah-Khan et al., 2019). Therefore, it also circumvents the problem regarding the restricted feed intake and lower utilization of diets provided with an excess of synthetic amino acids in fish diets (Liebert; Benkendorff, 2007; Marchão et al., 2020; Ullah-Khan, 2019). Studies on fish for the determination of amino acid requirements have increasingly used this technique (Bomfim et al., 2020; Liebert; Benkendorff, 2007; Liebert, 2009; Marchão et al., 2020).

Threonine is an important essential amino acid as it is vital for the digestive and immune systems of fish, being found in high concentrations in immunoglobulin and mucin, the latter of which is also widely used in the covering of the skin of fish (Alencar Araripe et al., 2011; Firmo et al., 2018). Moreover, threonine plays an important role in the growth and yield of fish fillets, as it is involved in the synthesis of body proteins and functions as a precursor to some non-essential amino acids, such as serine and glycine (Michelato et al., 2016). Considering the lack of information on the requirement of amino acids for tambatinga fingerlings, the levels of digestible threonine in the diets were evaluated.

MATERIAL AND METHODS

Facilities, experimental conditions, and design

The experiment was conducted at the Center for Agricultural and Environmental Sciences - CCAA of the Federal University of Maranhao - UFMA, located in the municipality of Chapadinha, MA, for around 45 days. The study was conducted according to the ethical standards of research using animals, after approval by the Animal Use Ethics Committee of the Federal University of Maranhao (Protocol No.: 23115.012034/2018–92).

The study used 700 fingerlings from the same batch that were acquired from a fish farm in the region, located in the city of Itapecuru Mirim-MA. The fish had an average initial weight of 2.39 ±0.02 g and were distributed in a completely randomized design comprising six treatments (levels of digestible threonine) with five replicates per treatment and twenty fish per experimental unit.

The fingerlings were kept in 35 polyethylene boxes of a capacity of 500 liters, equipped with individual water supply, drainage, and aeration systems. The water was obtained from an artesian well in the supply system at the Federal University of Maranhao - UFMA, and at least 10% of the water was renewed daily. The boxes were cleaned daily by siphoning after checking the water temperature.

Experimental fish feeds

To formulate the experimental diets, the total amino acid content of the soybean meal was analyzed and later converted into digestible amino acid content using the soybean digestibility coefficient described by Nascimento et al. (2020) for tambaqui (*Colossoma macropomum*) (Table 1).

Table 1: Total and digestible amino acid composition of soybean meal used in the experimental diets.

Amino acids (%)	Soybean meal	
	AAT[1]	AAD[2]
Lysine	2.73	2.58
Methionine	0.56	0.53
Methionine + cystine	1.18	1.16
Threonine	1.68	1.65
Isoleucine	2.01	1.95
Tryptophan	0.62	0.58

[1]Total Amino acids;
[2]Digestible amino acids, calculated based on the digestibility coefficients of corn and soybean meal proposed by Nascimento et al. (2020) for Tambaqui (*Colossoma macropomum*).

The experimental fish feeds were formulated using the technique of "dilution of diets" (Fisher; Morris, 1970). Two diets were formulated; one was protein-free (DIP) based on corn starch and soybean oil, while the other contained 1.600% digestible threonine (reference diet) based on soybean meal.

To obtain the experimental diets, the reference diet was diluted sequentially with the protein-free diet, resulting in isoenergetic, isocalcic, and isophophoric diets containing 0.600, 0.800, 1.000, 1.200, 1.400, and 1.600% digestible threonine.

In the experimental diets, the ratio of threonine to lysine was maintained at 60%, below on the values required for Nile tilapia reported by the NRC (2011) (69%) and the Brazilian Tables for Tilapia Nutrition (Furuya et al., 2010) (77%).

To confirm that threonine was at a suboptimal level in each experimental diet (first limiting amino acid), an additional treatment involving a diet with a lower level of digestible threonine tested (0.600%), plus 0.300% L-threonine (positive control) was used. The ratio of the other essential amino acids with lysine was maintained at least three percentage points above those recommended for tilapia by the NRC (2011) to prevent another amino acid from becoming limiting for each evaluated level of digestible threonine supplementation (Table 2).

The ingredients of the experimental diets were finely ground (Trf 60, Trapp®), weighed individually, and mixed (Horizontal Mixer 300 Kg, Branorte®). Subsequently, they were pelleted in a pelletizer/extruder with a 4 mm sieve (model MX 40, Inbramaq®, Laboratório de Nutrição e Alimentação de Organismos Aquáticos do Maranhão, Chapadinha, Brazil).

The experimental diets were pelleted to minimize the possibility of leaching of ingredients and were provided daily in six meals (8 h, 10 h, 12 h, 14 h, 16 h, and 18 h). In each meal, small amounts were provided with successive transfers, allowing maximum intake until apparent satiety.

Water quality parameters

The parameters of water quality were maintained within the recommended standards for the creation of species, as recommended by Gomes Simões and Araújo-Lima (2010) and Mendonça et al. (2012). The maximum and minimum water temperatures remained around 27.05 ±0.72 °C (8 h), and 24.91 ±0.72 °C (16 h), respectively, measured daily using a mercury bulb thermometer graduated between 0 and 50 °C. The concentration of dissolved oxygen in the water was around 9.02 ±1.21 ppm, pH 6.19 ±0.95, and total ammonia < 1.00 ppm, measured every three days using a digital pH meter (HI 8424, Hanna®), oximeter (HI 9146, Hanna®), and commercial colorimetric kit (Arcor®) for testing toxic ammonia, respectively.

Sample collection and chemical analysis

At the beginning of the experiment, an initial sample was composed of 50 g of fish, and at the end of the experimental period, all fish from each experimental unit were desensitized and euthanized using benzocaine overdose (500 mg L^{-1}). Fish samples (initial and final/experimental plot) were dried in a greenhouse using forced air circulation, pre-degreased, ground in a ball mill, and packed in containers for laboratory analysis. The analyses were performed according to the procedures described by Association of Official Analytical Chemists - AOAC (2002) at the Animal Nutrition Laboratory of the Federal University of Maranhao - UFMA.

At the end of the trial, the following indices of performance and feed efficiency were evaluated: feed intake (FI) Equation 1, digestible threonine intake (DTI) Equation 2, weight gain (WG) Equation 3, specific growth rate (SGR) Equation 4, feed conversion rate (FCR) Equation 5, and digestible threonine efficiency for weight gain (DTEG) Equation 6.

Table 2: Percentage and chemical composition of the protein-free and experimental diets (natural matter).

Ingredients (%)	Digestible threonine level (%)							
	DIP* 0.000	0.600	0.800	1.000	1.200	1.400	RD** 1.600	PCD*** 0.900
Soybean meal	0.00	33.30	44.40	55.50	66.60	77.70	88.80	33.30
Corn starch	79.17	49.52	39.63	29.75	19.86	9.98	0.09	49.22
Soybean oil	10.67	8.68	8.02	7.36	6.70	6.04	5.38	8.68
Rice husk	5.08	3.17	2.54	1.90	1.27	0.63	0.00	3.17
Lysine-HCl (78,4%)	0.00	0.17	0.22	0.28	0.33	0.39	0.44	0.17
DL-Methionine (99%)	0.00	0.29	0.39	0.49	0.59	0.69	0.79	0.29
L-Threonine (98,5)	0.00	0.06	0.08	0.10	0.12	0.14	0.16	0.06
L-Tryptophan (98%)	0.00	0.04	0.05	0.06	0.07	0.08	0.10	0.34
Calcitic limestone	0.13	0.08	0.07	0.05	0.03	0.02	0.00	0.08
Dicalcium phosphate	3.83	3.58	3.49	3.41	3.33	3.24	3.16	3.58
Vitamin and mineral premix [5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin C [4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.55	0.54	0.53	0.53	0.52	0.51	0.51	0.54
Antioxidant (BHT)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Dilution (%)							
DIP*	100.00	62.50	50.00	37.50	25.00	12.50	0.00	62.50
RR**	0.00	37.50	50.00	62.50	75.00	87.50	100.00	37.50
	Nutritional composition[1]							
Crude protein (%)	0.00	15.45	20.61	25.76	30.91	36.06	41.21	15.68
Digestible energy (kcal kg^{-1}) [3]	3000.0	3000.0	3000.0	3000.0	3000.0	3000.0	3000.0	3000.0
Crude fiber	4.70	4.70	4.70	4.70	4.70	4.71	4.71	4.70
Ca total (%)	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
P available (%) [2]	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Digestible lysine (%) [2]	0.000	1.000	1.333	1.667	2.000	2.333	2.667	1.000
Digestible Met. + cys (%) [2]	0.000	0.680	0.907	1.133	1.360	1.587	1.813	0.680
Total threonine (%)	0.000	0.653	0.870	1.088	1.305	1.523	1.740	0.653
Digestible threonine (%) [2]	0.000	0.600	0.800	1.000	1.200	1.400	1.600	0.900
Digestible tryptophan (%) [2]	0.000	0.230	0.307	0.383	0.460	0.537	0.613	0.230
Digestible isoleucine (%) [2]	0.000	0.651	0.868	1.085	1.303	1.520	1.737	0.651
	Relations (%)							
Digestib4zle met + cys/lysine	0.0	68	68	68	68	68	68	68
Digestible threonine/lysine	0.0	60	60	60	60	60	60	90
Digestible tryptophan/lysine	0.0	23	23	23	23	23	23	23
Digestible isoleucine/lysine	0.0	65	65	65	65	65	65	65
Digestible thr/energy (g Mcal^{-1})	0.000	0.200	0.267	0.333	0.400	0.467	0.533	0.300

* Protein-free diet; ** Reference diet (1.600% digestible threonine); *** Positive control diet - dilution 1 (0.600%) + 0.300% L-threonine; [1]Based on the values proposed by Rostagno et al. (2011); [2]Based on the digestibility coefficients for industrial amino acids proposed by Nascimento et al. (2020) and Rostagno et al. (2011) and for the amino acids and availability for soybean meal phosphorus proposed by Furuya (2010); [3]Based on the digestibility coefficients for corn starch. soybean meal and soybean oil proposed by Furuya (2010) for Nile tilapia; [4] Vit. C: Calcium L-Ascorbic acid 2-monophosphate, 42% of the active ingredient; [5] Vitamin and mineral supplements (5 kg t^{-1}), with guaranteed levels per kilogram of product: Vit. A, 1.200,000 IU; Vit. D3, 200.000 IU; 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, 48.00 mg; folic acid, 1.200 mg; Ca pantothenate, 12.000 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.

$$FI(g) = \text{feed consumed during the experimental period} \quad (1)$$

$$DTI\ (mg) = \frac{[\text{feed intake (mg)} \times \text{digestible threonine level in diet (\%)}]}{100} \quad (2)$$

$$WG\ (g) = \text{final mean weight (g) - initial mean weight (g)} \quad (3)$$

$$SGR\ (\%\ day\text{-}1) = \frac{[(\text{natural logarithm of final weight (g)} - \text{natural logarithm of initial weight (g)}) \times 100]}{\text{experimental period (days)}} \quad (4)$$

$$FCR\ (g\ g^{-1}) = \frac{\text{feed consumption (g)}}{\text{weight gain (g)}} \quad (5)$$

$$TEWG\ g\ g\text{-}1) = \frac{\text{wieght gain (g)}}{\text{digestible threonine intake (g)}} \quad (6)$$

The chemical composition of the fish (moisture, ash, protein, and fat content) was evaluated. Moisture was determined by oven-drying the fish at 105 °C until a constant weight was achieved. Crude protein (N * 6.25) was determined by the Kjeldahl method after acid digestion. Crude lipid was determined by the ether-extraction method using a Soxtec^{TM} System. Ash content was determined using a muffle furnace at 600 °C for 4 h. Based on body composition, the daily body deposition of protein, fat, and ash (BDP, BDF, and BDA), according to the Equations 7, 8 e 9 respectively, and retention efficiency of nitrogen (NRE), was determined according to the Equation 10.

Statistical analysis

The statistical analyses were performed using the statistical program SAEG version 9.1 (2007). The effects of digestible threonine levels in the diets were determined by decomposing the degrees of freedom of the digestible threonine levels in first and second-order orthogonal polynomials ($p<0.05$) according to the best fit. The adjustment was also verified for the Linear Response Plateau - LRP model. To choose the best fit model, the *P*-value (significance) and/or R^2 (model SQ/treatment SQ) was utilized. The effects of the positive diet control (0.900%) with the lowest dietary level of digestible threonine (0.600%) were evaluated using the Student Newman Keuls (SNK) test.

RESULTS AND DISCUSSION

The fish fed with the positive control diet (0.900% digestible threonine) showed superior response compared to those fed with the diet containing the lowest concentration of threonine (0.600%), except for the variables of FCR and BDF. These results indicated that threonine was the first limiting essential amino acid in all the experimental diets.

Variation in the dietetic levels of digestible threonine influenced ($p<0.05$) all the evaluated variables except feed intake ($p>0.05$). Consequently, the intake of energy and other nutrients by the fish did not vary, except for the intake of crude protein. As threonine was the first limiting amino acid in the protein component of each experimental diet, the observed effects on the other evaluated variables were caused by the difference in the intake of digestible threonine, which increased linearly ($p<0.01$) with an increase in its dietary concentration.

The increase in the dietary concentration of digestible threonine provided a quadratic increase in the weight gain and specific growth rate ($p<0.01$), with the digestible threonine requirement being estimated as 1.50 and 1.45%, respectively. However, the “Linear Response Plateau” - LRP model best fitted the data, estimating the threonine digestible requirement as 1.30 and 1.17%, beyond which a plateau was observed (Tables 3 and 4).

$$BDP(mg\ day^{-1}) = \frac{\{[(\text{final body protein, \%} \times \text{final weight, mg}) - (\text{initial body protein, \%} \times \text{initial weight, mg})] / 100\}}{\text{experimental period (days)}} \quad (7)$$

$$BDF(mg\ day^{-1}) = \frac{\{[(\text{final body fat, \%} \times \text{final weight, mg}) - (\text{initial body fat, \%} \times \text{initial weight, mg})] / 100\}}{\text{experimental period (days)}} \quad (8)$$

$$BDA\ (mg\ day^{-1}) = \frac{\{[(\text{final body ashes, \%} \times \text{final weight, mg}) - (\text{initial body ashes, \%} \times \text{initial weight, mg})] / 100}{\text{experimental period (days)}} \quad (9)$$

$$NRE\ (\%) = \frac{[\text{final body N (\%)} \times \text{final weight (g)}] - [\text{initial body N (\%)} \times \text{initial weight (g)}]}{[(\text{feed intake, g} \times \text{N level in ration, \%})/100]} \quad (10)$$

Table 3: Feed intake (FI), digestible threonine intake (DTI), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and digestible threonine efficiency for weight gain (TEWG) of tambatinga fingerlings as a function of the digestible threonine levels in the diet.

Digestible threonine level (%)	Variable					
	FI (g)	DTI (mg)	WG (g)	SGR (% day^{-1})	FCR (g g^{-1})	TEWG (g g^{-1})
PCD 0.900*	31.71 A	285.35 A	24,39 A	6.02 A	1.30 A	85.65 B
0.600	26.91 B	161.43 B	19.51 B	5.51 B	1.38 A	121.76 A
0.800	29.13	233.12	23.59	5.96	1.24	101.11
1.000	28.75	287.46	27.32	6.28	1.05	95.79
1.200	26.86	322.32	33.57	6.77	0.80	104.70
1.400	29.48	412.66	31.60	6.63	0.93	76.68
1.600	30.33	485.32	32.52	6.69	0.95	67.61
p>F	NS	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)	11.05	11.53	10.44	4.02	9.62	9.65

*Positive control diet - dilution 1 (0.600%) + 0.300% L-threonine; p>F - Significance of the "F" Test of analysis of variance; CV - Coefficient of variation; Averages on the same line, followed by the same letters do not differ by the SNK test ($p > 0.05$).

Table 4: Adjusted regression equations, determination coefficients (R^2), and requirement values for the variables digestible threonine intake (DTI), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and digestible threonine efficiency for weight gain (TEWG) of tambatinga fingerlings as a function of digestible threonine level in the diet.

Variable	Model	Equation	*p > F**	R^2	Requirement (%)
DTI (mg)	Linear	$\hat{Y} = 313.229x+27.385$	< 0.0001	0.99	-----
WG (g)	Linear	$\hat{Y} = 13.620x+13.034$	< 0.0001	0.83	-----
WG (g)	Quadratic	$\hat{Y} = -17.220x^2+51.505x-5.794$	0.0048	0.94	1.50
WG (g)	LRP	$\hat{Y} = 32.562-18.631(1.301-x)$	0.005	0.95	1.30
WG (g)	Quadratic + LRP	$\hat{Y} = 32.562 = -17.220x^2+51.505x-5.794$	-----	-----	1.40
SGR (% day^{-1})	Linear	$\hat{Y} = 1.197x+4.990$	< 0.0001	0.82	-----
SGR (% day^{-1})	Quadratic	$\hat{Y} = -1.691x^2 + 4.916x+3.142$	0.0019	0.97	1.45
SGR (% day^{-1})	LRP	$\hat{Y} = 6.659-2.046(1.169-x)$	< 0.0001	0.99	1.17
SGR (% day^{-1})	Quadratic + LRP	$\hat{Y} = 6.659 = -1.691x^2 +4.916x + 3.142$	-----	-----	1.27
FCR (g g^{-1})	Linear	$\hat{Y} = -0.478x+1.585$	< 0.0001	0.69	-----
FCR (g g^{-1})	Quadratic	$\hat{Y} = 0.921x^{2-}2.523x + 2.601$	0.0001	0.91	1.36
FCR (g g^{-1})	LRP	$\hat{Y} = 0.940+0.969(1.096 - x)$	0.0010	0.93	1.10
TEWG (g g^{-1})	Linear	$\hat{Y} = -47.874x + 147.272$	< 0.0001	0.83	-----

*p>F - Significance of the "F" Test of analysis of variance.

It is important to consider that the quadratic model can provide an overestimation of the requirement, as it assumes a bilateral symmetry of the response to the increase in nutrients, as the model describes the reduction in response to the same intensity of the addition (Siqueira et al., 2009). Although the LRP model provides a good statistical adjustment, it does not consider the animal's physiological aspects and the law of reduction of returns, which gives an underestimation of the optimal level since it does not consider the increases that could justify further improvements in the variables (Pianesso et al., 2015; Siqueira et al., 2009). On the other hand, the value of the first intersection of the LRP plateau with the quadratic has been used to estimate the requirements and circumvent the criticisms of the models, as it is an objectively obtained value and represents the intermediate level of both (Silva et al., 2018; Siqueira et al., 2009). Therefore, the digestible threonine levels in the diet, which were estimated to optimize WG and the SGR corresponding to the first intersection of the quadratic equation, with the plateau estimated by the LRP model, were 1.40% and 1.27%, respectively (Table 4, Figure 1).

These results indicate that at levels below 1.27%, the threonine deficient in the diets for tambatinga fingerlings. Importantly, no pathological symptoms were observed in fish fed diets containing lower threonine levels. These observations were in line with those reported by Yue et al. (2014) when evaluating the effect of threonine supplementation in the diet on the performance, body composition, and hematological and immunological parameters of Nile tilapia (*Oreochromis niloticus*), Alencar Araripe et al. (2011) on tambatinga fingerlings, and Firmo et al. (2018) on tambaqui juveniles (*Colossoma macropomum*).

The observed results differed from those obtained in similar studies on the same species and weight range conducted by Alencar Araripe et al. (2011). Those authors reported no improvement in the performance of tambatinga fingerlings when evaluating diets with different digestible threonine to lysine ratios (49.0 to 67.4%) and recommended the lowest ratio in the diet. This lack of effect on the growth performance may be related to the hypothesis that threonine may not be the first limiting essential amino acid in the experimental diets, as the relationships between methionine plus cystine (38%) and tryptophan (16%) with lysine in ratios used in that study were below the minimum of 60 and 19%, respectively, that were recommended for Nile tilapia and tambaqui by the NRC (2011), Brazilian Tables for Tilapia Nutrition (Furuya, 2010), and Souza et al. (2019), respectively.

On the other hand, similar effects were observed by Firmo et al. (2018) during research on the same weight range of tambaqui juveniles. The authors observed a quadratic improvement in the performance of tambaqui juveniles subjected to different digestible threonine levels (1.013 to 1.230%), up to the level of 1.102%, corresponding to a threonine: lysine ratio of 76%. Also, Yue et al. (2014), when working on Nile tilapia juveniles, observed an improvement in weight gain up to the level of 1.33% threonine after the fish were treated with different total threonine levels (0.73 to 1.72%).

The improvement in WG obtained in association with similar feed intake led to an improvement in the FCR, the value of which reduced in a quadratic manner ($p<0.01$) up to the digestible threonine level of 1.36%. However, the "Linear Response Plateau" - LRP model fitted the data the best, having estimated the requirement of digestible threonine at 1.10%, after which there was a plateau (Tables 3 and 4, Figure 2). On the other hand, the efficiency of digestible threonine for WG decreased linearly ($p<0.01$) with an increase in the dietary concentration of threonine.

Considering that feed and energy intake was similar between treatments, the improvement in FCR corroborated the results obtained for WG and SGR and occurred due to the improved amino acid adjustment of diets with increasing levels of threonine. Consequently, there was greater efficiency in energy use and the protein fraction of the feed, especially for the synthesis of lean tissue (body protein) that added higher water content to its composition (Bomfim et al., 2010; Silva et al., 2018).

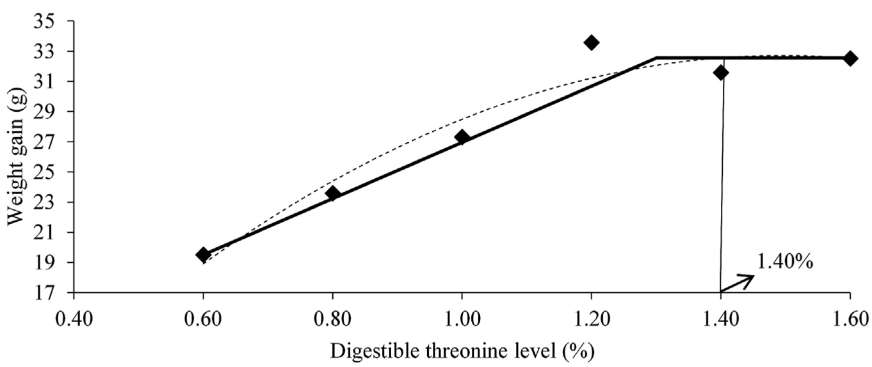

Figure 1: Graphical representation of the weight gain of tambatinga fingerlings as a function of the digestible threonine level in the diet.

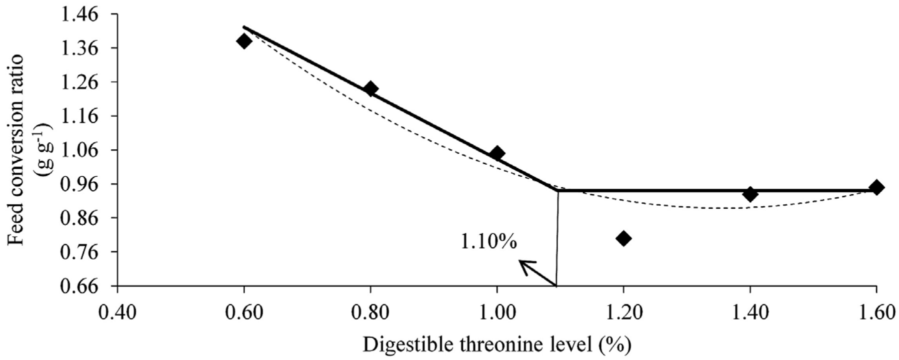

Figure 2: Graphical representation of the feed conversion fingerlings of tambatinga as a function of digestible threonine level in the diet.

The linear reduction in the efficiency of digestible threonine for WG ($p<0.01$) may be related to the proportional increase in threonine catabolism and other amino acids in non-limiting levels in diets (Alencar Araripe et al., 2011; Bomfim et al., 2008; Firmo et al., 2018; Lee et al., 2020).

The increase in digestible threonine level in diets increased the daily body depositions of protein ($p<0.01$), fat ($p<0.01$), and ash ($p<0.01$) in a quadratic manner, with the requirements being estimated at 1.41, 0.90, and 1.33%, respectively. However, the best-fit model for body deposition of protein and ash was the LRP ($p<0.01$), increasing these variables at the estimated digestible threonine levels of 1.11 and 1.07%, after which plateaus were observed. Using the first intersection of the quadratic equation with the plateau obtained using the LRP equation, the estimated digestible threonine level in the diet for the body deposition of protein was 1.20% (Tables 5 and 6, Figure 3).

The results obtained for BDP differed from those observed by Alencar Araripe et al. (2011) and Firmo et al. (2018), who did not observe any effect of the increase in digestible threonine level on this variable in their studies on tambatinga fingerlings and tambaqui juveniles, respectively. However, the digestible threonine value of 1.20%, which was estimated to optimize the BDP, was similar to the recommended value of 1.28% by Bomfim et al. (2008) for fingerlings of Nile tilapia (*Oreochromis niloticus*).

The best nutritional adjustment of the diets caused by an increase in digestible threonine up to the level of 1.20% was an increase in the deposition of lean tissue, highlighting the essentiality of threonine in diets for tambatinga and corroborating the improvement observed in the other parameters and food efficiency for the formation of lean tissue.

However, there was no increase in the BDP in fish fed with levels above 1.20% of digestible threonine. The increase in the levels of amino acids and consequently proteins increased the energy expenditure for the catabolism of excess amino acids; thus, justifying the observed reduction in the BDF (Tables 4 and 5) (Bomfim et al., 2010; Firmo et al., 2018). The increase in BDA at the digestible threonine level of 1.07% may be related to the formation of bone tissue to support muscle tissue, in line with the findings of Bomfim et al. (2020) and Sousa et al. (2018) on tambaqui fingerlings subjected to different levels of digestible phosphorus and tryptophan, respectively, in the feed.

The efficiency of nitrogen retention was also influenced in a quadratic manner ($P <0.01$), with a reduction, observed at the level of 0.82% (Tables 5 and 6). This reduction may be related to the proportional increase in the catabolism of non-limiting amino acids with an increase in the dietary level of digestible threonine, and consequently the crude protein level; thus, reducing the efficiency of protein use, since the experimental diets were formulated by the dilution technique (Bomfim et al., 2010; Firmo et al., 2018).

Weight gain has been used as a criterion to determine the nutritional requirements using the dose-response method (NRC, 2011). Another criterion that can be used is the BDP, as it only considers the increase in lean tissue, which is the determinant of amino acid requirements (Lee et al., 2020; Silva et al., 2018). Based on the data on WG and BDP, it was observed that the digestible threonine level of 1.20% was the most suitable for use in diets for tambatinga fingerlings. This value is above the 1.00% recommended for tambatinga fingerlings by Alencar Araripe et al. (2011) and 1.10% recommended for tambaqui juveniles by Firmo et al. (2018).

Table 5: Body deposition of protein (BDP), fat (BDF), and ash (BDA), and nitrogen retention efficiency (NRE) of the fingerlings of tambatinga, as a function of the digestible threonine level in the diet.

Digestible threonine level (%)	Variable			
	BDP (mg day^{-1})	BDF (mg day^{-1})	BDA (mg day^{-1})	NRE (%)
PCD 0.900*	79.30 ^{A}	66.70 ^{A}	20.17 ^{A}	63.94 ^{A}
0.600	63.20 ^{B}	68.30 ^{A}	15.40 ^{B}	56.99 ^{B}
0.800	73.50	59.90	17.00	48.85
1.000	93.10	63.00	21.20	50.61
1.200	120.60	72,00	28.60	59.51
1.400	108.60	58.70	25.70	40.57
1.600	107.80	49.20	23.00	33.74
p>F	< 0.0001	0.0015	<0.0001	<0.0001
CV (%)	12.66	12.49	11.80	9.56

*Positive control diet - dilution 1 (0.600%) + 0.300% L-threonine; p>F - Significance of the "F" Test of analysis of variance; CV - Coefficient of variation; Averages on the same line followed by the same letters do not differ by the SNK test (p > 0.05).

Table 6: Adjusted regression equations, determination coefficients (R^2), and requirement values for the variables body deposition of protein (BDP), fat (BDF), ash (BDA), and nitrogen retention efficiency (NRE) for fingerlings of tambaqui, as a function of the digestible threonine level in the diet.

Variable	Model	Equation	$p > F$*	R^2	Requirement (%)
BDP (mg day^{-1})	Linear	$\hat{Y} = 50.694x + 38.656$	<0.0001	0.73	-----
BDP (mg day^{-1})	Quadratic	$\hat{Y} = -81.051x^2 + 229.007x-49.960$	0.0011	0.89	1.41
BDP (mg day^{-1})	LRP	$\hat{Y} = 108.051-95.895(1.113-x)$	<0.0010	0.93	1.11
BDP (mg day $^{-1}$)	Quadratic+LRP	$\hat{Y} = 108.051 = -81.051x^2 + 229.007x-9.960$	-----	-----	1.20
BDF (mg day^{-1})	Linear	$\hat{Y} = -12.864x + 76.009$	0.0047	0.36	-----
BDF (mg day^{-1})	Quadratic	$\hat{Y} = -31.761x^2 + 57.010x + 41.284$	0.0341	0.55	0.90
BDA (mg day^{-1})	Linear	$\hat{Y} = 10.175x + 10.621$	<0.0001	0.57	-----
BDA (mg day^{-1})	Quadratic	$\hat{Y} = -22.588x^2 + 59.870x - 14.076$	0.0001	0.81	1,33
BDA (mg day^{-1})	LRP	$\hat{Y} = 24.311-21.989(1.070-x)$	<0.0087	0.79	1.07
NRE (%)	Linear	$\hat{Y} = -18.918x+69.155$	<0.0001	0.53	-----
NRE (%)	Quadratic	$\hat{Y} = -33.685x^2 + 55.190x + 32.326$	0.0006	0.67	0.82

*p>F - Significance of the "F" Test of analysis of variance.

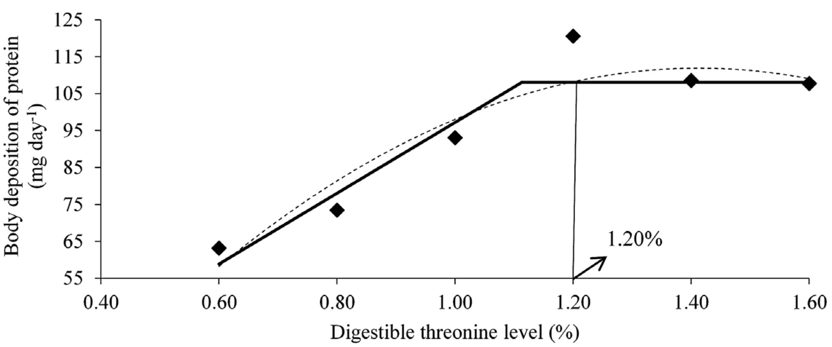

Figure 3: Graphical representation of the body deposition of protein in fingerlings tambatinga as a function of the digestible threonine level in the diet.

Another reason that may also influence the variation at the determined level is the choice of the statistical model (mean test, quadratic regression, or LRP), as already highlighted (Silva et al., 2018; Siqueira et al., 2009). Apart from the statistical model, health challenge and/or stress may be another factor that could contribute to the difference between the observed values, and they may involve additional demand for threonine for the production of mucin and immunoglobulins, as these compounds have a high concentration of this amino acid (Alencar Araripe et al., 2011; Bomfim et al., 2008).

CONCLUSION

The recommended level of digestible threonine in the diet for tambatinga fingerlings is between 1.20 to 1.40% (0.40 and 0.47% Mcal DE^{-1}, respectively), for providing greater body deposition of protein and weight gain, respectively.

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