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Lysine in optimal amounts in the diet of tambaqui (*Colossoma macropomum*) improves zootechnical performance and body protein deposition

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Abstract

Lysine is an essential amino acid for fish growth; however, its dietary requirement for tambaqui (*Colossoma macropomum*) weighing over 80 g remains poorly defined, limiting the optimization of diets during the grow-out phase. This study aimed to determine the digestible lysine requirement of tambaqui weighing between 87 and 340 g. A total of 210 fish (initial body weight: 87.07 ± 1.79 g) were randomly assigned to six dietary treatments with five replicates each. Experimental diets containing graded levels of digestible lysine (0.713, 0.988, 1.263, 1.538, 1.813, and 2.088%) were formulated using the diet dilution technique. Increasing dietary digestible lysine levels linearly increased lysine intake while reducing lysine utilization efficiency for weight gain and nitrogen retention efficiency. Weight gain, specific growth rate, body protein deposition, and body ash deposition increased up to dietary digestible lysine levels of 1.58%, 1.58%, 1.59%, and 1.92%, respectively. Conversely, feed intake, feed conversion ratio, and body fat deposition decreased up to estimated digestible lysine levels of 1.61%, 1.74%, and 1.86%, respectively. Based on body protein deposition, the dietary digestible lysine requirement for tambaqui weighing between 87 and 340 g was estimated at 1.59%, corresponding to 0.53 g of digestible lysine per Mcal of digestible energy.

Keywords: Dilution technique · Essential amino acid · Fish nutrition · Nutritional requirement

Introduction

Tambaqui (*Colossoma macropomum*) is one of the most economically important native fish species in the Amazon Basin and a cornerstone of Brazilian aquaculture. Despite its increasing production, nutrition remains one of the major constraints limiting the species' productive potential, largely because commercial diets are generally formulated based on the nutritional requirements of other fish species rather than the specific requirements of tambaqui (Hilsdorf et al. 2022).

Precision nutrition, supported by accurate estimates of essential amino acid requirements, is fundamental for improving growth performance, maximizing feed efficiency, reducing feed costs, and minimizing nitrogen discharge into the aquatic environment (Marchão et al. 2020; Silva et al. 2022; Martins et al. 2024). Among essential amino acids, lysine has received particular attention because it is the primary limiting amino acid in practical diets based on corn and soybean meal and plays a central role in protein accretion, growth, carcass composition, and nitrogen utilization (NRC 2011; Portz and Furuya 2012; Furuya et al. 2023).

Although digestible lysine requirements have been established for tambaqui during the early stages of development (Silva et al. 2018; Marchão et al. 2020; Teixeira et al. 2020; Liebl et al. 2022), There is limited information available for fish weighing over 80 g. This represents an important knowledge gap because nutritional requirements change throughout development (NRC 2011), and the grow-out phase is the longest and most economically important stage of commercial production. Consequently, the lack of species- and stage-specific nutritional recommendations may compromise growth performance, feed efficiency, and production profitability.

We hypothesized that the lysine requirement of fish during the grow-out phase differs from that reported for younger fish and that body protein deposition provides a robust criterion for estimating dietary lysine requirements. Therefore, this study aimed to determine the digestible lysine requirement of tambaqui weighing between 87 and 340 g using the diet dilution technique.

Material and methods

Ethical animal use

The experiment was conducted in accordance with the ethical principles for animal experimentation established by the Brazilian National Council for the Control of Animal Experimentation (CONCEA). All experimental procedures were approved by the Animal Ethics Committee of the Federal University of Maranhão (CEUA/UFMA; protocol no. 23115.011750/2018-52).

Experimental facility and fish

The experiment was conducted at the Laboratory of Aquatic Organism Nutrition (LANUMA), Center of Sciences of Chapadinha (CCCh), Federal University of Maranhão (UFMA), Chapadinha, Maranhão, Brazil, and lasted 60 days.

A total of 210 tambaqui with an initial body weight of 87.07 ± 1.79 g were used in a completely randomized design consisting of seven treatments (six dietary digestible lysine levels and one control treatment), five replicates per treatment, and six fish per experimental unit.

Fish were maintained in thirty-five 500-L polyethylene tanks connected to a closed recirculating water system with an average flow rate of 10 L h⁻¹ per tank. Each tank was equipped with an individual water supply, supplemental aeration, and drainage system using artesian well water.

Water quality parameter

The water quality parameters were maintained within the recommended range for tambaqui (Mendonça et al. 2009). Dissolved oxygen (7.43 ± 0.76 mg O₂ L⁻¹) and temperature (26.20 ± 0.52 °C in the morning and 28.70 ± 0.72 °C in the afternoon) were monitored daily using an oximeter (HI 9146, Hanna®, Woonsocket, USA), pH (5.90 ± 0.33) with the assistance of a pH meter (HI 8424, Hanna®, Woonsocket, USA), and total ammonia (<0.1 ppm mg N-NH₃ L⁻¹) using colorimetric reactions from a commercial kit (Alcon Ltda–Camboriú, SC, Brazil). Tanks were cleaned daily, 30 min after the final meal, to remove uneaten feed and fecal residues. Approximately 10% of the system water was replaced with fresh artesian well water after each cleaning.

Experimental diets

The total amino acid contents of corn and soybean meal were determined before diet formulation. Digestible amino acid values were estimated using the digestibility coefficients reported for tambaqui by Nascimento et al. (2020) (Table 1).

Table 1. Composition in total amino acids (TAA) and digestible amino acids (DAA) of soybean meal and corn used in experimental diets (based on natural matter)

Amino acids (%)	Soybean meal ^a		Corn ^a	
	TAA ^b	DAA ^c	TAA ^b	DAA ^c
Lys	2.75	2.60	0.27	0.25
Met	0.56	0.53	0.16	0.15
Met + Cyst	1.18	1.16	0.28	0.25
Thr	1.68	1.65	0.26	0.25
Trp	0.62	0.58	0.14	0.11
Ile	2.01	1.95	0.23	0.22
Val	2.28	2.21	0.40	0.38
His	1.28	1.22	0.25	0.23
Leu	3.54	3.35	0.83	0.82
Arg	3.46	3.43	0.43	0.42
Phe	2.40	2.36	0.36	0.35

^aDry matter Soybean meal: 95.20%

^aDry matter Corn: 93.90%

^bTotal amino acids.

^cDigestible amino acids, calculated based on the digestibility coefficients proposed by Nascimento et al. (2020) for tambaqui (*Colossoma macropomum*).

Experimental diets were formulated using the dilution technique (Fisher and Morris 1970; NRC 2011; Sousa et al. 2021). Initially, two diets were formulated, a reference diet (RD) based on corn and soybean meal,

containing 36.82% of crude protein (CP) and 2.088% of digestible lysine, and a protein-free diet (PFD) based on corn starch, containing the same levels of digestible energy, vitamins, and minerals. Subsequently, the RD was sequentially diluted with the PFD, to produce diets containing 0.713; 0.988; 1.263; 1.538; 1.813 and 2.088% digestible lysine (Table 2).

Table 2. Percentage and chemical composition of protein-free diet (PFD), reference diet (RD), experimental diets with increasing levels of digestible lysin, and control diet (CD)

Ingredients (%)	PFD	Digestible lysine levels (%)						
		0.713	0.988	1.263	1.538	1.813	2.088	0.988
							RD ^a	CD ^b
Soybean meal	0.000	26.720	37.024	47.329	57.634	67.938	78.243	26.720
Corn	0.000	2.886	3.999	5.112	6.225	7.338	8.451	2.886
Corn starch	79.016	54.413	44.925	35.436	25.948	16.460	6.972	54.062
Soybean oil	10.171	6.697	5.358	4.018	2.679	1.339	0.000	6.697
Cellulose	4.636	3.053	2.442	1.832	1.221	0.611	0.000	3.053
L-Lysine HCl (78.4%)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.351
DL-Methionine (99.0%)	0.000	0.176	0.244	0.312	0.379	0.447	0.515	0.176
L-Threonine (98.5%)	0.000	0.113	0.157	0.200	0.244	0.287	0.331	0.113
L-Tryptophan (98.0%)	0.000	0.0016	0.0023	0.0029	0.0035	0.0042	0.0048	0.0016
L-Valine	0.000	0.028	0.039	0.050	0.060	0.071	0.082	0.028
Calcite limestone	1.226	1.199	1.188	1.177	1.166	1.156	1.145	1.199
Dicalcium phosphat	3.827	3.605	3.519	3.433	3.347	3.261	3.176	3.605
Vit. and min. premix ^g	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Vitamin C ^f	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Salt	0.554	0.539	0.533	0.528	0.522	0.516	0.510	0.539
Antioxidant (BHT)	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Dilution (%)								
Protein-free diet	100.00	65.85	52.68	39.51	26.34	13.17	0.00	65.85
Reference diet	0.00	34.15	47.32	60.49	73.66	86.83	100.00	34.15
Nutritional Composition								
Crude protein (%)	0.00	12.57	17.42	22.27	27.12	31.97	36.82	12.57
Digest. energy(kcal kg ⁻¹) ^e	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Ethereal Extract (%)	10.21	7.29	6.16	5.03	3.90	2.77	1.64	7.29
Crude Fiber (%)	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29
Total Ca (%) ^c	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Available P (%) ^c	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Digestible Lys (%) ^d	0.00	0.713	0.988	1.263	1.538	1.813	2.088	0.988
Digest. Met + Cys (%) ^d	0.00	0.499	0.692	0.884	1.077	1.269	1.462	0.499
Digestible Thr (%) ^d	0.00	0.585	0.810	1.036	1.261	1.487	1.712	0.585

Digestible Trp (%) ^d	0.00	0.164	0.227	0.290	0.354	0.417	0.480	0.164
Digestible Ile (%) ^d	0.00	0.560	0.776	0.992	1.208	1.424	1.640	0.560
Relations (%)								
Digestible Lys/energy (g Mcal ⁻¹)								
¹)	0.00	0.238	0.329	0.421	0.513	0.604	0.696	0.329
Digestible Met + Cys/Lys	0.00	70.0	70.0	70.0	70.0	70.0	70.0	50.5
Digestible Thr/Lys	0.00	82.0	82.0	82.0	82.0	82.0	82.0	59.2
Digestible Trp/Lys	0.00	23.0	23.0	23.0	23.0	23.0	23.0	16.6
Digestible Ile/Lys	0.00	78.5	78.5	78.5	78.5	78.5	78.5	56.7

^a Reference diet (2.088% of lysine digestible).

^b Control diet = First level (0.713) + 0.351% L-Lysine-HCl (78.4%).

^c Based on the values proposed by Rostagno et al. (2011).

^d Based on the digestibility coefficients proposed by Nascimento et al. (2020).

^e Based on the digestibility coefficients proposed by Furuya et al. (2010).

^f Vit. C: Calcium L-ascorbic acid 2-monophosphate, 42% of active ingredient.

^g Vitamin and mineral supplement, amounts supplied per kg of diet: Vit. A, 6,000 IU; Vit. D3, 1,000 IU; Vit. E, 60.0 mg; Vit. K3, 12.0 mg; Vit. B1, 24.00 mg; Vit. B2, 24.00 mg; Vit. B6, 24.00 mg; Vit. B12, 24.00 mg; Vit. C, 24.00 mg; folic acid, 6.00 mg; Ca pantothenate, 60.00 mg; biotin, 0.24 mg; choline chloride, 108 g; niacin, 100.00 mg; Fe, 250.00 mg; Cu, 15.0 mg; Mn, 100.00 mg; Zn, 150.00 mg; I, 0.5 mg; Co, 0.05 mg; Se, 0.5 mg.

In all experimental diets, tryptophan/lysine, threonine/lysine, and methionine plus cystine/lysine digestible were maintained at least 5 percentage points above the ideal proportion proposed by Bomfim et al. (2020), Firmo et al. (2018) and Souza et al. (2019), respectively, for tambaqui. For the other amino acids, the amino acid/lysine ratio was maintained at least five percentage points above those estimated from the requirement values for Nile tilapia proposed by the NRC (2011), so that the responses obtained in the variables were only due to the variation of digestible lysine levels (first limiting amino acid in all experimental diets).

To confirm the hypothesis that lysine was the first limiting amino acid in all experimental diets, a seventh treatment (control diet-CD) was used, with five replicates and six animals per experimental unit. In this treatment, synthetic lysine (L-lysine-HCl 78.4%) was added to the first diet (0.713% digestible lysine) until reaching the level of the second diet (0.988% digestible lysine), as proposed by Nonis and Gous (2007) (Table 2).

The ingredients of the experimental diets were ground finely (Trf 60, Trapp®), weighed individually, mixed (Horizontal Mixer 300 kg, Branorte®), and subsequently extruded (Extruder model MX 40, Inbramaq), obtaining pellets with approximately 4 mm in diameter. After preparation, the experimental diets were analyzed, and the total amino acid contents were confirmed with the formulation targets.

Fish were hand-fed to apparent satiation six times daily (08:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h). Feed was offered in small portions to minimize nutrient leaching and ensure maximum voluntary feed intake

Growth performance

At the end of the 60-day experimental period, fish were fasted for 24 h before growth performance evaluation. The following variables were determined: weight gain (WG), feed intake (FI), digestible lysine intake (DLI), feed conversion ratio (FCR), specific growth rate (SGR), and lysine efficiency for weight gain (LEWG), calculated according to the following equations:

- $WG\ (g) = \text{final body weight (g)} - \text{initial body weight (g)}$;
- $FI\ (g) = \text{diet consumed during the experimental period}$;
- $DLI\ (mg) = [\text{feed intake (mg)} \times \text{digestible lysine content of the diet (\%)}] / 100$;
- $FCR\ (g\ g^{-1}) = \text{feed intake (g)} / \text{weight gain (g)}$;
- $SGR\ (\% \text{ day}^{-1}) = [(\text{natural logarithm of final weight (g)} - \text{natural logarithm of initial weight (g)}) \times 100] / \text{experimental period (days)}$;
- $LEWG\ (g\ g^{-1}) = \text{weight gain (g)} / \text{lysine intake (g)}$.

Sample collection and chemical analysis

At the beginning and end of the experimental period, all fish were individually weighed after a 24-h fasting period. For whole-body composition analysis (moisture, crude protein, crude fat, and ash), six fish were sampled at the beginning of the experiment, and two fish were randomly collected from each experimental unit at the end of the feeding trial. Fish were euthanized by immersion in a benzocaine solution (500 mg L⁻¹), ground, dried in a forced-air oven at 65 °C for 72 h, placed in airtight plastic containers, and stored at -20 °C until analysis.

Whole-body proximate composition was determined at the Animal Nutrition Laboratory of the Federal University of Maranhão according to the methods of the Association of Official Analytical Chemists (AOAC 2016). Moisture content was determined by oven drying at 105 °C until constant weight. Crude fat was determined by ether extraction using a fat extractor (LUCA-202, Lucadema®). Crude protein (N × 6.25) was determined using the Kjeldahl method, and ash content was determined by incineration in a muffle furnace (TE-1100-1P, Tecnal®) at 550 °C for 4 h.

Based on whole-body composition, body protein deposition (BPD), body fat deposition (BFD), body ash deposition (BAD), and nitrogen retention efficiency (NRE) were calculated using the following equations:

- $BPD\ (mg\ day^{-1}) = \{[(\text{final body protein, \%} \times \text{final weight, mg}) - (\text{initial body protein, \%} \times \text{initial weight, mg})] / 100\} / \text{experimental period (days)}$;
- $BFD\ (mg\ day^{-1}) = \{[(\text{final body fat, \%} \times \text{final weight, mg}) - (\text{initial body fat, \%} \times \text{initial weight, mg})] / 100\} / \text{experimental period (days)}$;
- $BAD\ (mg\ day^{-1}) = \{[(\text{final body ash, \%} \times \text{final weight, mg}) - (\text{initial body ash, \%} \times \text{initial weight, mg})] / 100\} / \text{experimental period (days)}$;
- $NRE\ (\%) = [(\text{final body N (\%)} \times \text{final weight (g)}) - (\text{initial body N (\%)} \times \text{initial weight (g)})] / [\text{feed intake (g)} \times \text{level of N in diet (\%)}] / 100$.

Statistical analysis

Statistical analyses were performed using the SAS 9.0 software (2002). The assumptions of normality and homoscedasticity were tested and met. Then, the data were submitted for analysis of variance at the 5% probability level.

The variables that showed a significant effect of digestible lysine levels by analysis of variance were submitted to regression analysis using linear and quadratic models according to the best fit. The adjustment was also verified for the discontinuous model Linear Response Plateau (LRP). The model providing the best fit was selected based on the coefficient of determination (R^2) and biological interpretability.

Additionally, for the variables that presented adjustment for both the LRP and the quadratic model, as an alternative to avoid the limitations of these models when used individually, the data were submitted to the procedure proposed by Baker et al. (2002), estimating the requirement by the first intersection of the quadratic equation with the plateau obtained by the LRP equation. To compare the effects of the control diet (0.988%) with the lowest level tested (0.713%) was used Dunnett's test ($P < 0.05$).

Results

Growth performance and feed efficiency

No mortality or external pathological signs were observed throughout the 60-day experimental period, including in fish fed the lowest dietary digestible lysine levels.

Fish fed the control diet showed higher digestible lysine intake ($P < 0.05$), lower lysine efficiency for weight gain ($P < 0.05$), and improved feed conversion ratio ($P < 0.05$) compared with fish receiving the lowest dietary digestible lysine level (0.713%) (Table 3).

Table 3 Feed intake (FI), digestible lysine intake (DLI), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and lysine efficiency for weight gain (LEWG) of tambaqui fed diets containing different levels of digestible lysine

Digestible lysine level (%)	Variable					
	FI (g)	DLI (g)	WG (g)	SGR (% day ⁻¹)	FCR (g g ⁻¹)	LEWG (g g)
0.713	433.02	3.09*	201.93	1.96	2.17*	65.24*
0.988	419.53	4.15	207.46	1.99	2.06	49.78
1.263	389.80	4.92	233.45	2.13	1.68	47.43
1.538	375.18	5.77	253.39	2.23	1.50	44.03
1.813	372.77	6.76	250.88	2.22	1.51	37.29
2.088	383.78	8.01	257.10	2.25	1.50	32.20
CD	414.21	4.09	222.72	2.08	1.86	54.34
<i>P > F</i>						
Lysine Level	0.008	< 0.001	0.039	0.028	< 0.001	< 0.001
Linear Effect	0.001	< 0.001	0.002	0.001	< 0.001	< 0.001

Quadratic Effect	0.051	0.306	0.999	0.999	0.026	0.163
LRP	< 0.001	0.999	0.003	0.002	< 0.001	0.999
SEM	6.21	0.31	6.75	0.03	0.06	2.17

CD – Control diet (0.713+0.351% L-Lysine- HCl).

SEM– Standard error of the mean (n= 5 tanks per treatment).

* Differs from the control diet (CD) by Dunnett's test (P<0.05).

Feed intake decreased ($P < 0.05$) as dietary digestible lysine increased. Although the quadratic model estimated the optimum dietary digestible lysine level at 1.78%, the Linear Response Plateau (LRP) model provided the best fit, estimating a breakpoint at 1.46%, beyond which feed intake reached a plateau (Tables 3 and 4). Based on the first intersection between the quadratic curve and the LRP plateau, the dietary digestible lysine requirement for feed intake was estimated at 1.61% (Table 4). In contrast, digestible lysine intake increased linearly ($P < 0.05$) with increasing dietary digestible lysine levels.

Table 4 Regression equations, coefficients of determination (R^2), and estimated digestible lysine requirements for feed intake (FI), digestible lysine intake (DLI), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and lysine efficiency for weight gain (LEWG) of tambaqui fed diets containing different levels of digestible lysine.

Variable	Model	Equation	R^2	Requirement (%)
FI (g)	L	$\hat{Y} = -41.6683X + 454.137$	0.75	-----
FI (g)	Q	$\hat{Y} = 54.7234X^2 - 194.948X + 549.301$	0.95	1.78
FI (g)	LRP	$\hat{Y} = 377.2 + 78.5658(1.4573 - X)$	0.96	1.46
FI (g)	Q + LRP	$377.2 = 54.7234X^2 - 194.948X + 549.301$	–	1.61
DLI (g)	L	$\hat{Y} = 3.46155X + 0.601735$	0.99	-----
WG (g)	L	$\hat{Y} = 44.2665X + 172.041$	0.89	-----
WG (g)	LRP	$\hat{Y} = 254 - 65.5921(1.5819 - X)$	0.96	1.58
SGR (%/dia)	L	$\hat{Y} = 0.233101X + 1.80148$	0.89	-----
SGR (%/dia)	LRP	$\hat{Y} = 2.2331 - 0.3439(1.5842 - X)$	0.96	1.58
FCR (g/g)	L	$\hat{Y} = -0.540301X + 2.49369$	0.84	-----
FCR (g/g)	Q	$\hat{Y} = 0.485959X^2 - 1.90147X + 3.33966$	0.95	1.96
FCR (g/g)	LRP	$\hat{Y} = 1.5029 + 0.8945(1.5113 - X)$	0.97	1.51
FCR (g/g)	Q + LRP	$1.5029 = 0.485959X^2 - 1.90147X + 3.33966$	–	1.74
LEWG (g/g)	L	$\hat{Y} = -21.4109x + 75.9780$	0.92	-----

Abbreviations: L, Linear; Q, Quadratic; LRP, Linear Response Plateau.

Weight gain and specific growth rate increased linearly ($P < 0.05$) as dietary digestible lysine increased. However, the LRP model provided the best fit for both variables, estimating a digestible lysine requirement of 1.58%, beyond which no further improvement was observed (Tables 3 and 4; Figure 1).

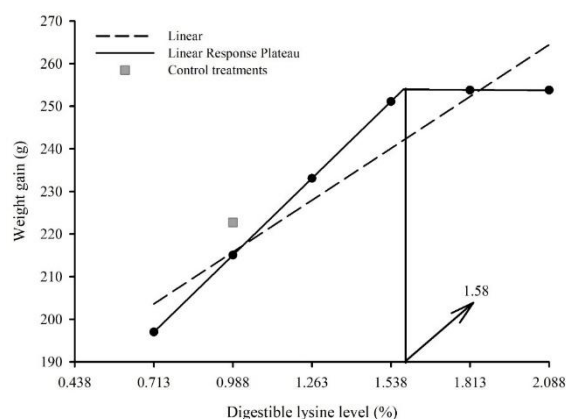


Fig. 1 Graphical representation of weight gain as a function of dietary digestible lysine levels in tambaqui

Feed conversion ratio decreased quadratically ($P < 0.05$) with increasing dietary digestible lysine levels. Although the quadratic model estimated an optimum level of 1.96%, the LRP model provided a better fit, estimating a breakpoint at 1.51%, after which the response reached a plateau (Tables 3 and 4). The first intersection between the quadratic model and the LRP plateau estimated the dietary digestible lysine requirement for optimal feed conversion ratio at 1.74% (Table 4; Figure 2).

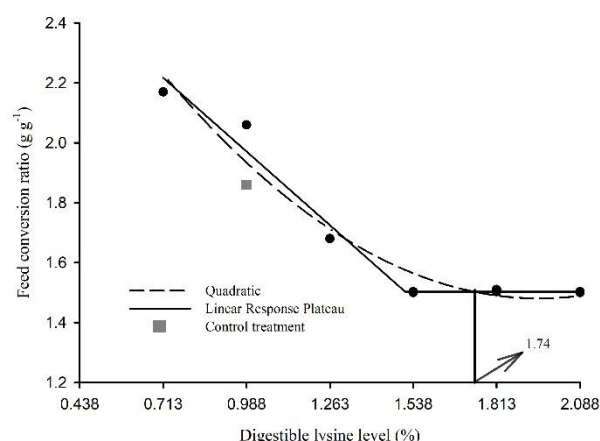


Fig. 2 Graphical representation of feed conversion ratio as a function of dietary digestible lysine levels in tambaqui

Lysine efficiency for weight gain decreased linearly ($P < 0.01$) with increasing dietary digestible lysine levels.

Body depositions and nitrogen retention efficiency

Fish fed the control diet exhibited higher body protein deposition and nitrogen retention efficiency ($P < 0.05$) than those fed the diet containing the lowest dietary digestible lysine level (0.713%) (Table 5).

Table 5 Whole-body composition, body protein deposition (BPD), body fat deposition (BFD), body ash deposition (BAD), and nitrogen retention efficiency (NRE) of tambaqui fed diets containing different levels of digestible lysine

Digestible lysine level (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	BPD (mg day ⁻¹)	BFD (mg day ⁻¹)	BAD (mg day ⁻¹)	NRE (%)
0.713	64.75	13.96	18.10	3.19	0.472*	0.747	0.094	52.89*
0.988	66.61	14.17	15.50	3.73	0.497	0.636	0.123	41.24
1.263	69.77	14.46	12.07	3.70	0.571	0.525	0.138	40.23
1.538	69.90	14.80	11.68	3.62	0.637	0.536	0.146	38.31
1.813	71.51	14.86	9.63	4.00	0.636	0.417	0.165	32.68
2.088	72.02	14.72	9.18	4.07	0.644	0.408	0.173	27.86
DC	64.56	14.53	17.53	3.38	0.552	0.779	0.115	64.46
<i>P > F</i>								
Lysine Level	<0.001	0.007	<0.001	0.005	0.001	<0.001	0.003	<0.001
Linear Effect	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic Effect	0.003	0.043	0.008	0.615	0.182	0.266	0.999	0.999
LRP	<0.001	0.001	<0.001	0.999	<0.001	<0.001	<0.001	0.999
SEM	0.53	0.10	0.63	0.08	0.02	0.03	0.01	1.59

CD – Control diet (0.713+0.351% L-Lysine- HCl).

SEM– Standard error of the mean (n= 5 tanks per treatment).

* Differs from the control diet (CD) by Dunnett's test (P<0.05).

230

231 Increasing dietary digestible lysine levels significantly affected whole-body composition ($P < 0.05$).

232 Whole-body moisture and crude protein increased linearly, whereas whole-body fat decreased linearly with
 233 increasing dietary digestible lysine levels. Except for whole-body ash content, the Linear Response Plateau
 234 (LRP) model provided the best fit according to the coefficient of determination (R^2), estimating dietary
 235 digestible lysine requirements of 1.72%, 1.56%, and 1.82% for moisture, crude protein, and body fat,
 236 respectively (Tables 5 and 6).

237 **Table 6** Regression equations, coefficients of determination (R^2), and estimated digestible lysine requirements
 238 for whole-body composition, body protein deposition (BPD), body fat deposition (BFD), body ash deposition
 239 (BAD), and nitrogen retention efficiency (NRE) of tambaqui fed diets containing different levels of digestible
 240 lysine

Variable	Model	Equation	R^2	Requirement (%)
Moisture (%)	L	$\hat{Y} = 5.32112X + 61.6415$	0.92	-----
Moisture (%)	LRP	$\hat{Y} = 71.7675 - 6.7700(1.7179 - X)$	0.96	1.72
Crude protein (%)	L	$\hat{Y} = 0.645610X + 13.5892$	0.82	-----
Crude protein (%)	LRP	$\hat{Y} = 14.7901 - 1.0167(1.5634 - X)$	0.98	1.56
Fat (%)	L	$\hat{Y} = -6.50007X + 21.7965$	0.93	-----
Fat (%)	LRP	$\hat{Y} = 9.1826 + 7.5455(1.8213 - X)$	0.97	1.82
ASH (%)	L	$\hat{Y} = 0.533091x + 2.97324$	0.77	-----
BPD (mg day ⁻¹)	L	$\hat{Y} = 0.139803x + 0.380273$	0.89	-----
BPD (mg day ⁻¹)	LRP	$\hat{Y} = 0.6401 - 0.2068(1.5900 - x)$	0.98	1.59

BFD (mg day ⁻¹)	L	$\hat{Y} = -0.242888x + 0.885198$	0.92	-----
BFD (mg day ⁻¹)	LRP	$\hat{Y} = 0.4084 + 0.2760 (1.8571 - x)$	0.95	1.86
BAD (mg day ⁻¹)	L	$\hat{Y} = 0.0550442x + 0.0626773$	0.96	-----
BAD (mg day ⁻¹)	LRP	$\hat{Y} = 0.1728 - 0.0603 (1.9205 - x)$	0.97	1.92
NRE (%)	L	$\hat{Y} = -15.8729x + 61.0992$	0.92	-----

Abbreviations: L, Linear; LRP, Linear Response Plateau.

Similarly, body deposition variables were significantly affected by dietary digestible lysine levels. Body protein deposition (BPD) and body ash deposition (BAD) increased linearly ($P < 0.01$), whereas body fat deposition (BFD) decreased linearly ($P < 0.01$) with increasing dietary digestible lysine levels (Table 5). According to the coefficient of determination (R^2), the LRP model provided the best fit for these variables, estimating dietary digestible lysine requirements of 1.59%, 1.92%, and 1.86% for BPD, BAD, and BFD, respectively (Table 6; Figure 3).

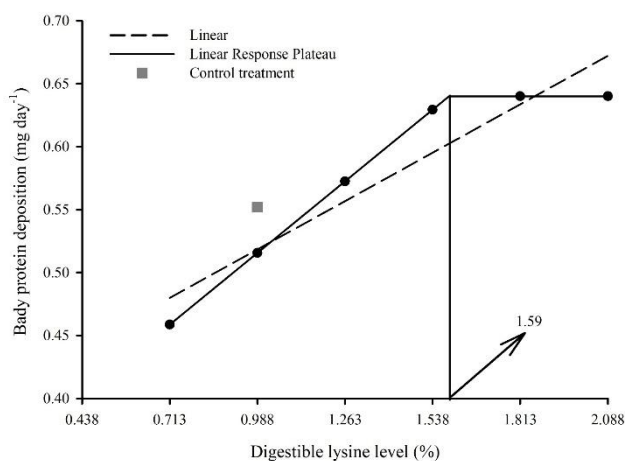


Fig. 3 Graphical representation of body protein deposition as a function of dietary digestible lysine levels in tambaqui

Nitrogen retention efficiency decreased linearly ($P < 0.01$) as dietary digestible lysine levels increased (Table 5).

Discussion

Lysine was confirmed as the first limiting amino acid in all experimental diets, as demonstrated by the superior feed conversion ratio, lower lysine efficiency for weight gain, and greater body protein deposition and nitrogen retention efficiency observed in fish fed the control diet (0.713% digestible lysine + 0.351% L-lysine-HCl) compared with those fed the diet containing only 0.713% digestible lysine. Because dietary protein utilization depends on the supply of the first limiting amino acid, the responses observed in the present study can be attributed to differences in dietary digestible lysine rather than to differences in crude protein concentration among the diets.

Although increasing dietary digestible lysine levels reduced feed intake and, consequently, the intake of energy, vitamins, and minerals, growth performance, feed efficiency, and body protein deposition improved

as the dietary amino acid profile progressively approached the fish's lysine requirement, thereby increasing the availability of digestible lysine for protein synthesis. Fish fed diets containing less than 1.61% digestible lysine exhibited higher feed intake, probably as a compensatory response to inadequate lysine supply, as previously reported for jundiá (*Rhamdia quelen*) (Montes-Girão and Fracalossi 2006) and pirapitinga (*Piaractus brachipomus*) (Vásquez-Torres and Arias-Castellanos 2013).

The improvement in weight gain observed in the present study can be attributed to the progressive correction of the dietary amino acid imbalance through lysine supplementation, which increased the availability of amino acids for protein synthesis and consequently promoted greater lean tissue accretion. Similar responses have been reported for tambaqui by Silva et al. (2018) and Marchão et al. (2020), and for tambatinga (♀ *Colossoma macropomum* × ♂ *Piaractus brachipomus*) by Sousa et al. (2021), highlighting the essential role of lysine in maximizing growth performance when deficient diets are supplemented.

The dietary digestible lysine requirement estimated for maximum weight gain (1.58%) was lower than that reported by Silva et al. (2018) for tambaqui weighing 0.34–5 g (1.73%) and by Sousa et al. (2021) for tambatinga weighing 0.82–15 g (1.80%). This difference is likely associated with the lower amino acid requirements of larger fish, whose lower relative growth rate reduces the demand for protein accretion compared with fish in the early stages of development. In contrast, the requirement estimated in the present study was similar to those reported by Teixeira et al. (2020) and Liebl et al. (2022) for tambaqui weighing 1.67–38 g (1.61%) and 33–70 g (1.56%), respectively. Consistent with the response observed for weight gain, the estimated digestible lysine requirement for maximum specific growth rate was also 1.58%, reflecting the close relationship between these growth variables. Nevertheless, differences in dietary lysine requirements among studies may also reflect variations in species, body weight, statistical model, nutrient digestibility, and experimental conditions (Martins et al. 2024).

Feed conversion ratio improved up to a dietary digestible lysine level of 1.74% due to the combined effects of reduced feed intake and increased weight gain. As dietary digestible lysine levels increased, the amino acid profile became more balanced, enhancing the efficiency of dietary protein utilization for body protein deposition (Xing et al. 2024). Because lean tissue contains a higher proportion of water than adipose tissue, protein deposition contributes more efficiently to body weight gain than fat deposition. A similar digestible lysine requirement for optimal feed conversion (1.75%) was reported for pacu (*Piaractus mesopotamicus*) weighing 9–50 g (Abimorad et al. 2010).

Despite the improvement in growth performance, lysine utilization efficiency decreased as dietary digestible lysine levels increased, indicating that a progressively smaller proportion of the ingested lysine was converted into body weight gain. According to Heger and Frydrych (2019), amino acid utilization efficiency is strongly influenced by dietary concentration. At suboptimal intake levels, amino acids are used more efficiently for protein synthesis, with only a small proportion being catabolized. As the dietary concentration of the limiting amino acid approaches or exceeds the physiological requirement, a greater proportion is diverted to catabolic pathways rather than retained for body protein deposition, resulting in reduced utilization efficiency (Heger and Frydrych 2019).

The increase in body protein deposition (BPD) up to the dietary digestible lysine level of 1.59% was consistent with the requirements reported by Marchão et al. (2020) and Teixeira et al. (2020) for tambaqui (1.58% and 1.56%, respectively). This response was consistent with the improvements observed in weight gain

and feed conversion ratio, confirming that body protein deposition closely reflects growth performance. The greater BPD observed with increasing dietary lysine levels is attributed to the improved balance of dietary amino acids, which enhances protein synthesis and lean tissue accretion (Ebenezzar et al. 2019; Lee et al. 2020). Conversely, dietary digestible lysine levels below the physiological requirement limit protein deposition by restricting the availability of lysine for muscle protein synthesis (Lee et al. 2020; Sousa et al. 2021).

As dietary digestible lysine levels approached the requirement, body protein deposition increased, whereas body fat content and body fat deposition decreased. The reduction in body fat may be associated with the greater energy expenditure required for amino acid metabolism (anabolism and catabolism) in fish fed diets containing higher lysine levels, thereby reducing the amount of energy available for lipid deposition (Zehra and Khan 2014). Similar responses have been reported for tambaqui (Marchão et al. 2020; Teixeira et al. 2020), tambatinga (Sousa et al. 2021), pacu (*Piaractus mesopotamicus*) (Bicudo et al. 2009), and Nile tilapia (Furuya et al. 2013).

Body ash deposition is closely associated with lean tissue accretion because muscle tissue contains a substantial proportion of the body's mineral content (Pianesso et al. 2015). Accordingly, the lower body ash deposition observed in fish fed the lysine-deficient diet is likely a consequence of reduced muscle growth. As dietary digestible lysine levels increased, body ash deposition also increased, reflecting the greater accretion of lean tissue, as previously reported for tambaqui (Marchão et al. 2020) and Sousa et al. (2018).

The reduction in nitrogen retention efficiency observed at higher dietary digestible lysine levels may be attributed to a greater proportion of absorbed amino acids being directed toward catabolic pathways rather than retained for body protein deposition (Bomfim et al. 2010). Similar reductions in nitrogen retention efficiency were reported by Sousa et al. (2021) for tambatinga and by Marchão et al. (2020) for tambaqui, both of whom formulated experimental diets using the diet dilution technique.

Traditionally, amino acid requirements in fish have been estimated based on the dietary level that optimizes growth performance, particularly weight gain (NRC 2011). However, body weight gain reflects the accretion of protein, lipids, minerals, and water (NRC 2011), whereas amino acids are required primarily for protein synthesis rather than for the deposition of lipid or mineral reserves (Burnham and Gous 1992; Gous 2007).

Therefore, body protein deposition appears to be a more biologically appropriate criterion for estimating amino acid requirements in fish (Bomfim et al. 2020; Lee et al. 2020; Sousa et al. 2021), particularly for lysine, which is the essential amino acid incorporated in the greatest proportion into body proteins (Furuya et al. 2010; NRC 2011)

Conclusion

The dietary digestible lysine requirement for tambaqui (*Colossoma macropomum*) weighing between 87 and 340 g is 1.59%, corresponding to 0.53 g of digestible lysine per Mcal of digestible energy, as determined by body protein deposition.

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Author contributions: AFLC: conducted the experiments, chemical analyses, statistical analysis, and discussion contribution. MADB and FBR: collaboration on carrying out the experiments, data analysis, and discussion. MLS, YLF and VCS: collaboration on data sampling, analysis conduction, and results. JCS and RSM: statistical analysis, supervised the findings, discussion, and final text. All the authors have read and approved the final manuscript.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval This study was carried out by the norms of ethical principles for animal experimentation described by the Conselho Nacional de Controle de Experimentação Animal (CONCEA), with procedures approved by the Committee on Ethics in the Use of Animals (CEUA) of the Universidade Federal do Maranhão, registered with No. 23115.011750/2018-52.

Competing interests The authors declare no competing interests.

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