Lysine requirements for maintenance and utilization efficiency of Tambaqui (Colossoma macropomum) at different body weights

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Abstract

Background

Lysine is an essential amino acid and, generally, the first limiting in diets for tambaqui (*Colossoma macropomum*), main native fish produced in continental aquaculture from South America. However, there is a lack of information on their amino acids requirements, especially for maintenance and efficiency of utilization of lysine.

Results

Diets with increasing levels of digestible lysine significantly increased final weight and the weight gain of tambaqui, for all studied ages. Feed intake decreased significantly only for fish weighing 121 g ($p < 0.05$), and was not affected for other ages. However, digestible lysine consumption significantly increased in all body weights groups evaluated ($p < 0.05$). In addition, digestible lysine consumption provided sufficient intervals to obtain of values negative, near zero and positive of retention body protein and lysine, allowing estimation of maintenance requirements without extrapolation. The maintenance lysine requirement was determined at the intersection of the point to maintain body protein retention equal to zero, and the efficiency of lysine utilization was the slope of the line between the digestible lysine consumption and its retention, for different tambaqui body weights (121, 235 and 596 g). Linear responses ($p < 0.05$) were observed between protein retention and body lysine in function on the consumption of digestible lysine for all evaluated body weights, and the parallelism test showed influences of body weight on the values of the maintenance requirement and efficiency of utilization for lysine, with an increase in the maintenance requirement as the fish grow. However, the efficiency of using lysine decreases with the increase in fish body weight.

Conclusions

The requirement of tambaqui for maintenance lysine and efficiency utilization are directly related to the body weight. The requirement for digestible lysine for tambaqui maintenance was 82.03 mg $kg^{-0.7}$ day^{-1} for body weights 121 to 235 g and utilization efficiency of 55%. For weight of 596 g, the requirement of maintenance was 106.85 mg $kg^{-0.7}$ day^{-1} and 40% of utilization efficiency of lysine.

Background

Tambaqui (*Colossoma macropomum*) is a species of fish found widely in the Amazon basin and is one of the most cultivated fishes in Brazil and other countries of South and Central America, owing to its great robustness, ease of reproduction in captivity and the organoleptic characteristics of its meat [1, 2]. Despite the great importance of tambaqui in these regions, diet formulations for this species generally use available data on the nutritional requirements of other tropical fish species, which may not ensure satisfactory performance due to the physiological particularities existing in each species.

Lysine stands out as one of the first limiting essential amino acids in practical fish diets, especially when formulated with ingredients of plant origin [3]. In addition, lysine is used almost exclusively for protein synthesis, being found at a higher proportion in muscle tissues [4–6]. Therefore, precise estimation of requirement this amino acid is necessary to formulate efficient and low-cost diets.

The methodology for determining the lysine requirement that has been gaining popularity in studies with fish is the factorial method, which estimates the requirements on the principle that fish need lysine for the maintenance of vital processes and growth. The lysine requirement for maintenance can be defined as the balance between lysine intake and body protein (nitrogen) excretion, with no change in body protein content. The efficiency of lysine utilization for growth can be determined by the slope coefficient of a straight line between consumption of lysine and its retention [7–9].

The factorial method allows the elaboration of models capable of predicting the nutritional requirements of fish, as it contemplates differences in weight, body composition, growth potential, in different breeding conditions. Body weight can have a major influence on the requirement for maintenance and efficiency of utilization of lysine in fish [10–12].

However, the application of the factorial method depends on the determination of the parameters that express the maintenance requirements, and the efficiency of utilization of the amino acids in the diet, and this information can be obtained from dose-response studies, specifically planned. Given that there are no studies with tambaqui, the objective was to determine the requirement for lysine for maintenance and the efficiency of utilization for tambaqui with different body weights.

Methods

Chemical analysis of food and experimental diets

To formulate the experimental diets, analysis of corn, soybean meal, corn starch and soy oil were performed (Table 1). The crude protein (CP) of the food was determined by the standard method [13], and the percentage of nitrogen was obtained by the Kjeldahl method after acid digestion. Gross energy (GE) content was measured by combustion in a Parr bomb calorimeter (model: ECO-CAL2K). The analysis of the total amino acid (TAA) content of corn and soybean meal was performed by high performance liquid chromatography (HPLC). To obtain the digestible essential amino acids (DAA), the values of TAA were converted using the digestibility coefficients of corn and soybean meal proposed by [14] for tambaqui.

Table 1
Composition of the main ingredients used in experimental diets (on the natural matter bases)

Nutrientes	Corn		Soybean meal		Corn starch		Soy oil	
Dry matter	93.90		95.20		88.90		99.61	
Crude protein (%)	7.62		46.65		—		—	
Gross energy (kcal kg^{-1})	3941.00		4092.00		3821.00		9333.00	
Amino acids (%)	TAA[1]	DAA[2]	TAA[1]	DAA[2]	TAA[1]	DAA[2]	TAA[1]	DAA[2]
Lysine	0.27	0.25	2.76	2.61	—	—	—	—
Methionine	0.17	0.15	0.59	0.55	—	—	—	—
Threonine	0.26	0.25	1.71	1.68	—	—	—	—
Tryptophan	0.02	0.02	0.78	0.72	—	—	—	—
Valine	0.39	0.38	2.28	2.20	—	—	—	—
Histidine	0.24	0.22	1.25	1.20	—	—	—	—
Leucine	0.75	0.74	3.41	3.31	—	—	—	—
Isoleucine	0.27	0.26	2.20	2.13	—	—	—	—
Arginine	0.42	0.41	3.42	3.39	—	—	—	—
Phenylalanine	0.33	0.32	2.37	2.33	—	—	—	—
Cystine	0.13	0.12	0.68	0.61	—	—	—	—

[1]Total amino acids;

[2]Digestible amino acids.

The experimental diets were formulated using the “dilution” technique [15]. Initially, a reference diet (RD) was formulated containing 18.06% crude protein (CP) and 0.901% digestible lysine, based on corn and soybean meal (Table 2). Subsequently, this was diluted with another protein-free diets (PFD) based on corn starch, containing the same levels of energy, vitamins, and minerals, enabling levels of 0.225, 0.450, 0.675 and 0.901% digestible lysine (Table 2). After preparing the experimental diets, the total amino acids were analyzed and their contents confirmed with the formulation targets.

To confirm that lysine was the first limiting nutrient in experimental diets, a fifth treatment (CD: control diet) was added, with five replications, to which synthetic lysine was added at the first level (0.225%) until reaching the second level (0.450%). Thus, it is possible to confirm that the response obtained in the study occurred due to the variation in lysine and not due to the variation between crude proteins (4.51–18.06%) of the experimental diets.

In experimental diets, the ratios of methionine plus cystine / lysine, threonine / lysine and tryptophan / lysine were maintained above the ideal protein ratio proposed by [16–18], respectively, for tambaqui (Table 2), in order to prevent another amino acid from becoming limited for each level of digestible lysine evaluated.

Table 2
Diets formulated by dilution method with increasing digestible lysine levels for tambaqui with different weights

Ingredients (%)	Digestible lysine levels (%)					
	PFD	0.225	0.450	0.675	0.901 (RD)	CD
Soybean	0.00	7.13	14.26	21.39	28.53	7.13
Corn	0.00	15.60	31.20	46.80	62.40	15.60
Corn starch	81.80	61.35	40.90	20.45	0.00	61.35
Soy oil	9.85	8.36	6.88	5.39	3.91	8.36
Cellulose	2.90	2.17	1.45	0.72	0.00	2.17
L-lysine HCl (78.4%)	0.00	0.00	0.00	0.00	0.00	0.29
DL-Methionine (99%)	0.00	0.03	0.06	0.09	0.11	0.03
L-Threonine (98.5%)	0.00	0.03	0.05	0.08	0.11	0.03
Calcitic limestone	1.05	1.10	1.16	1.21	1.26	1.10
Dicalcium phosphate	3.28	3.11	2.94	2.77	2.60	3.11
Premix Vit. and Min.[1]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin C[2]	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.55	0.54	0.53	0.52	0.51	0.54
Antioxidant (BHT)	0.02	0.02	0.02	0.02	0.02	0.02
Diets	Dilution (%)					
PFD	100.00	75.00	50.00	25.00	—	75.00
RD	0.00	25.00	50.00	75.00	100.00	25.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional Composition (%)						
Dry matter	89.40	90.73	91.71	91.90	92.26	90.86
Crude protein	0.00	4.51	9.03	13.54	18.06	4.51
Gross energy (kcal kg^{-1})	4044.88	4030.98	4018.01	4004.11	3991.51	4030.98
Digestible energy (kcal kg^{-1})[3]	3000.00	3000.00	3000.00	3000.00	3000.00	3000.00
Crude fiber	2.81	2.81	2.81	2.81	2.861	2.81

Ingredients (%)	Digestible lysine levels (%)					
	PFD	0.225	0.450	0.675	0.901 (RD)	CD
Available P[4]	0.60	0.60	0.60	0.60	0.60	0.60
Total Ca[4]	1.20	1.20	1.20	1.20	1.20	1.20
Total lysine	0.00	0.239	0.478	0.717	0.956	0.466
Digestible lysine[5]	0.00	0.225	0.450	0.675	0.901	0.450
Total methionine + cystine	0.00	0.167	0.334	0.501	0.658	0.167
Digestible methionine + cystine[5]	0.00	0.153	0.306	0.466	0.612	0.153
Total threonine	0.00	0.192	0.374	0.566	0.758	0.192
Digestible threonine[5]	0.00	0.185	0.369	0.561	0.739	0.185
Total tryptophan	0.00	0.059	0.117	0.176	0.235	0.059
Digestible tryptophan[5]	0.00	0.043	0.086	0.129	0.173	0.043
Relation based on the ideal protein concept						
Methionine + Cys/digestible lysine	0.00	68	68	68	68	34
Threonine/digestible lysine	0.00	82	82	82	82	41
Tryptophan/digestible lysine	0.00	24	24	24	24	10

[1]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.

[2]Vit. C: Calcium L-Ascorbic acid 2-monophosphate, 42% of active ingredient.

[3]Values calculated based on the digestibility coefficients determined by [19, 20].

[4]Values calculated based by [21].

[5]Values calculated based on the digestibility coefficients determined by [14].

For the extrusion process of experimental diets, the ingredients of PDF and RD were finely ground (Trf 60, Trapp®) and weighed individually and mixed (Horizontal Mixer 300 Kg, Branorte®). To obtain intermediate diets (0.225, 0.450 and 0.675) mixtures were performed by dilution (Table 2). Subsequently, they were pelleted in equipment with a 4–5 mm sieve (Extruder model MX 40, Inbramaq®, Laboratório de Nutrição e Alimentação de Organismos Aquáticos do Maranhão, Chapadinha, in Brazil).

The experimental diets were provided daily in four meals (08:00, 11:00, 14:00 and 17:00 h), until apparent satiation. At each meal, they were supplied in small quantities with successive passes, allowing maximum intake.

Fish, rearing conditions and experimental design

Three experimental trials, using different tambaqui body weights, were conducted at the Laboratory of Nutrition and Food of Aquatic Organisms of Maranhão, located at the Center for Agricultural and Environmental Sciences of the Federal University of Maranhão, Chapadinha, in Brazil (03°44'33"S, 43°21'21"W; altitude 105 m). The experimental procedures were approved by Animal Use Ethics Committee of the Federal University of Maranhão (Protocol Nº 23115.005476/2017-00).

Each experimental trial lasted 20 days, and five days before the beginning of the experimental period, the fish were acclimated to the experimental conditions.

In each experiment, 100 tambaqui with different initial mean weights of 121 ± 1.35; 235 ± 1.23 and 596 ± 47.57 g were used, distributed in a completely randomized design, consisting of four treatments (0.225; 0.450; 0.675 and 0.901% of digestible lysine in the diets) and five replicates, with 5 fish per experimental unit. A fifth treatment (CD: control diet) was added, with five replications, to confirm that the response obtained was due to the variation of digestible lysine and not to the variation between crude proteins (4.51 - 18.06%).

During the experimental period, the fish were kept in polyethylene boxes, with a capacity of 1000 L each, equipped with individual water supplies, drainage systems, and aeration systems, the water supply for the boxes was derived from an artesian well, with flow rate of 40 L h^{-1} per box.

Water quality parameters

The water temperature was measured daily at 7:00 and 16:00 h with a mercury bulb thermometer graduated from 0 to 50 °C. Controls for pH and the content of dissolved oxygen and ammonia in the water were measured every three days using a pH meter (HI 8424, Hanna®), oximeter (HI 9146, Hanna®) and commercial kit (Arcor®) for toxic ammonia test, respectively.

Carcass preparation and analysis

The comparative slaughter technique was used to study maintenance requirements and efficiency of utilization of lysine of tambaqui. At the beginning of each experiment, 15 fish from the same population used in each experiment were euthanized (benzocaine 100 mg L^{-1}, after fasting for 24 h) and frozen in an ultra-freezer with temperature -70 °C for later determination of the initial body protein content. At the end of the study, after a 24 h fast, all fish in each experimental unit were weighed, euthanized (benzocaine

100 mg L^{-1}) and frozen in an ultra-freezer with temperature -70 ° C to determine the final body protein content.

Subsequently, the initial and final samples were lyophilized for 72 h (-50 °C, -80 kPa) in L108 freeze-drying equipment (LIOTOP®) and then processed in an analytical mill (IKA® A11 basic).

The crude protein of whole body tambaqui was determined by the standard method [13], and the percentage of nitrogen was obtained by the Kjeldahl method after acid digestion.

The analysis of lysine content of whole body of tambaqui was performed using high performance liquid chromatography (HPLC) at the CBO laboratory (Valinhos, Brazil).

Evaluated variables

The variables evaluated were feed intake (FI; g $fish^{-1}$ day^{-1}), digestible lysine intake (DLI; mg $kg^{-0.7}$ day^{-1}), final weight (FW; g), weight gain (WG; %), body protein retention (BPR; mg $kg^{-0.7}$ day^{-1}), and body lysine retention (BLR; mg $kg^{-0.7}$ day^{-1}).

For the calculate weight gain (%) the following formula was used:

- WG (%) = (Final Weight (g) - Initial Weight (g)) / Initial Weight (g) × 100.

For the calculate body protein retention (BPR) were used analyzes of the initial body protein content (initial fish samples) and analyzes of the final body protein content, calculated using the following formula:

- BPR (mg $kg^{-0.7}$ day^{-1}) = (final body protein (%) × final weight (g)) - (initial body protein (%) × initial weight (g) / experimental period / final weight ($kg^{-0.7}$).

For the calculate body lysine retention (BLR) (mg $kg^{-0.7}day^{-1}$), lysine analysis was performed, which corresponds to 7.41, 6.55 and 6.35% of whole body protein of tambaqui, for body weight 121, 235 and 596, respectively, and calculated by the following formula:

- BLR (mg $kg^{-0.7}$ day^{-1}) = {[(body protein retention (%) × protein lysine content (%)) / 100] / final weight ($kg^{-0.7}$)}.

Maintenance lysine requirements were determination based on digestible lysine intake (mg $kg^{-0.7}day^{-1}$) that provided zero protein retention (mg $kg^{-0.7}day^{-1}$).The efficiencies for using lysine for growth were obtained by the slope coefficient of the line between the digestible lysine consumption (mg $kg^{-0.7}$ day^{-1}) as a function of body lysine retention (mg $kg^{-0.7}day^{-1}$) [8, 12].

Statistical analysis

The data obtained, in each experimental trial, were submitted to an analysis of variance. For those with significant effects as a function of digestible lysine levels, the means of each treatment were compared using the Duncan test. Linear regression analysis of protein and body lysine deposition as a function of digestible lysine consumption was also performed.

Additionally, to compare the responses of the different body weights, the body protein retention (BPR) and body lysine retention (BLR) data obtained in the experimental tests were compared using the parallelism test [22] between equations two by two. The weight was a categorical variable and DLI (mg $kg^{-0.7}$ day^{-1}) was a covariate in the model:

- BPR / BLRij = β_0 + BWi + β_1*DLIij + $\Sigma i \beta_2 i$ (BW* DLI)ij + e_{ij};

Where BPR / BLRij = body protein retention / body lysine retention corresponding to the j body weight observation i;

BWi = effect of body weight;

DLIij = digestible lysine intake;

β0, β1, and β2i = regression parameters;

(BW*DLI)ij = effect of the interaction between the categorical variable and covariate;

e_{ij} = random error associated with observation j of body weight i.

In this case, the hypotheses tested were:

a) H0: Gi = 0 for all i, there is no effect on body weight;

H1: Gi ≠ 0 for at least one i; there is an effect of body weight;

b) H0: β1 = 0, general slope is zero, non-significant regression;

H1: β1 ≠ 0, the overall slope differs from zero, significant regression;

c) H0: β2i = 0, the inclination of body weight i does not differ from the average inclination.

All analyses were performed with the aid of SAS software (Statistical Analysis System, version 9.0) considering a significance level of up to 5%.

Results

Water quality

For body weights of 121, 235 and 596 g, the minimum and maximum water temperature values observed during the experimental tests were 25.3 ± 0.6, 25.8 ± 0.6, and 25.6 ± 0.3°C in the morning (8:00 h) and 27.1 ± 0.2, 27.5 ± 0.2, and 27.5 ± 0.1°C in the afternoon (17:00 h), respectively. The pH values were 7.02 ± 0.3, 7.03 ± 0.3, and 7.01 ± 0.2 and dissolved oxygen levels were 10.80 ± 0.7, 10.90 ± 0.7, and 10.50 ± 0.4 mg L^{-1}, respectively. Total ammonia was ≤ 1.00 ppm.

Performance

There was no mortality during the experimental periods, nor were any external pathological signs observed, even in animals fed with at the low-lysine level.

The animals submitted to the control diet (CD) were superior to those submitted to a diet with the first level of lysine (0.225%) for all variables analyzed in the experiments (different body weights), with the exception of FI for body weights from 235 to 596 g. The lowest weight gains were observed in fish fed with the first level of digestible lysine (0.225%).

In addition, the mean final weight and weight gain of tambaqui were influenced ($P < 0.05$) by the increasing digestible lysine levels in the diets for all body weights evaluated (Table 3), with the highest final weight and weight gain obtained by tambaqui fed the highest digestible lysine level evaluated (0.901%). Regarding the body weights evaluated, the greatest weight gain was obtained by the 121 g animals, with a value of 26.06%.

Table 3
Initial weight, final weight and weight variation of tambaqui fed different diets

Body weight (g)	Digestible lysine levels (%)	Weight (g)		Weight gain (%)
		Initial	Final	
121	0.225	121.68 ± 0.69	132.79 ± 1.57 a	9.12 ± 0.83 a
	0.450	120.83 ± 0.28	146.24 ± 1.63 bc	21.03 ± 1.42b c
	0.675	121.03 ± 0.50	150.51 ± 4.05 bc	24.31 ± 2.86 c
	0.901	121.27 ± 0.67	152.86 ± 2.42 c	26.06 ± 2.08 c
	CD[1]	121.38 ± 0.91	143.91 ± 1.27 b	18.57 ± 0.74 b
235	0.225	234.20 ± 0.53	231.68 ± 1.42 a	− 1.08 ± 0.47 a
	0.450	235.00 ± 0.38	261.52 ± 3.65 bc	11.28 ± 1.46b c
	0.675	235.34 ± 0.73	264.23 ± 2.84 bc	12.87 ± 1.24b c
	0.901	234.23 ± 0.71	266.54 ± 4.57 c	13.30 ± 1.76 c
	CD[1]	234.93 ± 0.39	254.65 ± 2.48 b	8.40 ± 1.12 b
596	0.225	584.72 ± 7.88	607.12 ± 8.73 a	3.86 ± 1.34 a
	0.450	604.78 ± 24.57	659.00 ± 28.10 ab	9.04 ± 2.28 abc
	0.675	594.28 ± 25.40	653.10 ± 24.27 ab	10.03 ± 1.45 bc
	0.901	599.94 ± 27.98	678.38 ± 26.78b	13.36 ± 2.77c
	CD[1]	600.17 ± 5.97	640.70 ± 7.17ab	6.77 ± 1.07ab

[1] Control diet;

The values presented correspond to the mean (± standard error) of 25 fish for each body weight evaluated in each treatment;

Means with different superscripts in the same column, for each experiment, differed by the Duncan test ($P < 0.05$).

Digestible lysine levels influenced feed intake ($P < 0.05$) only for fish weighing 121 g, where there was an increase in diet intake up to 0.450% of digestible lysine. As expected, digestible lysine intake increased in all body weights groups evaluated ($P < 0.05$) because the diets presented increasing digestible lysine levels.

Table 4
Feed intake (FI) and digestible lysine (DLI), protein retention (BPR) and lysine (BLR) of tambaqui

Body weight (g)	Digestible lysine levels (%)	FI	DLI	BPR	BLR
		g fish^{-1} day^{-1}	mg kg$^{-0.7}$ day^{-1}	mg kg$^{-0.7}$ day^{-1}	mg kg$^{-0.7}$
121	0.225	6.74 ± 0.35 c	62.06 ± 3.00 a	-72.53 ± 9.55 a	-5.37 ± 0.71 a
	0.450	6.49 ± 0.28 c	111.96 ± 3.75 b	168.36 ± 19.40 b	12.48 ± 1.44 b
	0.675	5.07 ± 0.28 a	128.56 ± 1.41 c	338.14 ± 15.08 c	25.06 ± 1.12 c
	0.901	4.41 ± 0.26 a	147.62 ± 3.62 d	484.40 ± 27.24 d	35.89 ± 2.02 d
	CD¹	6.47 ± 0.16 c	115.20 ± 0.76 b	172.84 ± 10.83 b	12.81 ± 0.80 b
235	0.225	5.69 ± 0.18	35.61 ± 0.98 a	-361.92 ± 13.71 a	− 26.82 ± 1.02 a
	0.450	5.73 ± 0.21	65.69 ± 1.96 c	-200.28 ± 35.78 b	− 14.84 ± 2.65 b
	0.675	5.36 ± 0.20	91.34 ± 2.61 d	129.67 ± 31.39 c	9.61 ± 2.33 c
	0.901	5.16 ± 0.23	116.84 ± 3.84 e	97.98 ± 43.82 d	20.43 ± 3.25 d
	CD¹	4.75 ± 0.09	56.25 ± 1.08 b	-248.84 ± 19.48 b	-18.44 ± 1.44 b
596	0.225	15.62 ± 1.51	49.86 ± 4.91 a	-330.91 ± 28.02 a	-24.52 ± 2.08 a
	0.450	19.16 ± 1.60	115.08 ± 8.72 b	87.21 ± 45.90 b	6.46 ± 3.40 b
	0.675	15.45 ± 0.82	137.55 ± 7.18 c	136.39 ± 45.40 b	10.11 ± 3.36 b
	0.901	14.64 ± 1.52	176.65 ± 7.31 d	385.31 ± 38.20 c	28.55 ± 2.83 c
	CD¹	19.98 ± 1.68	103.69 ± 1.198 b	60.66 ± 40.77 b	4.50 ± 0.35 b

Body weight (g)	Digestible lysine levels (%)	FI	DLI	BPR	BLR
		g fish^{-1} day^{-1}	mg kg$^{-0.7}$ day^{-1}	mg kg$^{-0.7}$ day^{-1}	mg kg$^{-0.7}$ day^{-1}

[1] Control diet;

The values presented correspond to the mean (± standard error) of 25 fish for each body weight evaluated in each treatment;

Means with different letters in the same column, for each experiment, differed by the Duncan test ($P < 0.05$).

Maintenance requirements and efficiency of utilization of lysine

Linear regressions performed between body protein retention and body lysine retention as a function of digestible lysine consumption had a linear effect ($P < 0.05$) on all body weights evaluated.

There was no difference ($P > 0.05$) between the parameters of the equations obtained by the parallelism test, indicating the need for only one equation to describe the responses for body weights of 121 and 235 g. However, there was a difference ($P < 0.05$) between the parameters of the equation obtained for the body weight of 596 g and the other body weights (121 and 235 g), with the need to adjust the specific equation for tambaqui with body weight of 596 g. The requirement for digestible lysine for maintenance of tambaqui with body weights from 121 to 235 g was 82.03 mg kg$^{-0.7}$day^{-1}, which was obtained through the equation Y = 7.361 (± 0.292) x − 603.785. (± 29.620) ($P < 0.0001$; $r^2 = 0.9437$) and for the body weight of 596 g was 106.85 mg kg$^{-0.7}$day^{-1} obtained by the equation Y = 5.373 (± 0.367) x − 574.104 (± 47.403) ($P < 0.0001$; $r^2 = 0.9225$). Both results were based on protein retention equal to zero, as shown in Figs. 1a and 1b, respectively, indicating an increase in lysine requirement for maintenance as tambaqui body weight increased.

The efficiency of lysine utilization with weight gain was obtained through the slope of the coefficient of the line, which was 55% for body weight from 121 to 235 g and 40% for the body weight of 596 g, as obtained through the equations Y = 0, 545 (± 0.022) x − 447 (± 2.195) ($P < 0.0001$; $r^2 = 0.9437$) and Y = 0.3982 (± 0.027) x − 42.543 (± 3.513) ($P < 0.0001$; $r^2 = 0.9225$), respectively. The linear regressions between digestible lysine consumption and body lysine retention are shown in Figs. 2a and 2b. Lysine utilization efficiency decreased with increasing tambaqui body weight.

Discussion

In the comparison by means test, the animals submitted to the CD differed from all levels and presented similar responses to those fed with a diet containing 0.450% digestible lysine in all analyzed variables (Tables 3 and 4), with the exception of FI for body weights from 235 to 596 g, confirming that lysine was the first amino acid limiting in experimental diets.

The highest weight gain was obtained for tambaqui fed 0.901% lysine in the diet, for all weight ranges evaluated, evidencing the essentiality of this amino acid for the species.

In addition, digestible lysine intake provided sufficient intervals to obtain protein retention values and negative, near zero, and positive body lysine, allowing estimation of maintenance requirements without extrapolation.

Normally, in trials to determine the amino acid requirement for maintenance, animals are expected to lose weight because of the use of diets extremely deficient in the amino acid under study, especially at lower levels [8, 23]. In the present study, this effect was observed only for tambaqui with a 235 g body weight, fed 0.255% level of digestible lysine in the diet. Similar effects were observed by [8] in studies to determine the requirement of maintenance of lysine and methionine for Nile tilapia (*Oreochromis niloticus*).

However, weight gain is not a good parameter for determining amino acid requirements for maintenance because fish gain weight through protein, fat, and mineral deposition [24], and there is no demand for amino acids to maintain lipid and mineral reserves. Thus, protein (nitrogen) or amino acid deposition under study is a more accurate indicator for achieving amino acid requirements for maintenance than weight gain [7].

Studies of requirements maintenance of digestible lysine for tambaqui are lacking in the literature. The values obtained in the present study, for body weights from 121 to 235 g (82.03 mg $kg^{-0.7}$ day^{-1}) and 596 g (106.85 mg $kg^{-0.7}$ day^{-1}), are above the recommendations described for other fish species already studied.

In studies with juvenile and adult Nile tilapia, tropical and omnivorous species, with initial mean weights of 20.70 and 165 g, values of 16.90 and 68 mg $kg^{-0.7}$ day^{-1} of digestible lysine were obtained for maintenance, respectively [8]. For Atlantic salmon (*Salmo salar*), with initial mean weights of 62.80 and 95.70 g, the values obtained were of 21.70 and 18 mg $kg^{-0.7}$ day^{-1} of digestible lysine, respectively [9, 25]. Fish species may present differences in protein turnover [26], as well as differences in the metabolism of amino acid utilization for maintenance [3].

In addition, breeding programs with Nile tilapia and Atlantic salmon seek to maximize weight gain over a shorter period and improve feed efficiency [27, 28], may have reduced the requirement of amino acids for the maintenance of these species compared to that tambaqui. Thus, this explains the higher requirement for maintenance lysine for tambaqui observed in the present study.

The requirement for maintenance lysine was positively correlated with tambaqui body weight, i.e., there was an increased requirement for maintenance lysine as the fish grew. This effect was also reported by [8], who observed that adult tilapia (165 g) had a higher demand for maintenance lysine compared to that of juveniles (20.7 g), with values of 68.80 and 16.90 mg $kg^{-0.7}$ day^{-1}, respectively.

In young animals, amino acid requirements for body maintenance represent a small fraction of the total requirement, but this situation is reversed as the animal increases in size [29]. In the early stages of animal life, protein deposition occurs at a high rate compared to that of adult animals; therefore, caution is needed when determining the requirement for amino acids for maintenance early in life because the relationship between amino acids destined for maintenance and growth change rapidly with the growth rate of the animal [10].

The length of the experimental period is another factor that interferes with the variation of the maintenance values of an amino acid. Very long trial period (greater than 30 days) metabolic adaptation and changes in maintenance conditions can occur [10]. In this study, each experiment lasted 20 days, being enough to guarantee changes in the deposition of body protein content (retained nitrogen) and to determine the requirement to maintenance of lysine for tambaqui.

The efficiency of lysine utilization for fish has received great attention in the formulation of diets, especially when formulated with a protein of plant origin. The lysine utilization efficiency obtained for tambaqui was 55% for body weight 121 to 235 g and 40% for body weight 596 g, which indicated a negative relationship with tambaqui body weight. Thus, there was a reduction in the efficiency of use with the increasing body weight of tambaqui, this demonstrated the direct influence of body weight on this variable.

The results obtained in this study indicated a reduction in amino acid utilization efficiency for body protein deposition and a simultaneous increase in amino acid utilization for replacement of inevitable protein losses (maintenance) as tambaqui grows. Moreover, in young animals, the growth rate and protein turnover are higher compared to that of older animals [10].

The results obtained in the present study corroborated those reported by [8] for Nile tilapia, where the efficiency of lysine utilization was 72 and 52% for animals with initial mean weight of 20.70 and 165 g, respectively. [12], with tilapia of different body weights fed the same diet, also observed a reduction in the efficiency of lysine utilization with increasing body weight, with values of 68, 63 and 47% for the mean body weights of 10, 58 and 248 g, respectively.

Lysine utilization efficiency has also been determined for other fish species. For Atlantic salmon ranging from 62 to 85% [9, 25 and 30], rainbow trout (*Oncorhynchus mykiss*) with variation of 63 to 75% [31–33] and Atlantic cod (*Gadus morhua*) ranging from 71 to 85% [7].

The low efficiency of lysine utilization by tambaqui (55 and 40%), compared to that of other species, may be related to its slow growth. Slow-growing fish probably use a higher proportion of dietary amino acids for body tissue maintenance than do fast-growing fish, which use amino acids for body protein deposition. Thus, this increases the requirement of lysine for maintenance, which is an amino acid used almost exclusively in protein synthesis.

However, because of the limited amount of information available in the literature on the efficiency of lysine utilization and that of other amino acids by different species, it is difficult to properly assess the influence of species on amino acid utilization efficiency until there have been a sufficient number of species studied.

In addition to the differences among species and body weights, other factors may influence the efficiency of lysine utilization, such as dietary lysine concentration [25]. Low values of efficiency of utilization of lysine are obtained when using high concentrations of lysine in experimental diets. Probably due to the reduction in the rate of lysine absorption [34] or the need for excretion of excess amino acids in diets, which requires energy expense.

The source of protein used to formulate experimental diets also influences the efficiency of lysine utilization [31, 33], when a source of low biological value protein is used and ingested by fish, there may be a reduction in the body protein website due to the limitation of essential amino acids.

The values obtained in the present study for lysine maintenance requirements and utilization efficiency for tambaqui are crucial for understanding the basal metabolic needs of lysine, allowing the production of tambaqui at a minimal cost. Additionally, they will serve as beginning parameters for the elaboration of an effective factorial model, able to predict the lysine requirements for tambaqui under several environmental and/or physiological conditions, and will thereby serve as a guide for further studies.

Conclusions

Body weight is a variable that influences the maintenance requirement and the utilization efficiency of lysine, and must be taken into account in mathematical models that estimate the lysine requirement by the factorial method.

The requirement for digestible lysine for tambaqui maintenance was 82.03 and 106.85 mg $kg^{0.7}$ day^{-1} for body weights 121 to 235 and 596 g, with 55 and 40% utilization efficiencies, respectively.

Declarations

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Availability of data and materials

The datasets produced and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RSM, JCS, TJRS conducted the animal trial, RSM, FBR, MADB and JCS analyzed and interpreted the data and wrote the paper; RSM, JCS and DCNN provided their assistance for chemical analysis and revised the manuscript; FBR, MADB and JCS supervised the design of the study and data analysis, and revised the manuscript. All authors critically reviewed the manuscript for intellectual content and gave final approval for the version to be published.

Ethics approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Dairiki, J.K., Silva, T.B.A., 2011. Revisão de literatura: exigências nutricionais do tambaqui-compilação de trabalhos, formulação de ração adequada e desafios futuros. Embrapa Amazônia Ocidental-Documentos (INFOTECA-E).
2. Woynárovich A, Van Anrooy R. Field guide to the culture of tambaqui (*Colossoma macropomum*, Cuvier, 1816). FAO Fisheries and Aquaculture Technical Paper, Roma. 2019; 1-121.
3. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, D.C., USA; 2011.
4. Abimorad EG, Favero GC, Squassoni GH, Carneiro D. Dietary digestible lysine requirement and essential amino acid to lysine ratio for pacu (*Piaractus mesopotamicus*). Aquac Nutr. 2010;16:370-7. https://doi.org/10.1111/j.1365-2095.2009.00674.x.
5. Michelato M, de Oliveira Vidal LV, Xavier TO, de Moura LB, de Almeida FLA, Pedrosa VB, Furuya WM. Dietary lysine requirement to enhance muscle development and fillet yield of finishing Nile tilapia. 2016; 457:124-30. https://doi.org/10.1016/j.aquaculture.2016.02.022.

6. Silva JC, Bomfim MAD, Lanna EAT, Ribeiro FB, Siqueira JC, de Sousa, TJR, Nascimento DCN. Lysine requirement for tambaqui juveniles. Semina: Ciênc Agrár. 2018;39:2157-68. http://dx.doi.org/10.5433/1679-0359.2018v39n5p2157.
7. Grisdale-Helland B, Hatlen B, Mundheim H, Helland SJ. Dietary lysine requirement and efficiency of lysine utilization for growth of Atlantic cod. 2011a; 315:260-8. https://doi.org/10.1016/j.aquaculture.2011.02.015.
8. He JY, Tian LX, Lemme A, Gao W, Yang HJ, Niu J, Liang GY, Chen PF, Liu YJ. Methionine and lysine requirements for maintenance and efficiency of utilization for growth of two sizes of tilapia (*Oreochromis niloticus*). Aquac Nutr. 2013; 19:629-40. https://doi.org/10.1111/anu.12012.
9. Helland SJ, Hatlen B, Grisdale-Helland B. Energy, protein and amino acid requirements for maintenance and efficiency of utilization for growth of Atlantic salmon post-salmons determined using increasing ration levels. 2010; 305:150-58. https://doi.org/10.1016/j.aquaculture.2010.04.013.
10. Abboudi T, Mambrini M, Ooghe W, Larondelle Y, Rollin X. Protein and lysine requirements for maintenance and for tissue accretion in Atlantic salmon (*Salmo salar*) fry. Aquaculture. 2006;261:369-83. https://doi.org/10.1016/j.aquaculture.2006.07.041.
11. Hauler RC, Carter CG. Reevaluation of the quantitative dietary lysine requirements of fish. Rev Fish Sci. 2001; 9:133-63. https://doi.org/10.1080/20016491101735.
12. Hua K, Suwendi E, Bureau DP; Effect of body weight on lysine utilization efficiency in Nile Tilapia (*Oreochromis niloticus*). 2019;505:47-53. https://doi.org/10.1016/j.aquaculture.2019.02.030.
13. Official Method of Analysis. 21th Edn., Association of Official Analytical Chemists Inc., Rockville, USA; 2019.
14. Nascimento TMTD, Buzollo H, Sandre, LCGD, Neira, LM, Abimorad EG, Carneiro DJ. Apparent digestibility coefficients for amino acids of feed ingredients in tambaqui (*Colossoma macropomum*) diets. R Bras Zootec. 2020;49. https://doi.org/10.37496/rbz4920190032.
15. Fisher C, Morris TR. The determination of the methionine requirements of laying pullets by a diet dilution technique. Brit Poult Sci. 1970;11:67-82. https://doi.org/10.1080/00071667008415793.
16. Souza FO, Bomfim MAD, Ribeiro FB, Lanna EAT, Sousa TJRD, Costa DDCD. Methionine plus cystine to lysine ratio in diets for tambaqui juveniles. Rev Caatinga. 2019; 32: 243-50. http://dx.doi.org/10.1590/1983-21252019v32n124rc.
17. Firmo, DS, Bomfim MAD, Ribeiro FB, Siqueira JC, Lanna EAT, Takishita SS, Porto NG. Threonine to lysine ratio in diets of tambaqui juveniles (*Colossoma macropomum*). Semina: Ciênc Agrár. 2018; 39:2169-80. https://doi: 10.5433/1679-0359.2018v39n5p2169.
18. Bomfim MAD, Marchão RS, Ribeiro FB, Siqueira JCD, Silva LR, Takishita SS. Digestible tryptophan requirement for tambaqui (*Colossoma macropomum*) fingerlings. Rev Ciênc Agron. 2020;51. http://doi: 10.5935/1806-6690.20200025.
19. Furuya, W.M.Tabelas brasileiras para a nutrição de tilápias. 2010;100.
20. Buzollo H, Nascimento TMTD, Sandre LCGD, Neira LM, Jomori RK, Carneiro DJ. Apparent digestibility coefficients of feedstuff used in tambaqui diets. Bol Inst Pesca. 2018;44. http://doi: 10.20950/1678-

2305.2018.316.

21. Rostagno HS, Albino, LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, Ferreira AS, Barreto SLT, Euclides RF. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3. ed. Viçosa, MG: UFV - Departamento de Zootecnia, 2011.
22. Kaps M, Lamberson WR. Biostatistics for Animal Science. Wallingford: CABI Publishing. 2004;365-83.
23. Siqueira JC, Sakomura NK, Rostagno HS, Bonato MA, Pinheiro SRF, Nascimento DCN. Exigência de lisina para mantença determinada com galos de diferentes genótipos. R Bras Zootec. 2011;40:812-20. http://dx.doi.org/10.1590/S1516-35982011000400015.
24. Bureau DP, Azevedo PA, Tapia-Salazar M, Cuzon G. Pattern and cost of growth and nutrient deposition in fish and shrimp: Potential implications and applications. Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola. 2000;19:111-40.
25. Grisdale-Helland B, Gatlin DM, Corrent E, Helland SJ. The minimum dietary lysine requirement, maintenance requirement and efficiency of lysine utilization for growth of Atlantic salmon smolts. Aquacult Res. 2011b; 42:1509-29. https://doi.org/10.1111/j.1365-2109.2010.02743.x.
26. Lupatsch I, Kissil GW, Sklan D. Comparison of energy and protein efficiency among three fish species gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aeneus*): energy expenditure for protein and lipid deposition. 2003;225:175-89. https://doi.org/10.1016/S0044-8486(03)00288-6.
27. Gjedrem T. Genetic improvement for the development of efficient global aquaculture: a personal opinion review. Aquaculture. 2012;344:12-22. https://doi.org/10.1016/j.aquaculture.2012.03.003.
28. Gupta MV, Acosta BO. From drawing board to dining table: the success story of the GIFT project. NAGA, WorldFish Center Quarterly. 2004;27:4-14.
29. Bonato MA, Sakomura NK, Siqueria JC, Fernandes JBK, Gous RM. Maintenance requirements for methionine and cysteine, and threonine for poultry. S Afr J Anim Sci. 2011;41:209-22. http://dx.doi.org/10.4314/sajas.v41i3.3.
30. Carter CG, Hauler RC. Effect of high digestible protein to digestible energy ratio on lysine utilization by Atlantic salmon, *Salmo salar*, parr. Aquaculture. 2011;311:209-14. https://doi.org/10.1016/j.aquaculture.2010.11.045.
31. Nang Thu TT, Parkouda C, de Saeger S, Larondelle Y, Rollin X. Comparison of the lysine utilization efficiency in different plant protein sources supplemented with L-lysine.HCl in rainbow trout (*Oncorhynchus mykiss*) fry. 2007; 272:477-88. https://doi.org/10.1016/j.aquaculture.2007.06.032.
32. Nang Thu TT, Parkouda C, de Saeger S, Larondelle Y, Rollin X. Protein level does not affect lysine utilization efficiency at marginal lysine intake in growing rainbow trout (*Oncorhynchus mykiss*) fry. 2009;288:312-20. https://doi.org/10.1016/j.aquaculture.2008.12.007.
33. Rodehutscord M, Borchert F, Gregus Z, Pack M, Pfeffer E. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. Aquaculture. 2000;187:163-76. https://doi.org/10.1016/S0044-8486(99)00388-9.

34. Peres, H, Oliva-Teles, A. Lysine requirement and efficiency of lysine utilization in turbot (*Scophthalmus maximus*) juveniles. Aquaculture. 2008:275:283-90. https://doi.org/10.1016/j.aquaculture.2007.12.015.

Figures

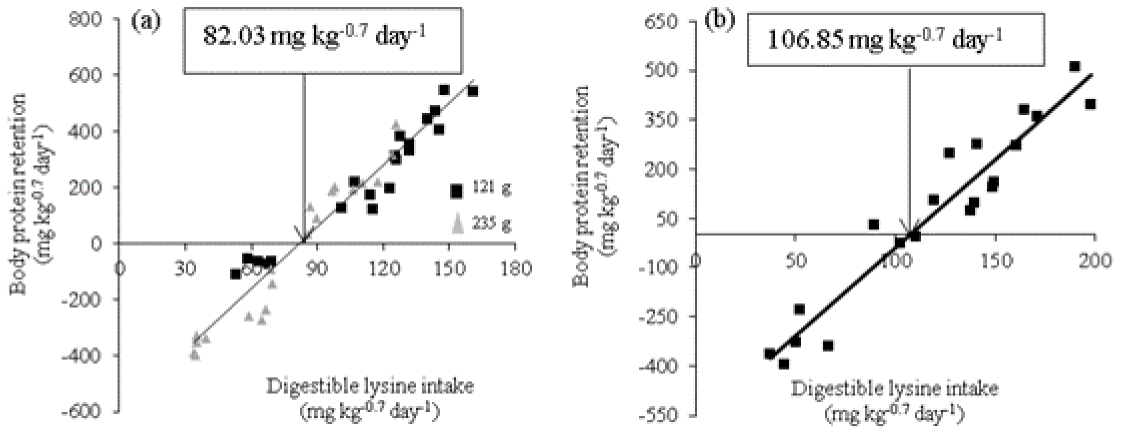

Figure 1 Graphical representation of linear regression of body protein retention as a function of digestible lysine consumption for tambaqui with body weight 121 to 235 (a) and 596 g (b).

Figure 1

Caption found in figure.

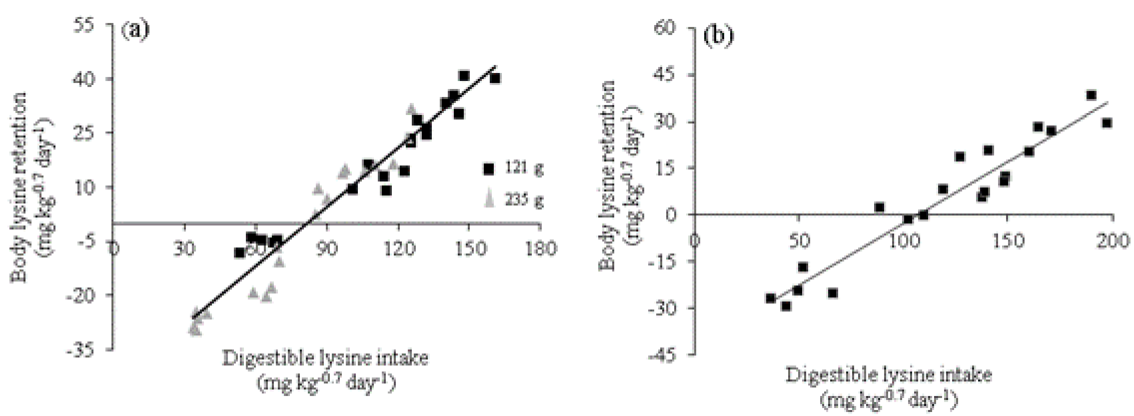

Figure 2 Graphical representation of linear regression of body lysine retention as a function of digestible lysine consumption for tambaqui with body weight 121 to 235 (a) and 596 g (b).

Figure 2

Caption found in figure.