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Dietary valine requirement of tambaqui (*Colossoma macropomum*) with different body weights

Exigência de valina dietética para tambaqui (*Colossoma macropomum*) com diferente peso corporal

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ABSTRACT

Tambaqui (*Colossoma macropomum*) is one of the highly produced freshwater fish in Brazilian fish farming. However, its production efficiency associated with the refinement of diet formulations is limited due to a lack of information on nutritional requirements for certain essential amino acids such as valine. This study was designed to estimate the valine requirement of tambaqui having different body weights. A total of 720 juveniles were used in the feed trials. The first trial consisted of 360 fish with an average initial weight of 33.28 ±0.19 g, and the second trial comprised 360 fish (121.19 ±1.29 g). The trials consisted of dose–response experiments with five levels of valine (5.0, 7.0, 9.0, 11.0, and 13.0 g/kg diet) and four replicate tanks per treatment. The control diet was obtained by adding L-valine to the diet containing 5.0 g/kg diet (the first level of valine tested) to ensure it reached the diet containing 7.0 g/kg diet, corresponding to the second level tested. Analysis of variance, Dunnett's test for comparison with the control diet, and regression analysis were performed. The results showed that the valine intake, weight gain, specific growth rate, and feed conversion ratio were significantly improved by the dietary valine ($P < 0.05$) in both trials. Dietary levels of valine exerted quadratic effects on body protein, body fat, body protein deposition, and nitrogen retention efficiency of tambaqui with 33 g and 121 g as body fat deposition was affected by the dietary valine level only in tambaqui weighing 33 g. Quadratic regression analysis of body protein deposition data revealed the optimum dietary valine requirement of tambaqui (33.0 g–83.0 g) and (121.0 g–277.0 g) as 11.9 g/kg diet and 9.1 g/kg diet, respectively.

Index terms: Amazon fish; branched-chain amino acid; essential amino acid; fish nutrition.

RESUMO

O tambaqui (*Colossoma macropomum*) é um dos peixes de água doce mais produzidos na piscicultura brasileira. No entanto, sua eficiência produtiva associada ao refinamento das formulações de dietas é limitada devido à falta de informações sobre as exigências nutricionais de determinados aminoácidos essenciais, como a valina. Objetivou-se estimar a exigência de valina para o tambaqui com diferentes pesos corporais. Um total de 720 juvenis foi utilizado nos experimentos, o primeiro com 360 peixes com peso médio inicial de 33,28 ±0,19 g e o segundo com 360 peixes (121,19 ±1,29 g). Os experimentos foram conduzidos em dose-resposta com cinco níveis de valina (5,0; 7,0; 9,0; 11,0 e 13,0 g/kg de dieta) e quatro repetições por tratamento. A dieta controle foi obtida pela adição de L-valina à dieta contendo 5,0 g/kg de dieta (primeiro nível de valina testado), de modo que atingiu a dieta contendo 7,0 g/kg de dieta, correspondente ao segundo nível testado. Foram realizados análise de variância dos dados, teste de Dunnett's para comparação com dieta controle e análise de regressão. Os resultados para a ingestão de valina, ganho de peso, taxa de crescimento específico, taxa de conversão alimentar foram significativamente melhorados pela valina dietética ($P<0,05$) em ambos os experimentos. Os níveis dietéticos de valina exerceram efeitos quadráticos sobre a proteína corporal, gordura corporal, deposição de proteína corporal e eficiência de retenção de nitrogênio do tambaqui com 33 g e 121 g, sendo a deposição de gordura corporal afetada pelo nível de valina somente no tambaqui com 33 g. Com base na análise de regressão quadrática dos dados de deposição de proteína corporal, as exigências dietéticas de valina de tambaqui (33,0 g-83,0 g) e (121,0 g-277,0 g) foram estimadas em 11,9 g/kg da dieta e 9,1 g/kg da dieta, respectivamente.

Termos para indexação: Peixe da Amazônia; aminoácido de cadeia ramificada; aminoácido essencial; nutrição de peixe.

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INTRODUCTION

In addition to being the most expensive component of aquatic feeds, proteins are an important source of essential amino acids (EAAs) and non-essential amino acids (non-EAAs) and play a crucial role in the growth and physiological functions of fish (National Research Council - NRC, 2011). However, fish do not have metabolic requirements for protein, necessitating the need for an adequate balance of EAAs and non-EAAs (Costa et al., 2021; Wilson, 2002). Diets formulated solely based on crude protein content may not meet the needs of all amino acids (AA), especially essential ones, thereby compromising performance and increasing the release of nitrogen compounds into the environment due to over-supplementation of nitrogen (Marchão et al., 2020; Silva et al., 2018).

Valine is an EAA and potentially limiting in conventional fish feeds (Rodrigues et al., 2020). Oliveira et al. (2021), in their study with pacu fish, reported dietary valine, lysine, and arginine as the most limiting EAAs, and signified the importance of the composition of these AAs during the preparation of diets for this species. In juvenile Nile tilapia, methionine, threonine, and valine are the most limiting dietary EAAs (Diógenes et al., 2016).

Valine majorly participates in the synthesis of body proteins, in addition to performing other important physiological functions, including the repair and growth of muscle tissue and the maintenance of nitrogen balance in the body (Suryawan et al., 2011). In addition, valine has been reported to increase fish growth by increasing digestive and absorption capacity, thus, influencing the balance of intestinal microflora (Dong et al., 2013). Therefore, it is necessary to understand valine requirements according to fish species and their development stages.

The tambaqui (*Colossoma macropomum*), produced in 25 of the 26 Brazilian states, is considered one of the most important species for Brazilian aquaculture (Instituto Brasileiro de Geografia e Estatística - IBGE, 2019). It is particularly known for the flavor of its meat in the consumer market. The tambaqui is an omnivorous species of freshwater from the Amazon and Orinoco rivers, which feeds on fruits, seeds, and small organisms. It displays desirable characteristics for fish farming and can easily adapt to conditions and rearing systems. However, high nutritional value feeds are required when raised in captivity to maximize production (Woynárovich; Van Anrooy, 2019).

The requirement of EAAs for tambaqui has been studied for lysine (Marchão et al., 2020), methionine plus cysteine (Souza et al., 2019), threonine (Firmo et al., 2018), and tryptophan (Bomfim et al., 2020). The requirement of valine for other fish species has also been determined (Abidi; Khan, 2004; Castillo; Gatlin, 2018; Dong et al., 2013; Ren et al., 2015; Xiao et al., 2018; Zehra; Khan, 2014; Zhou et al., 2020). However, the optimal valine requirements have not been established for tambaqui, especially requirements at different body weights and using the diet dilution technique (Fisher; Morris, 1970).

Therefore, considering the lack of information about the valine requirement for the tambaqui diet and the importance of specific nutrition for the species, we estimated the valine requirement for tambaqui with different body weights.

MATERIAL AND METHODS

Experimental diets

To formulate the experimental diets, we first analyzed the total AA contents of corn and soybean meal. Next, we converted these into digestible AAs using digestibility coefficients of ingredients (Table 1). The total AA contents of corn and soybean meal used in the experimental diets were obtained using high-performance liquid chromatography (HPLC).

Table 1: Total and digestible amino acid compositions of corn and soybean meal used in experimental diets (based on natural matter).

Amino acid (%)	Corn[a]		Soybean meal[a]	
	TAA[b]	DAA[c]	TAA[b]	DAA[c]
Lysine	0.27	0.25	2.76	2.61
Methionine	0.12	0.12	0.50	0.48
Methionine + Cystine	0.38	0.22	1.58	0.99
Threonine	0.25	0.25	1.71	1.68
Tryptophan	0.23	0.18	0.59	0.56
Isoleucine	0.24	0.24	2.09	2.03
Valine	0.35	0.35	2.10	2.04
Leucine	0.67	0.66	3.30	3.21
Arginine	0.40	0.39	3.31	3.28
Crude protein	7.61		46.66	

[a]Dry matter soybean meal: 95.20% and corn: 93.90%.
[b]Total amino acids, determined using high-performance liquid chromatography (HPLC) by the CBO laboratory, Valinhos, SP.
[c]Digestible amino acids, calculated based on the digestibility coefficients presented by NASCIMENTO et al. (2020) for tambaqui (*Colossoma macropomum*).

Diets were formulated using the dilution technique to obtain increased levels of digestible valine (Fisher; Morris, 1970). The dilution technique, an alternative technique for formulating experimental diets, was recently used by Nascimento et al. (2020) in a study on determining the optimum dietary EAA profile of Nile tilapia and Marchão et al. (2020) in a study on the requirement for digestible lysine for tambaqui juveniles. This technique mixes two diets, one containing a high content of the tested AA and another protein-free diet (PFD), to obtain a diet with intermediate levels of AAs tested and reduced levels of AAs.

Initially, a reference diet (RD) was formulated containing a 287.0 g/kg diet of crude protein (CP) and a 13.0 g/kg diet of digestible valine (77% digestible valine: lysine ratio) based on corn and soybean meal (Table 2). Subsequently, this diet was diluted with another PFD based on corn starch, containing the same levels of energy, vitamins, and minerals, to obtain diets with increasing dietary valine concentrations (5.0, 7.0, 9.0, 11.0, and 13.0 g/kg diet) (Table 2).

Other AAs exceeded the ideal ratios to digestible lysine recommended by the NRC (2011) for Nile tilapia by at least two percentage points to prevent another AA from becoming limiting.

Crude protein levels ranged from (110.4 to 287.0 g/kg diet) because the diets were formulated using the dilution technique. In this technique, the AA balance of the treatments remained constant because it is based on the use of a high-protein diet deficient in the evaluated AA. The evaluated AA is diluted in a protein-free isoenergetic diet, resulting in its intermediate levels (NRC, 2011; Siqueira et al., 2013). To confirm valine as the first-limiting nutrient and that the responses obtained were a function of valine and not crude protein in the diet, a sixth diet (control diet) was used. The control diet was obtained by adding synthetic valine (L-valine 98.5%) to the diet containing the first level of digestible valine (5.0 g/kg diet) to reach a concentration of 7.0 g/kg diet, corresponding to the second level tested, as proposed by Nonis and Gous (2008).

Initially, the ingredients of experimental diets were ground (Trf 60, Trapp), sifted, and mixed (Horizontal Mixer 300 kg, Branorte). To obtain intermediate diets (5.0, 7.0, 9.0, 11.0, and 13.0 g/kg diet), mixtures were diluted (Table 2). Subsequently, they were extruded in an equipment with a 3–5 mm sieve (Extruder model MX 40, Inbramaq, Laboratório de Nutrição e Alimentação de Organismos Aquáticos do Maranhão, Chapadinha, Brazil).

Table 2: Diet formulation and chemical composition of the protein-free diet (PFD), reference diet (RD), control diet (CD), and experimental diets (g/kg natural matter bases).

Ingredients (g/kg)	Digestible valine Levels						
	PFD	5.0	7.0	9.0	11.0	13.0(RD)	CD
Soybean meal	0.00	206.9	289.7	372.5	455.3	538.1	206.9
Corn	0.00	136.5	191.1	245.7	300.3	354.9	136.5
Corn starch	802.9	494.1	370.5	247.0	123.5	0.00	494.1
Soybean oil	103.2	80.6	71.6	62.5	53.5	44.5	80.6
Cellulose	37.5	23.1	17.3	11.5	5.8	0.00	23.1
Lysine-HCl (78.4%)	0.0	0.9	1.3	1.7	2.0	2.4	0.9
DL-Methionine (99%)	0.0	2.0	2.8	3.6	4.5	5.3	2.0
L-Threonine (98.5%)	0.0	0.9	1.3	1.7	2.1	2.4	0.9
L-Tryptophan (98%)	0.0	0.1	0.1	0.2	0.2	0.3	0.1
L-Valine (99%)	0.0	0.3	0.4	0.6	0.7	0.8	2.3
L-Leucine (99%)	0.0	0.6	0.8	1.0	1.2	1.4	0.6
Calcitic limestone	7.0	7.2	7.3	7.4	7.5	7.6	7.2
Dicalcium phosphate	38.2	35.7	34.7	33.6	32.6	31.6	35.7
Vitamin and mineral premix[f]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin C[e]	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Continue...

Table 2: Continuation.

Ingredients (g/kg)	Digestible valine Levels						
	PFD	5.0	7.0	9.0	11.0	13.0(RD)	CD
Salt	5.5	5.4	5.3	5.2	5.2	5.1	5.4
Antioxidant (BHT)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dilution (%)		D1	D2	D3	D4	D5*	**
Protein-free diet	100.00	61.54	46.15	30.77	15.38	0.00	
Reference diet	0.00	38.46	53.85	69.23	84.62	100.00	
Chemical composition (g/kg in the natural matter)							
Crude protein	0.00	110.4	154.6	198.7	242.9	287.0	110.4
Digestible energy (kcal kg^{-1})[d]	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Crude fiber[a]	34.7	34.7	34.7	34.7	34.7	34.7	34.7
Total Ca[a]	12.0	12.0	12.0	12.0	12.0	12.0	12.0
P available[a]	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Total Na[a]	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Digestible Valine[b]	0.00	5.0	7.0	9.0	11.0	13.0	7.0
Digestible Lysine[b]	0.00	6.5	9.0	11.6	14.2	16.8	6.5
Digestible Met. + Cist[b]	0.00	4.3	6.1	7.8	9.5	11.3	4.3
Digestible Threonine[b]	0.00	4.7	6.6	8.5	10.4	12.3	4.7
Digestible Tryptophan[b]	0.00	1.4	1.9	2.5	3.1	3.6	1.4
Digestible Arginine[b]	0.00	7.3	10.3	13.2	16.1	19.1	7.3
Digestible Leucine[b]	0.00	8.1	11.3	14.5	17.8	21.0	8.1
Relationships							
Digestible Met + Cys/Lys (0.63)[c]	0.00	0.66	0.67	0.67	0.67	0.67	0.66
Digestible Thr/Lys (0.69)[c]	0.00	0.72	0.73	0.73	0.73	0.73	0.72
Digestible Typ/Lys (0.19)[c]	0.00	0.21	0.21	0.21	0.21	0.21	0.21
Digestible Arg/Lys (0.75)[c]	0.00	1.12	1.12	1.12	1.12	1.12	1.12
Digestible Val/Lys (0.94)[c]	0.00	0.77	0.77	0.77	0.77	0.77	1.07
Digestible Leu/Lys(1.19)[c]	0.00	1.25	1.25	1.25	1.25	1.25	1.25

* Reference diet (13.0 g digestible valine/kg of dry diet).
** Control diet = D1 + 0.20% L-valine.
[a] Based on the values proposed by Rostagno et al. (2011).
[b] Based on the digestibility coefficients proposed by Nascimento et al. (2020).
[c] Amino acid:lysine ratio based on NRC values (2011).
[d] Based on the digestibility coefficients proposed by Furuya et al. (2010).
[e] Vit. C: ascorbic acid 2-monophosphate calcium salt, 42% active ingredient.
[f] Vitamin and mineral supplement, amounts supplied per kg 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.

The experimental diets were provided daily as six meals (08:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h) until satiation. At each meal, they were supplied in small quantities with successive passes, allowing the maximum intake.

Ethics Statement, experimental facility, fish, and water quality parameters

The study was conducted at the Laboratory of Nutrition and Food for Aquatic Organisms of Maranhão, located at the Center of Agrarian and Environmental Sciences of the Federal University of Maranhão, Chapadinha, in Brazil (03◦ 44′ 33′ ′ S, 43◦ 21′ 21′ ′ W; altitude 105 m) and lasted for 50 days for each trial. It was conducted using experimental procedures approved by the Animal Use Ethics Committee of the Federal University of Maranhão (Protocol N° 23115.031736/2020–83).

The study was divided into two trials. In total, 720 tambaqui (*C. macropomum*) were used, being 360 fish, with an average initial weight of 33.3 ±0.2 g, in the first trial and 360 fish, with an average initial weight of 121.2 ±1.3 g, in the second trial. In each trial, the fish were randomly distributed into 24 experimental units, with 15 fish in each unit.

During the trials, 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 of boxes was derived from an artesian well, with a flow rate of 30 L h^{-1} per box.

The temperature of the water was measured daily at 7:30, and 17:30 h using a mercury bulb thermometer graduated from 0 to 50 °C. Controls for pH, the content of dissolved oxygen, and ammonia in the water were measured every 3 days using a pH meter (HI 8424, Hanna), oximeter (HI 9146, Hanna), and a commercial kit (Arcor) for toxic ammonia test, respectively. The temperature of water ranged from 24.7 ±0.6 °C to 27.1 ±0.2 °C (first trial) and 26.4 ±0.7 °C to 27.5 ±0.1 °C (second trial). The pH was kept at 7.02 ±0.3, dissolved oxygen was maintained above 10.5 ±0.4 mg/L, and ammonia nitrogen was kept below 1.0 ppm.

Sample collection and analysis

At the beginning of the experiment, 20 fish were sampled, euthanized by subjecting them to the benzocaine solution (500 mg L^{-1}), and stored at −20 °C to determine the whole-body composition. At the end of each trial period, fish were not fed for 24 h before sampling. All fish from each experimental unit were individually weighted, euthanized with a benzocaine solution (500 mg L^{-1}), and frozen in a freezer at −20 °C. Subsequently, the fish samples were thawed, and the whole fish was crushed and dehydrated (dry) in an oven with forced-air circulation, dried for 72 h at 65 °C, and then ground in a micro mill (IKA A11 basic) and stored in plastic containers at -20 °C until body composition was analyzed.

The samples were analyzed for whole-body composition (dry matter, moisture content, crude protein, fat, and ash) according to the (Association of Official Analytical Chemists - AOAC, 2019) standard procedures.

At the end of each trial, the following performance and feed efficiency indexes were evaluated: feed intake (FI), digestible valine intake (DVI), weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR), according to the equations given below:

- FI (g) = dry feed consumed during the experimental period
- DVI (g) = [feed intake (g) × digestible valine level in feed (%)]/100
- WG (g) = final mean weight (g)−initial mean weight (g)
- SGR (% day^{-1}) = [In final weight (g) −In initial weight (g)] × 100]/experimental period (days)
- FCR (g^{-1}) = feed intake (g)/weight gain (g).

Based on the body composition (moisture, ash, protein, and fat), the daily deposition rates of body protein (BPD), body fat (BFD), and body ash (BAD), as well as the nitrogen retention efficiency (NRE), were calculated according to the equations given below:

- BPD (mg day^{-1}) = {[FBP (%) × FW (mg)] − [IBP (%) × IW (mg)]/100}/experimental period (days)
- IBP = initial body protein, FBP = final body protein, IW = initial weight, FW = final weight.
- BFD (mg day^{-1}) = {[FBF (%) × FW (mg)]−[IBF (%) × IW (mg)]/100}/experimental period (days)
- FBW = final body fat, IBW = initial body fat, IW = initial weight, FW = final weight.
- BAD (mg day^{-1}) = {[FBA (%) × FW (mg)]−[IBA (%) × IW (mg)]/100} experimental period (days)
- FBA = final body ash, IBA = initial body ash, IW = initial weight, FW = final weight.
- NRE (%) = [FBN (%) × FW(g)]−[IBN (%) × IW(g)]/[feed intake (g) × N level in ratio (%)]/100
- FBN = final body nitrogen, IBN = initial body nitrogen, IW = initial weight, FW = final weight.

Statistical analysis

The data were submitted to the Shapiro–Wilk and Levene tests to test for normality and homoscedasticity. Once the assumptions met, the data were then submitted to analysis of variance. The variables affected by valine levels were subjected to regression analysis performed using linear and quadratic models. To compare the effects of the CD (1.125%) with other treatments, Dunnett's test was used. All statistical analyses were performed using the SAS 9.0 software (Statistical Analysis System - SAS, 2002), with a value of $P < 0.05$ indicating significance.

RESULTS AND DISCUSSION

No dead fish were observed during the 50 days of each trial. No evidence of outward pathological signs was noted in fish, considering the low levels of dietary valine. The growth performance, body composition, and body deposition are shown in Tables 3, 5, and 7, respectively.

In both trials, the fish raised on the control diet (CD) with 7.0 g/kg valine in the diet showed superior responses to those raised on a diet containing 5.0 g/kg valine in the diet, with emphasis on body protein deposition, feed conversion ratio, and nitrogen retention efficiency. The experimental diets in our study were formulated using the dilution technique (Fisher; Morris, 1970), and despite the variations in protein intake among treatments, the results obtained in animals submitted to the CD confirmed that valine was the first limiting AA in experimental diets. In addition, the results were related only to variations in the valine intake and not to the crude protein content of the diets.

Table 3: Mean values ±standard error of feed intake (FI), digestible valine intake (DVI), weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) of tambaqui with different body weights as a function of digestible valine levels in the diet.

		Variables				
Body weight (g)	Digestible valine levels (g/kg diet)	FI (g fish $^{-1}$)	DVI (g fish $^{-1}$)	WG (g)	SGR (% day^{-1})	FCR (g g^{-1})
33 g	CD	103.74 ±9.18	257.20 ±8.77	47.08 ±1.76	4.21 ±0.02	2,23 ±0.25
	5.0	116.31 ±4.17	193.84 ±6.94*	36.57 ±2.50	4.07 ±0.04*	3.21 ±0.14 *
	7.0	113.22 ±3.61	264.19 ±8.43	47.13 ±3.23	4.21 ±0.04	2.42 ±0.10
	9.0	110.87 ±4.61	332.60 ±13.84*	56.02 ±5.66	4.31 ±0.06	2.02 ±0.13
	11.0	108.45 ±2.85	397.63 ±10.44*	59.12 ±0.97 *	4.35 ±0.01	1.84 ±0.02
	13.0	114.75 ±5.69	497.26 ±24.66*	63.61 ±1.95*	4.40 ±0.02*	1.80 ±0.06
	P>F[1]	0.7167	<0.0001	0.0003	0.0002	<0.0001
	Linear effect	0.5699	<0.0001	<0.0001	<0.0001	<0.0001
	Quadratic effect	0.2621	0.3200	0.1636	0.0900	0.0004
	SEp	10.84	27.14	6.14	0.072	0.27
121 g	CD	224.28 ±2.53	341.11 ±4.62	98.16 ±1.36	4.10 ±0.00	2.40 ±0.15
	5.0	220.12 ±1.89	244.58 ±2.10*	79.84 ±2.24	4.03 ±0.01	2.76 ±0.09
	7.0	220.24 ±2.04	342.60 ±3.17	106.9 ±15.13	4.09 ±0.05	2.15 ±0.28
	9.0	222.14 ±4.61	444.29 ±9.22*	124.84 ±8.51	4.19 ±0.03	1.81 ±0.14
	11.0	220.40 ±1.99	538.76 ±4.87*	102.77 ±9.22	4.11 ±0.03	2.19 ±0.18
	13.0	220.13 ±0.52	635.92 ±1.49*	91.92 ±3.63	4.08 ±0.01	2.41 ±0.11
	P>F[1]	0.9760	<0.0001	0.0147	0.0316	0.0094
	Linear effect	0.9844	<0.0001	0.4442	0.2289	0.2002
	Quadratic effect	0.6524	0.6404	0.0017	0.0068	0.0012
	SEp	5.14	0.054	14.35	0.054	0.30

CD – Control diet (D1 + 0.20% L-valine).
P > F – Significance of the "F" test of the analysis of variance.
Sep-Pooled standard error of the mean.
*Means in the same column differ from the control diet (CD) by the Dunnett test (P < 0.05).

The use of the diet dilution technique provides more accurate results with respect to the performance of animals than the supplementation technique because the dilution technique provides constant levels of AAs in all treatments, uses a lower concentration of synthetic AAs, and provides better efficiency in the use of dietary nutrients (Siqueira et al., 2013). This formulating diet approach avoided the need to include large quantities of crystalline AAs in the diet, similar to ours during the supplementation technique. Moreover, according to Cowey (1995), the presence of large amounts of crystalline AAs in fish diets reflects lower growth rates, possibly due to the more rapid assimilation of crystalline AAs than AAs present in the diet as a component of protein.

Regarding the tambaqui with a bodyweight of 33 g (trial one), the feed intake was not influenced ($P > 0.05$) by the increasing valine levels in the diets, whereas the valine intake, weight gain, and specific growth rate increased by an increment in the valine level from 5.0 to 13.0 g/kg diet ($P < 0.05$). The feed conversion ratio was reduced in a quadratic manner, estimating the optimum valine requirement at 11.7 g/kg diet (Tables 3 and 4 and Figure 1).

Table 4: Adjusted regression equations, coefficients of determination and requirement values for the variables, digestible valine intake (DVI), weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) of tambaqui with different body weights as a function of digestible valine levels in the diet.

Body weight (g)	Variable	Model	Equation	R^2	P>*F*	Level (g/kg diet)
33 g	DVI (g)	Linear	$\hat{Y} = 37.014x + 3.9789$	0.99	<0.0001	-
	WG (g)	Linear	$\hat{Y} = 3.3043x + 22.752$	0.94	0.0003	-
	SGR (% day^{-1})	Linear	$\hat{Y} = 0.04x + 3.908$	0.93	0.0002	-
	FCR (g g^{-1})	Quadratic	$\hat{Y} = 0.030893x^2 - 0.72657x + 6.0457$	0.99	<0.0001	11.7
121 g	DVI (g)	Linear	$\hat{Y} = 48.941x + 0.7547$	0.99	<0.0001	-
	WG (%)	Quadratic	$\hat{Y} = -2.0702x^2 + 38.261x - 58.831$	0.87	0.00017	9.2
	SGR (% day^{-1})	Quadratic	$\hat{Y} = -0.00639x^2 + 0.12061x + 3.5828$	0.79	0.0068	9.4
	FCR	Quadratic	$\hat{Y} = 0.042604x^2 - 0.80044x + 5.6765$	0.90	0.0012	9.3

P > F-Significance of the "F" test of the analysis of variance.

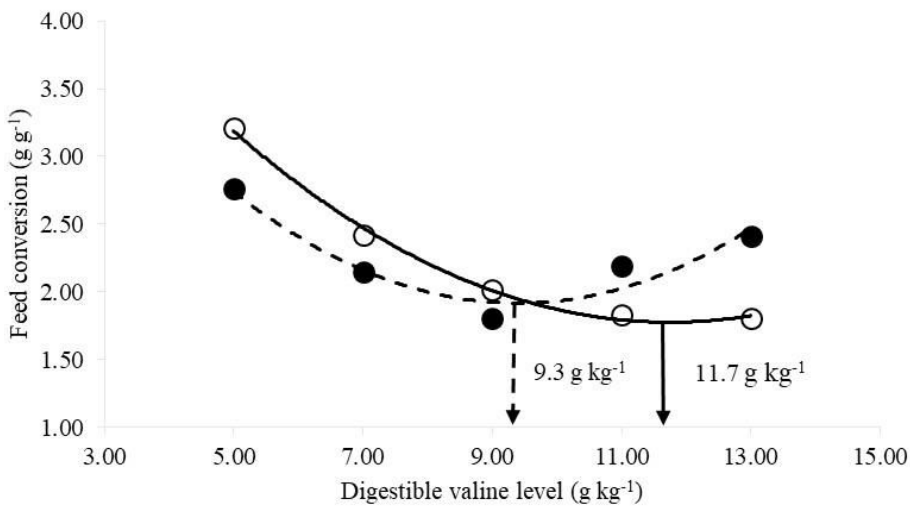

Figure 1: Graphic representation of feed conversion ratio of 33 g (solid line) and 121 g (dotted line) tambaqui fed with diets containing different levels of digestible valine.

Similar to trial one, the tambaqui with an initial body weight of 121 g (trial two) showed an increase in the valine intake with valine supplementation ($P < 0.05$) and a quadratic reduction in feed conversion rate up to the valine level of 9.3 g/kg diet. However, the weight gain and specific growth rate of trial one differed by showing a quadratic response, which increased up to the levels of dietary valine of 9.2 and 9.4 g/kg diet, respectively, and gradually decreased thereafter (Tables 3 and 4 and Figure 1).

The optimal valine requirement to maximize the weight gain and specific growth rate of tambaqui with an initial weight of 121 g was recorded in 9.2 and 9.4 g/kg of dry diet, respectively; these values were lower than the valine requirements of tambaqui with an initial weight of 33 g, which showed a linear increase in weight gain and specific growth rate with the supplementation of dietary valine. As different stages of growth have metabolic or physiological requirements for specific AAs, the requirement for AAs may change during life stages, as observed in the present study (Wilson; Poe; Robinson, 1985).

In this study, improvements in weight gain, specific growth rate, feed conversion ratio, body protein, body protein deposition, and nitrogen retention efficiency of tambaqui fed with increasing dietary valine levels indicated the importance of valine for the productive performance of this species and showed its ability to assimilate the valine crystal for growth. Previous reports on feeding trials with different dietary valine levels showed that growth performances in fish were improved by supplementation of dietary valine (Ahmad; Ahmed; Dar, 2021; Dong et al., 2013; Rollin et al., 2006; Xiao et al., 2018).

Xiao et al. (2018) conducted a study with juvenile Nile tilapia (initial weight = 6.48 g) and estimated the optimum valine requirement in 11.5 g/kg diet for weight gain, which was higher than the dietary valine values reported in our study for weight gain in fish with an initial weight of 121 g. Although Nile tilapia is also an omnivorous species, fingerlings are expected to have a higher metabolic rate and higher AA requirements than post-juvenile fish. Values higher than those presented in the present study have also been reported by Dong et al. (2013), which estimated the valine requirement in 13.7 g/kg diet for improving the specific growth rate of juvenile Jian carp, characterized by being the first artificial breed Chinese carp, which grows 30% faster than the common carp (Dong; Yuan, 2002).

Rahimnejad and Lee (2013) evaluated the digestible valine requirement of juvenile red sea bream *Pagrus major* and estimated 9.0 g/kg diet for the maximum weight gain, equivalent to 20.0 g/kg of dietary protein, which was lower than the dietary valine values mentioned in our study to maximize weight gain (45.3 g/kg of dietary protein). The data here were also expressed in g/kg of dietary protein because the red sea bream *Pagrus major* is a strictly carnivorous species and tambaqui is omnivorous. In addition, Abimorad et al. (2010) reported that comparisons should not be made between AA requirement values in species with distinct feeding behaviors, except when they are expressed as dietary proteins.

The higher level determined to optimize the feed conversion ratio of tambaqui with 33 g as compared to the level determined for tambaqui with 121 g of body weight can be explained by a change in AA requirements during the growth, which decreases throughout an animal's life due to the reduction in the growth rate (NRC, 2011). The improvement in feed conversion ratio was provided by a higher concentration of valine because no feed intake differences were observed in any of the trials.

Abidi and Khan (2004) and Xiao et al. (2018) reported an improved feed conversion ratio with an increase in the dietary concentration of valine up to 15.0 and 12.7 g/kg diet, respectively. These values were lower than those mentioned in our study. Several factors, such as fish size, developmental stages, type of diet, dietary protein and energy concentrations, feeding frequency, and environmental conditions, interfere with AA requirements (Abidi; Khan, 2004; Abimorad et al., 2010; Yue et al., 2014; Zehra; Khan, 2014). Therefore, comparisons between digestible valine values reported in the literature and those described in our study are difficult, even among species considered omnivorous.

For body composition and body deposition in tambaqui with an initial body weight of 33 g, the increased dietary valine levels influenced the body protein (%), body protein deposition, and nitrogen retention efficiency in a quadratic way ($P < 0.05$), which increased up to the levels of dietary valine of 10.7, 11.9, and 9.5 g/kg diet, respectively. In addition, body fat and body fat deposition showed a quadratic response ($P < 0.05$), which decreased until the dietary valine levels of 10.7 and 9.4 g/kg diet, respectively (Tables 5, 6, 7 and 8 and Figure 2).

In trial two (with an initial weight of 121 g), the levels of dietary valine influenced body protein (%), body protein deposition, and nitrogen retention efficiency in a quadratic manner ($P < 0.05$), which increased up to 9.0, 9.1, and 8.2 g/kg diet, respectively. For body fat (%), a decrease was observed until the valine level of 10 g/kg of the diet with a subsequent increase in fat and no effect of valine supplementation on body fat deposition ($P > 0.05$). For dry matter, a linear reduction was observed ($P < 0.05$) in this trial (Tables 5, 6, 7 and 8 and Figure 2).

Table 5: Mean values ±standard error of dry matter, protein, fat, moisture, and body ash contents of tambaqui with different weights as a function of digestible valine levels in the diet.

		Variable				
Body weight (g)	Digestible valine levels (g/kg diet)	Dry matter (%)	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
33 g	CD	30.00 ± 0.67	16.33 ± 0.18	10.69 ± 0.15	70.00 ± 0.67	3.23 ± 0.18
	5.0	28.13 ± 1.21	12.42 ± 0.32 *	13.02 ± 0.41*	71.87 ± 1.21	2.69 ± 0.16
	7.0	30.09 ± 1.27	16.04 ± 0.25	11.20 ± 0.25	69.91 ± 1.27	3.22 ± 0.30
	9.0	26.50 ± 1.23	15.05 ± 0.41*	8.83 ± 0.47*	73.50 ± 1.23	2.86 ± 0.13
	11.0	28.75 ± 0.27	18.21 ± 0.26*	7.51 ± 0.30 *	71.25 ± 0.27	3.03 ± 0.15
	13.0	26.21 ± 0.62	15.92 ± 0.19	9.57 ± 0.13	73.79 ± 0.62	3.22 ± 0.17
	P > F[1]	0.0803	<0.0001	<0.0001	0.8303	0.2598
	Linear effect	0.1235	<0.0001	<0.0001	0.1235	0.1681
	Quadratic effect	0.4134	<0.0001	<0.0001	0.4134	0.8300
	SEp	1.91	0.56	0.62	1.91	0.38
121 g	CD	39.95 ± 0.79	16.28 ± 0.46	19.08 ± 0.58	60.05 ± 0.79	4.59 ± 0.06
	5.0	40.63 ± 0.82	15.74 ± 0.49	19.93 ± 0.66	59.37 ± 0.82	4.97 ± 0.25
	7.0	40.50 ± 0.74	16.81 ± 0.23	18.93 ± 0.49	59.51 ± 0.74	4.75 ± 0.13
	9.0	37.60 ± 1.08	19.11 ± 0.52*	13.50 ± 0.50*	62.41 ± 1.08	4.99 ± 0.33
	11.0	37.61 ± 0.82	16.96 ± 0.41	15.49 ± 0.55*	62.39 ± 0.82	5.16 ± 0.30
	13.0	37.89 ± 1.11	15.73 ± 0.45	17.08 ± 0.64	62.12 ± 1.11	5.08 ± 0.31
	P>F[1]	0.0587	0.0004	<0.0001	0.0587	0.8589
	Linear effect	0.0119	0.9300	0.0001	0.0119	0.4649
	Quadratic effect	0.2983	<0.0001	<0.0001	0.2983	0.8406
	SEp	1.80	0.87	1.14	1.80	0.49

CD – Control diet (D1+0.20% L-valine).
P > F – Significance of the "F" test of the analysis of variance.
Sep-Pooled standard error of the mean.
*Means in the same column differ from the control diet (CD) by the Dunnett test (*P* < 0.05).

Table 6: Regression equations, coefficients of determination, and requirement values for variables fat, protein, and dry matter in tambaqui with different body weights as a function of the levels of digestible valine in the diet.

Body weight (g)	Variable	Model	Equation	R^2	P>F	Level (g/kg diet)
33 g	Protein (%)	Quadratic	$\hat{Y}$ =-0.1369 × ^{2} + 2.9239x+1.4031	0.72	<0.0001	10.7
	Fat (%)	Quadratic	$\hat{Y}$ = 0.15732 × ^{2} + 3.3613x+26.276	0.91	<0.0001	10.7
121 g	Protein (%)	Quadratic	$\hat{Y}$ = –0.16194 × ^{2} +2.9208x+4.9953	0.77	<0.0001	9.0
	Fat (%)	Quadratic	$\hat{Y}$ = 0.225 × ^{2} + 5.4067x + 37.519	0.73	<0.0001	10.0
	Dry matter (g g^{-1})	Linear	$\hat{Y}$ =-0.4189x + 42.613	0.70	0.0119	-

P > F-Significance of the "F" test of the analysis of variance.

Table 7: Mean values ±standard error of body protein (BPD), fat (BFD), and ash (BAD) deposition and nitrogen retention efficiency (NRE) in tambaqui with different body weights as a function of digestible valine levels in the diet.

Body weights (g)	Digestible valine levels (g/kg diet)	BPD (mg day^{-1})	BFD (mg day^{-1})	BAD (mg day^{-1})	NRE (%)
33 g	CD	162.99 ± 6.82	155.68 ± 14.76	26.17 ± 3.41	40.24 ± 1.83
	5.0	64.22 ± 7.91*	154.32 ± 7.45	10.19 ± 2.89	22.41 ± 2.39*
	7.0	158.05 ± 13.56	153.13 ± 8.18	26.50 ± 7.44	40.46 ± 2.45
	9.0	168.70 ± 12.70	128.76 ± 17.37	25.50 ± 5.29	34.34 ± 1.24
	11.0	245.37 ± 6.87*	107.03 ± 7.12*	30.77 ± 3.52	41.93 ± 0.77*
	13.0	213.96 ± 4.91*	158.80 ± 2.92	37.62 ± 2.27	29.43 ± 1.41*
	P>F[1]	<0.0001	0.0090	0.0120	<0.0001
	Linear effect	<0.0001	0.2511	0.0012	0.0148
	Quadratic effect	<0.0001	0.0099	0.4810	<0.0001
	SEp	18.71	21.62	8.97	3.57
121 g	CD	281.66 ± 36.85	395.79 ± 13.06	55.82 ± 9.72	36.35 ± 1.00
	5.0	151.70 ± 15.93*	314.42 ± 35.30	42.64 ± 9.02	28.06 ± 2.84*
	7.0	266.90 ± 63.00	343.99 ± 76.32	53.07 ± 21.64	36.06 ± 2.32
	9.0	492.54 ± 38.02*	160.66 ± 39.64*	95.43 ± 27.85	50.25 ± 2.33*
	11.0	295.07 ± 48.42	193.20 ± 26.84*	78.43 ± 18.38	24.64 ± 1.54*
	13.0	193.38 ± 27.90	232.33 ± 38.40	61.70 ± 16.56	13.77 ± 1.98*
	P>F[1]	0.0004	0.0626	0.3794	<0.0001
	Linear effect	0.4135	0.0592	0.3238	<0.0001
	Quadratic effect	<0.0001	0.1974	0.1435	<0.0001
	Sep	82.25	85.66	36.80	4.18

CD – Control diet (D1 + 0.20% L-valine).
P > F – Significance of the "F" test of the analysis of variance.
Sep-Pooled standard error of the mean.
*Means in the same column differ from the control diet (CD) by the Dunnett test (P < 0.05).

Table 8: Adjusted regression equations, determination coefficients, and requirement values for variables body protein deposition (BPD), body fat deposition (BFD), and body ash deposition (BAD), and nitrogen retention efficiency (NRE) in tambaqui with different body weights as a function of the level of digestible valine in the diet.

Body Weight (g)	Variable	Model	Equation	R^2	P>F	Level (g/kg diet)
33 g	BPD (mg day^{-1})	Quadratic	$\hat{Y} = -3.2942 \times^2 + 78.634x - 244.47$	0.92	<0.0001	11.9
	BFD (mg day^{1})	Quadratic	$\hat{Y} = 1.9384 \times^2 - 36.748x + 298.62$	0.50	0.0099	9.4
	BAD (mg day^{-1})	Linear	$\hat{Y} = 2.957x - 0.49$	0.86	0.0012	-
	NRE (%)	Quadratic	$\hat{Y} = -0.8465 \times^2 + 16.012x - 35.06$	0.71	<0.0001	9.5
121 g	BPD (mg day^{-1})	Quadratic	$\hat{Y} = -15.302 \times^2 + 281.01x - 887.31$	0.77	<0.0001	9.1
	NRE (%)	Quadratic	$\hat{Y} = -1.3845 \times^2 + 22.921x - 52.511$	0.79	<0.0001	8.2

P > F-Significance of the "F" test of the analysis of variance.

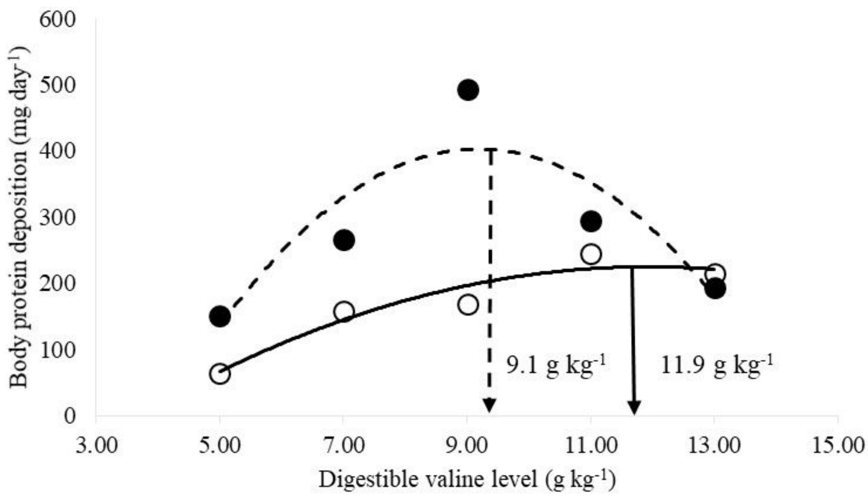

Figure 2: Graphic representation of body protein deposition of tambaqui with 33 g (solid line) and 121 g (dotted line) fed diets containing different levels of digestible valine.

Regarding body composition, the responses of BP, body protein deposition, and nitrogen retention efficiency to the increased dietary valine levels in tambaqui with an initial body weight of 33 g were a little higher than those reported for tambaqui with an initial weight of 121 g due to different growth rates, which is directly related to different depositions of muscle tissue. The optimum valine requirement for tambaqui (33 g and 121 g) in our study was lower than the dietary valine values reported for juvenile hybrid grouper (15.8 g/kg diet; Zhou et al., 2020), juvenile tilapia (12.7 g/kg diet; Xiao et al., 2018), Indian major carp (15.8 g/kg diet; Abidi; Khan, 2004). Despite differences in valine levels to maximize body protein deposition, studies have shown an improvement in protein deposition with dietary valine supplementation, suggesting that a better dietary AA balance probably prevents the selective catabolism of AAs and consequently increases protein synthesis (Tantikitti; Chimsung, 2001).

In addition, the quadratic nitrogen retention efficiency response to increasing levels of valine in the diet showed that at excessive levels, the percentage of retained nitrogen decreased, consequently increasing the excretion of nitrogen compounds into the environment and contributing to contamination and eutrophication of the aquatic environment (Furuya et al., 2013).

A better balance of AAs in the diet can contribute to an increase in protein synthesis, with a concomitant decrease in the accumulation of lipid reserves (Tantikitti; Chimsung 2001), as demonstrated in our studies, in which the whole-body fat composition decreased with supplementation of dietary valine, and subsequently increased. This result is consistent with previous reports of feeding trials with red sea bream (Rahimnejad; Lee, 2013) and Jian carp (Dong et al., 2013). The body fat deposition for tambaqui weighing 121 g was not affected by increased dietary valine; however, the highest body fat deposition was reported at the two lowest dietary valine levels.

Only the tambaqui weighing 33 g showed a significant increase in body ash deposition with an increase in the dietary valine level. As previously discussed, a better balance of AAs improves the body's protein deposition, leading to better use of dietary minerals for the formation of osseous tissues to support muscle tissues (Pianesso et al., 2015). A close functional relationship exists between osseous, and muscle tissues, Bomfim et al. (2020) evaluated the growth curve of tambaqui (*C. macropomum*) and reported that the maximum development of total length was reached at 243.4 days, whereas the maximum development of body weight was reached at 333.9 days, suggesting an earlier bone development than muscle tissue because that fish weight gain is associated with the accretion of proteins.

The AA requirement for fish has been determined by the level that optimizes performance variables, mainly weight gain (NRC, 2011). However, weight gain consists of increases in proteins, lipids, minerals, and water (NRC, 2011), and there is no metabolic demand for AAs to maintain lipid and mineral reserves (Marchão et al., 2020). In this sense, body protein deposition is the most suitable variable to determine the AA requirement for fish (Bomfim et al., 2020; Lee et al., 2020; Silva et al., 2018; Costa Sousa et al., 2021), especially valine because it is used in greater proportion for protein synthesis (Rodrigues et al., 2018).

CONCLUSIONS

The results of our study demonstrated that dietary valine provides optimal growth and increases the body protein of tambaqui with different body weights. Using a quadratic regression analysis of body protein deposition data, the optimum dietary valine requirement of tambaqui (33.0 g–83.0 g) and (121.0 g–277.0 g) was estimated to be 11.9 g/kg diet and 9.1 g/kg diet, respectively, corresponding to 4.53% of dietary crude protein. These values are within the range of valine requirements of similar fish species reported by the NRC, 2011.

AUTHOR CONTRIBUTION

Conceptual idea: Silva, J.V.; Ribeiro, F.B.; Siqueira, J.C.; Bomfim, M.A.D.; Methodology design: Silva, J.V; Ribeiro, F.B; Siqueira, J.C.; Bomfim, M.A.D.; Marchão, R.S.; Data collect: Silva, J.V; Nascimento, D.C.N.; Marchão, R.S.; Data analysis and interpretation: Siqueira, J.C.; Silva, J.V; Ribeiro, F.B.; Writing and editing: Silva, J.V; Ribeiro, F.B.; Siqueira, J.C.; Nascimento, D.C.N.; Marchão, R.S.

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