

Apparent digestibility coefficients for amino acids of feed ingredients in tambaqui (*Colossoma macropomum*) diets

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ABSTRACT - This study evaluated the apparent digestibility coefficients (ADC) of essential (EAA) and non-essential (NEAA) amino acids of 13 ingredients for tambaqui (*Colossoma macropomum*) diets. Proteic and energetic ingredients were analyzed separately. The trial with energetic and proteic ingredients were arranged in a randomized block design, with four replicates: energetic ingredients (corn, wheat bran, broken rice, and sorghum) with four treatments, whereas proteic ingredients (corn gluten meal, soybean meal, poultry byproduct meal, salmon meal, fish meal [tilapia processing residue], wheat gluten meal, feather meal, cottonseed meal, and alcohol yeast [spray dried]) with nine treatments. Each block was considered as one round of fecal collection. A total of 420 tambaqui juveniles (mean initial weight: 70±8.58 g) were used. Among energetic ingredients, corn (94.6%) and wheat bran (91.9%) had the highest ADCEAA, followed by broken rice (75.7%), and sorghum (72.8%). On average, ADCEAA and ADCNEAA values of proteic ingredients were 79.5-98.5%, except for alcohol yeast (ADCEAA: 68.4 and ADCNEAA: 76.7%). Tryptophan was the first limiting amino acid in most ingredients tested and had the lowest chemical scores (0.06-0.51), except for wheat bran, corn gluten meal, and soybean meal, in which lysine was the first limiting amino acid. Soybean meal had the highest digestible essential amino acid index (EAAI: 1.02) and the most balanced amino acid profile, whereas wheat gluten meal had the lowest EAAI (0.48). Overall, tambaqui was very efficient to digest proteic and energetic ingredients.

Keywords: amino acids, chemical score, digestibility, feed ingredients, fish, nutrition

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Introduction

Tambaqui (*Colossoma macropomum*) is one of the most widely produced freshwater species in South America (Araújo-Lima and Gomes, 2005) and the most produced native species in continental aquaculture, with great growth in 2016 with 136.99 thousand tons (IBGE, 2016). Interest in the species has risen due to its adaptability to intensive production systems and artificial feeding, fast growth, omnivorous feeding habit, high feed efficiency, and excellent taste and desirable texture (Araújo-Lima and Gomes, 2005).

The use of balanced and highly digestible diets is crucial for sustainable fish production. Digestibility coefficients provide an indication of the amount of the nutrient that is absorbed; the higher the

digestibility of the nutrient, the better it will be utilized by fish, resulting in higher production performance and reducing excretion of nutrients in the production environment (Oliveira Filho and Fracalossi, 2006).

A previous study (Buzollo et al., 2018) highlighted the importance of studies on nutrient digestibility of conventional feed ingredients used by the aquafeed industry to maximize production and reduce operating costs and levels of nitrogen, phosphorus, and organic matter released into effluents of fisheries. However, no study has evaluated the digestibility of amino acids in ingredients used in commercial tambaqui diets. Thus, limited information is available for the formulation of balanced diets for commercial tambaqui production.

We aimed to determine the apparent digestibility coefficients of amino acids of 13 ingredients, which were divided into energetic (corn, wheat bran, broken rice, and sorghum) and proteic (corn gluten meal, soybean meal, poultry byproduct meal, salmon meal, fish meal [tilapia processing residue], wheat gluten meal, feather meal, cottonseed meal, and alcohol yeast [spray dried]) ingredients. The chemical score of each amino acid was calculated, and the amino acid profile of each ingredient compared to that of tambaqui white muscle to determine the limiting digestible amino acids of each ingredient.

Material and Methods

The experimental trial was conducted in Jaboticabal, SP, Brazil (21°15'07.5" S, 48°19'46.0" W), in accordance with the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Use (case no. 016114/11).

In total, 420 tambaqui juveniles (mean initial weight: 70±8.58 g) were used in the study. The animals were kept in 28 tanks (430 L) provided with continuous aeration and water from a flowing artesian well (renewal rate: ~10 times per day). The physicochemical parameters of the water were within the acceptable range for the species (Aride et al., 2004; Araújo Lima and Gomes, 2005): mean±SD, pH: 7.85±0.17; temperature: 29.72±0.34 °C; dissolved oxygen: 5.71±0.34 mg/L; electrical conductivity: 150.75±17.62 µS/cm; alkalinity: 88.67±0.82 µg/L; ammonia: 189.17±59.29 µg/L; nitrate: 419.96±100.28 µg/L; nitrite: 28.68±39.09 µg/L; and total phosphorus: 200.89±61.00 µg/L.

To determine the ADC of each ingredient, a reference diet was prepared to contain 237 g/kg of crude protein and 16.32 MJ/kg of gross energy (Table 1). The 13 test ingredients used in the experimental diets were obtained from four Brazilian industries: Guabi® (sorghum, corn gluten meal, poultry byproduct meal, wheat gluten meal, feather meal, cottonseed meal, and alcohol yeast [spray dried]), Coplana® (corn, wheat bran and soybean meal), Agromix® (broken rice), and Grupo Ambar Amaral® (fish meal [tilapia processing residue]), with exception of salmon meal that was imported from Chile, and were divided into two groups: energetic = corn, wheat bran, broken rice, and sorghum; and proteic = corn gluten meal, soybean meal, poultry byproduct meal, salmon meal, fish meal (tilapia processing residue), wheat gluten meal, feather meal, cottonseed meal, and alcohol yeast (spray dried). With these ingredients (Table 2), 13 test diets were formulated to contain 695 g/kg of the reference diet, 300 g/kg of the test ingredient (100 g/kg for wheat gluten meal due to the cohesive and viscoelastic properties of gluten that may provide result in a rubbery, dry pellet (Day et al., 2006), and 5 g/kg of chromium-III oxide (Cr₂O₃) used as the inert digestibility marker. For the preparation of diets, the ingredients were ground, manually mixed, moistened, and extruded using an Exteec extruder (Ex Micro model). Pellets were dried in an oven with forced-air ventilation at 55 °C for 24 h.

The digestibility coefficients of amino acids from the test ingredients were determined with the use of an inert marker (chromium-III oxide), according to Nose (1966). For fecal collection, 14 glass fiber collectors (80-L each) provided with continuous aeration and water circulation were constructed according to the modified Guelph system described by Abimorad and Carneiro (2004). Fecal collection from the four replicates of the 14 treatments (13 test diets and a reference diet) was divided into two periods. First period – distribution of replicates 1 and 2 in 28 feeding tanks. The adaptation to the diets was carried out for seven days. On day 8, feces were collected from replicate 1 (first 14 feeding tanks),

and on day 9, from replicate 2 (another 14 feeding tanks). Second period – redistribution of the diets of replicates 3 and 4 in 28 feed tanks. The adaptation to the diets was carried out for seven days. On day 8, feces were collected from replicate 3 (first 14 feeding tanks), and on day 9, from replicate 4 (another 14 feeding tanks). In both periods, the fish from each replicate were fed to apparent satiation and transferred after the last feeding of the day (18.00 h) to the conical tanks and, therefore, the collections were performed during the night. Feces were collected into Falcon conical tubes (kept on ice to reduce feces degradation), every 3 h, for ease of animal management, according to previous project, until

Table 1 - Composition and proximate analysis of reference diet (values on a dry matter basis, g/kg)

Item	g/kg
Ingredient	
Fish meal (tilapia processing residue)	202.0
Soybean meal	88.9
Corn	335.1
Wheat bran	220.0
Broken rice	140.0
Dicalcium phosphate	8.0
Limestone	1.0
Vitamin and mineral supplement ¹	5.0
Analyzed composition	
Dry matter	885.4
Crude protein	209.8
Digestible protein ²	187.9
Lipids	53.2
Digestible lipids ²	45.2
Gross energy (MJ/kg)	14.4
Digestible energy (MJ/kg) ²	12.2
Crude fiber	66.1
Mineral matter	72.1
Non-nitrogen extractive ³	382.8
Calcium ⁴	13.2
Phosphorus ⁴	6.6
Arginine	13.9
Histidine	3.8
Isoleucine	6.6
Leucine	12.7
Lysine	9.8
Methionine	5.5
Phenylalanine	7.9
Threonine	6.1
Tryptophan	1.2
Valine	8.3
Aspartic acid	13.2
Glutamic acid	29.6
Alanine	14.0
Cystine	10.3
Glycine	16.8
Serine	8.1
Proline	13.4
Tyrosine	5.2

¹ Vitamin and mineral supplement (IU or mg/kg): folic acid, 1250 mg; calcium pantothenate, 1200 mg; Cu, 2500 mg; Fe, 15 g; I, 375 mg; Mn, 12.5 mg; Se, 87.5 mg; Zn, 12.5 mg; Co, 125 mg; vitamin A, 2500 IU; vitamin B12, 4000 mg; thiamine B1, 4000 mg; riboflavin B2, 4000 mg; pyridoxine B6, 4000 mg; vitamin C, 50,000 mg; vitamin D3, 6,000,000 IU; vitamin E, 37,500 IU; vitamin K3, 3750 mg; niacin 122,500 mg; biotin, 15 mg.

² Values calculated based on the digestibility coefficients determined by Buzollo et al. (2018).

³ NNE = DM - (CP + LP + MM + CF).

⁴ Values calculated according to Rostagno et al. (2011).

Table 2 - Composition of ingredients used in experimental diets offered to juvenile tambaqui (values on dry matter basis, g/kg)

	Ingredient (g/kg)													
	CO	WB	BR	SO	CGM	SBM	PM	SAM	TPR	WGM	FM	CM	AY	
IFN ¹	4 - 02 - 948	4 - 05 - 190	4 - 02 - 948	4 - 20 - 893	5 - 28 - 242	5 - 20 - 637	5 - 03 - 798	5 - 02 - 012	5 - 01 - 974	-	5 - 03 - 795	5 - 07 - 873	7 - 05 - 500	
Dry matter	881.6	900.7	887.2	891.4	919.1	896.2	968.7	919.1	980.3	944.6	949.2	913.7	952.3	
Crude protein	92.6	184.2	92.3	103.7	687.6	529.3	679.5	721.9	614.4	852.7	803.2	488.1	380.9	
Lipids	40.9	44.0	17.2	44.4	44.1	32.1	146.7	90.7	106.9	44.7	135.1	14.3	23.2	
Mineral matter	14.1	56.4	9.0	14.5	22.8	73.2	169.2	163.2	257.1	6.0	34.8	62.5	76.3	
Gross energy (MJ/kg)	18.4	18.1	17.7	18.5	16.6	19.5	21.6	20.0	18.2	22.7	24.9	18.6	19.0	
EAA														
Arginine	4.88	11.88	8.12	4.49	25.13	41.84	55.13	65.28	53.45	29.22	63.21	51.33	20.48	
Histidine	2.27	3.89	1.92	1.91	13.06	12.61	21.68	22.63	10.30	15.03	6.53	9.63	7.56	
Isoleucine	2.95	5.44	3.61	3.93	30.57	24.88	27.98	25.46	20.40	30.70	38.35	13.46	20.48	
Leucine	11.00	11.21	7.33	13.57	115.66	39.72	46.87	46.24	36.83	58.76	59.95	25.28	25.10	
Lysine	2.72	8.10	3.72	3.14	14.58	35.04	54.92	50.16	46.11	11.01	37.08	13.79	43.89	
Methionine	2.50	2.55	2.82	2.13	15.45	6.92	21.16	29.05	18.67	13.44	9.06	6.57	7.88	
Phenylalanine	4.20	6.99	4.62	4.26	40.15	25.66	21.68	22.52	16.83	39.70	37.51	19.15	16.59	
Threonine	3.40	5.55	3.83	4.04	23.83	20.42	28.70	31.55	25.30	19.69	38.56	14.45	21.21	
Tryptophan	0.23	2.11	0.11	0.56	1.52	4.80	2.68	4.35	3.37	0.84	0.63	1.89	1.47	
Valine	3.86	7.55	4.96	4.71	29.81	22.76	27.98	30.03	24.07	29.22	48.78	18.50	20.79	
NEAA														
Aspartic acid	6.69	12.77	9.13	7.96	49.94	62.71	46.35	59.08	31.62	28.90	59.31	37.21	41.90	
Glutamic acid	18.26	40.19	17.36	23.22	165.38	102.54	97.04	87.80	72.12	320.45	99.14	81.43	48.30	
Alanine	6.92	8.10	5.07	9.42	62.89	22.99	46.45	51.90	48.86	20.86	37.72	18.06	24.36	
Cystine	6.35	8.22	6.09	5.61	27.85	15.40	23.33	31.12	14.08	46.37	95.24	18.82	15.12	
Glycine	3.29	8.33	3.83	3.25	18.06	21.87	54.20	69.09	73.96	25.94	59.63	14.67	16.49	
Serine	4.08	7.33	4.17	4.38	35.47	26.89	25.50	31.66	22.75	37.58	82.49	15.54	20.37	
Proline	7.83	11.99	3.94	7.85	63.87	25.33	36.75	40.91	44.58	98.45	85.02	16.20	12.92	
Tyrosine	3.06	4.66	3.83	3.93	36.34	18.52	18.58	20.35	15.81	26.68	27.71	12.81	13.23	

CO - corn; WB - wheat bran; BR - broken rice; SO - sorghum; CGM - corn gluten meal; SBM - soybean meal; PM - poultry byproduct meal; SAM - salmon meal; TPR - tilapia processing residue; WGM - wheat gluten meal; FM - feather meal; CM - cottonseed meal; AY - alcohol yeast; EAA - essential amino acid; NEAA - non-essential amino acid.

¹ International feed number.

6.00 h of the next day. All feces collected from each replicate were lyophilized using a Thermo Electron Corporation Fisher®, freeze-dried, and analyzed.

Chromium-III oxide concentrations in diets and feces were determined by nitric-perchloric digestion according to the method described by Furukawa and Tsukahara (1966). The amino acids were analyzed using liquid chromatography in cationic exchange resin columns and post-column derivation with ninhydrin and an autoanalyzer. For the amino acid count, the samples were hydrolyzed with HCl 6 N for 22 h at 110 °C according to the method described by Moore and Stein (1963). Tryptophan was determined after the enzymatic hydrolysis with Pronase at 40 °C for 24 h, followed by a colorimetric reaction with 4-(dimethylamino)benzaldehyde in sulfuric acid 21.2 N and read at 590 nm. The tryptophan content was calculated according to Spies (1967).

The apparent digestibility coefficient (ADC) for a test ingredients was calculated from the amount of marker and amino acid in the reference diet, test diet, and feces according to the equation of Nose (1966):

$$ADC = \left[1 - \left(\frac{\% \text{ marker in diet}}{\% \text{ marker in feces}} \times \frac{\% \text{ aa in feces}}{\% \text{ aa in diet}} \right) \right] \times 100$$

The ADC for an amino acid in a test ingredient was calculated according to the following equation of Forster (1999):

$$ADC_{\text{ingredient}} = \frac{[(a + b) \times ADC_{\text{test diet}} - (a) \times ADC_{\text{reference diet}}]}{b}$$

in which a = AA contribution of the reference diet to the AA content of the test diet (% AA in reference diet × 0.695), and b = AA contribution of test ingredient to AA content of test diet (% AA in test ingredient).

Amino acid limitations in test ingredients were estimated by calculating the chemical score index (CSI) for each amino acid according to the following equation of Sgarbieri (1987):

$$CSI = \left[\frac{\% \text{ EAA in ingredient protein}}{\% \text{ corresponding EAA in muscle protein}} \right] \times 100$$

The essential amino acid index (EAAI) of the test ingredients were calculated according to the following equation of Oser (1959):

$$EAAI = \sqrt[n]{\frac{100a}{a_p} \times \frac{100b}{b_p} \times \frac{100c}{c_p} \times \dots \times \frac{100j}{j_p}}$$

in which a, b, c...j are the % digestible EAA of test ingredient protein; ap, bp, cp...jp are the % EAA in tambaqui muscle; and n = number of amino acids considered.

The two methods compare the amount of digestible AA in the ingredients with the amino acid profile of fish white muscle (Hepher, 1988). For these calculations, nine fish (mean weight: 42.0±5.76 g) from the same population were killed by ice-slurry immersion and, white muscle samples were taken for amino acid analysis.

The essential amino acid (EAA) with the lowest chemical score index was considered the first limiting amino acid of the ingredient. The EAAI was calculated from the geometric mean of all EAA scores. Protein quality is high in ingredients with higher EAAI values.

Proteic and energetic ingredients were analyzed separately. The trial with energetic and proteic ingredients were arranged in a randomized block design, with four replicates; energetic ingredients with four treatments (ingredients) and four replicates, whereas proteic ingredients with nine treatments (ingredients) and four replicates. Each block was considered as one round of fecal collection. The ADC values were subjected to ANOVA using the PROC GLM procedure of SAS (Statistical Analysis System, version 9.2). When significant differences were detected, treatment means were compared using Tukey's test at 5% significance level.

Results

No fish mortality was observed during the experimental period. No effect of the fecal collection period (round) was observed; therefore, the ADC data of all test diets were analyzed together. Of the 13 proteic and energetic ingredients tested, only six had digestibility coefficients <70% for some amino acids, and significant differences in the ADC of amino acids were observed across ingredients ($P < 0.05$).

High ADC values (>70%) were observed for most amino acids (Table 3), and only the ADC of arginine, phenylalanine, threonine, serine, and tyrosine for sorghum and threonine, serine, and tyrosine for broken rice were <70%. Additionally, corn (95%) and wheat bran (92%) had the highest ADC values, whereas broken rice (78.7%) and sorghum (74.6%) had the lowest ADC values.

Mean ADC_{EAA} and ADC_{NEAA} values of most proteic ingredients were high (>70%; Table 4). Only wheat gluten meal, feather meal, cottonseed meal, and alcohol yeast had ADC <70% for a few amino acids. Amino acid digestibility varied across proteic ingredients, but some EAA had low ADC common to a few ingredients: arginine (wheat gluten meal, feather meal, and alcohol yeast), isoleucine (alcohol yeast), lysine (wheat gluten meal and cottonseed meal), threonine (wheat gluten meal, cottonseed meal, and alcohol yeast), and valine (alcohol yeast).

The non-essential amino acids (NEAA) glycine and serine also had low ADC values for wheat gluten meal and alcohol yeast. Conversely, alanine had the highest ADC_{NEAA} values (>90%) in all proteic ingredients, whereas the other NEAA had a wide variation in ADC values. Additionally, corn gluten meal and soybean meal had the highest ADC_{EAA} (96.9 and 96.6%, respectively) and overall ADC_{AA} (corn gluten meal: 97.6%, soybean meal: 96.6%) values.

Table 3 - Apparent digestibility coefficients (ADC) for essential (EAA) and non-essential (NEAA) amino acids of energetic ingredients offered to tambaqui (%)

Amino acid	Apparent digestibility coefficient (%)				ANOVA P-value
	Corn	Wheat bran	Broken rice	Sorghum	
EAA					
Arginine	97.1±0.36a	94.9±0.38a	71.4±0.41b	61.2±0.33c	<0.001
Histidine	94.1±0.49a	89.2±0.36a	71.9±0.61b	73.3±0.57b	<0.001
Isoleucine	96.8±0.58a	91.8±0.49a	81.2±0.61b	71.7±0.43b	<0.001
Leucine	98.5±0.39a	93.2±0.47a	81.2±0.42b	78.6±0.43b	<0.001
Lysine	91.4±0.40a	89.4±0.51a	84.1±0.39a	74.9±0.39b	<0.001
Methionine	96.9±0.23a	90.2±0.69ab	80.1±0.67b	98.7±0.51a	<0.010
Phenylalanine	98.8±0.49a	91.4±0.52a	78.2±0.57b	63.9±0.66c	<0.001
Tryptophan	75.3±0.22c	99.7±0.27a	90.9±0.14b	77.6±0.16c	<0.001
Threonine	99.6±0.59a	88.1±0.66a	58.2±0.63b	41.0±0.70c	<0.001
Valine	97.3±0.60a	91.2±0.51a	76.6±0.45b	70.2±0.41b	<0.001
EAA mean	94.6±0.43a	91.9±0.49a	75.7±0.40b	72.8±0.43b	<0.001
NEAA					
Aspartic acid	97.5±0.24a	96.0±0.58a	93.2±0.74a	79.1±0.50b	<0.001
Glutamic acid	98.3±0.29a	98.2±0.21a	91.1±0.38b	78.6±0.36c	<0.001
Alanine	97.0±0.41ab	91.0±0.49bc	87.4±0.48c	98.9±0.25a	<0.001
Cystine	84.3±0.38ab	87.9±0.30a	82.7±0.28ab	80.6±0.37b	<0.010
Glycine	94.3±0.38a	89.9±0.40ab	90.1±0.35ab	87.2±0.31b	<0.001
Serine	96.1±0.55a	90.6±0.57a	66.1±0.52b	60.7±0.28b	<0.001
Proline	97.4±0.36a	94.8±0.39a	85.6±0.44b	73.5±0.29c	<0.001
Tyrosine	98.6±0.48a	88.5±0.57a	63.8±0.79b	56.5±0.84b	<0.001
NEAA mean	95.5±0.39a	92.0±0.37a	82.5±0.32b	76.9±0.32c	<0.001
Overall AA mean	95.0±0.41a	92.0±0.43a	78.7±0.32b	74.6±0.34b	<0.001

Mean (n = 4) ± standard error.

Values with different letters in the same row are statistically different by Tukey's test ($P < 0.05$).

Table 4 - Apparent digestibility coefficients (ADC) for essential (EAA) and non-essential (NEAA) amino acids of proteic ingredients offered to tambaqui (%)

Amino acid	Apparent digestibility coefficient (%)										ANOVA P-value	
	Corn gluten meal	Soybean meal	Poultry byproduct meal	Salmon meal	Fish meal (tilapia processing residue)	Wheat gluten meal	Feather meal	Cottonseed meal	Alcohol yeast			
EAA												
Arginine	99.1±0.22a	99.2±0.20a	80.2±0.31b	81.6±0.50b	92.2±0.26a	51.3±0.50d	69.1±0.41c	81.3±0.48b	53.8±0.55d	<0.001		
Histidine	98.3±0.20a	95.8±0.23a	99.8±0.24a	99.8±0.35a	84.0±0.37c	99.1±0.27a	85.3±0.46bc	85.3±0.25bc	89.7±0.37b	<0.001		
Isoleucine	98.8±0.19a	97.1±0.22ab	93.3±0.18c	93.8±0.21bc	84.7±0.35e	95.4±0.20abc	88.6±0.36d	84.4±0.25e	69.4±0.30f	<0.001		
Leucine	99.9±0.17a	97.2±0.26ab	93.6±0.23abc	90.9±0.47bcd	87.0±0.30d	94.9±0.55ab	87.2±0.25cd	84.6±0.49d	73.5±0.43e	<0.001		
Lysine	93.5±0.35a	94.5±0.35a	91.4±0.24a	92.9±0.21a	85.9±0.35ab	65.4±0.81d	78.7±0.60bc	67.2±0.68cd	72.3±0.30cd	<0.001		
Methionine	99.5±0.34a	95.1±0.35abc	97.6±0.27ab	90.3±0.58bcd	89.8±0.42cd	99.6±0.24a	83.4±0.52d	91.9±0.34abcd	86.2±0.49d	<0.001		
Phenylalanine	99.9±0.21a	98.2±0.22ab	91.5±0.23bcd	90.3±0.48bcd	85.5±0.36d	93.9±0.61abc	85.8±0.57cd	87.0±0.53cd	71.9±0.39e	<0.001		
Tryptophan	81.2±0.52ab	94.2±0.15a	92.8±0.65a	84.8±0.20ab	85.4±0.70ab	92.1±0.59a	72.2±0.80b	90.8±0.67a	72.5±0.50b	<0.001		
Threonine	99.4±0.24a	98.1±0.25a	77.9±0.50b	84.7±0.28b	82.5±0.29b	60.2±0.33c	82.1±0.53b	44.9±0.35d	44.4±0.41d	<0.001		
Valine	99.3±0.21a	97.0±0.28ab	87.1±0.22cd	87.5±0.28bcd	84.5±0.36cd	88.6±0.69bc	88.7±0.58bc	77.4±0.32d	50.7±0.47e	<0.001		
EAA mean	96.9±0.26a	96.6±0.22a	90.5±0.25b	89.7±0.34b	86.1±0.35bc	84.0±0.49cd	82.1±0.45cd	79.5±0.40d	68.4±0.34e	<0.001		
NEAA												
Aspartic acid	99.9±0.23a	99.5±0.21ab	96.9±0.28bc	96.2±0.19c	92.4±0.26d	76.3±0.30g	81.1±0.24f	86.7±0.25e	76.2±0.20g	<0.001		
Glutamic acid	99.8±0.16a	98.9±0.20ab	95.2±0.27c	96.1±0.29bc	94.6±0.26c	99.1±0.24ab	86.9±0.09e	91.4±0.36d	74.4±0.35f	<0.001		
Alanine	99.9±0.21a	97.0±0.27ab	98.7±0.58ab	99.3±0.61ab	90.3±0.34b	99.4±0.63ab	99.9±0.30a	99.6±0.39a	99.8±0.50a	<0.050		
Cystine	91.3±0.39abc	90.9±0.30abc	97.1±0.38a	94.3±0.41ab	83.9±0.41d	87.9±0.65bcd	81.3±0.40d	72.1±0.49e	85.1±0.09cd	<0.001		
Glycine	90.3±0.33a	93.9±0.33ab	87.2±0.35c	90.0±0.50bc	86.5±0.34c	55.3±0.41e	85.6±0.39c	83.6±0.39c	71.3±0.45d	<0.001		
Serine	99.5±0.18a	96.2±0.25a	99.9±0.35a	99.4±0.35a	85.1±0.27b	97.3±0.58a	85.5±0.58b	75.9±0.47c	63.3±0.36d	<0.001		
Proline	99.8±0.17a	97.9±0.31ab	89.4±0.30cd	93.9±0.15bc	86.8±0.35d	95.1±0.49ab	86.0±0.38d	73.0±0.41e	73.1±0.41e	<0.001		
Tyrosine	99.0±0.22a	98.2±0.27a	93.5±0.47ab	93.5±0.25ab	85.2±0.47c	73.3±0.46de	87.8±0.46bc	81.4±0.36cd	70.3±0.31e	<0.001		
NEAA mean	98.5±0.18a	96.6±0.26ab	94.7±0.30b	95.3±0.25ab	88.1±0.29c	85.1±0.39cd	86.9±0.34c	83.0±0.26d	76.7±0.31e	<0.001		
Overall AA mean	97.6±0.23a	96.6±0.24ab	92.4±0.26bc	92.2±0.30c	87.0±0.31d	84.5±0.45de	84.3±0.40de	81.0±0.30e	72.1±0.31f	<0.001		

AA - amino acid.

Mean (n = 4) ± standard error.

Values with different letters in the same row are statistically different by Tukey's test (P<0.05).

Table 5 - Chemical score and essential amino acid index (EAAI) of ingredients relative to juvenile tambaqui white muscle protein

Essential amino acid	Juvenile tambaqui white muscle (g kg ⁻¹ CP) ¹	Chemical score												
		Corn	Wheat bran	Broken rice	Sorghum	Corn gluten meal	Soybean meal	Poultry byproduct meal	Salmon meal	Fish meal (tilapia processing residue)	Wheat gluten meal	Feather meal	Cottonseed meal	Alcohol yeast
Arginine	53.7	0.96	1.14	1.16	0.49	0.67	1.46	1.21	1.37	1.49	0.33	1.04	1.59	0.54**
Histidine	22.0	1.06	0.88	0.78	0.59	0.86	1.05	1.35	1.34	0.65	0.76	0.33**	0.75	0.63
Isoleucine	38.5	0.83	0.72**	0.82	0.79	1.16	1.19	1.01	0.83	0.75	0.89	1.08	0.61	1.02
Leucine	71.4	1.53	0.76	0.94	1.38	2.21	0.99	0.88	0.83	0.72	0.63	0.82	0.49	0.67
Lysine	105.5	0.27**	0.37*	0.30**	0.29**	0.20*	0.60*	0.75**	0.59**	0.64**	0.12**	0.36	0.25**	0.94
Methionine	28.7	1.96	1.11	1.79	1.21	1.43	0.91	1.58	1.97	1.24	1.00	1.76	0.89	0.89
Phenylalanine	35.2	1.22	0.96	1.01	0.86	1.63	1.32	0.91	0.89	0.66	1.31	1.13	0.95	1.11
Threonine	31.9	0.88	0.95	1.19	0.95	0.88	1.14	1.23	1.16	1.10	0.66	1.07	0.84	1.26
Tryptophan	10.0	0.24*	1.02	0.12*	0.22*	0.22**	0.88**	0.31*	0.51*	0.45*	0.06*	0.06*	0.18*	0.16*
Valine	43.2	0.94	0.86	0.96	0.75	0.99	0.97	0.83	0.84	0.77	0.70	0.97	0.68	0.68
EAAI		0.84	0.84	0.75	0.65	0.82	1.02	0.93	0.96	0.80	0.48	0.66	0.61	0.70

¹ Mean values analyzed (n = 9).

* First limiting amino acid; ** Second limiting amino acid.

The crude and digestible amino acid compositions of proteic and energetic test ingredients were used to calculate the chemical scores and EAAI of ingredients relative to tambaqui white muscle protein (Table 5).

Tryptophan was the first limiting amino acid in ten ingredients (CSI: 0.06-0.51) and lysine was the second limiting amino acid in eight ingredients (CSI: 0.25-0.75).

Soybean meal had the highest EAAI (1.02) and was the most complete ingredient relative to the amino acid profile of juvenile tambaqui white muscle. Conversely, wheat gluten meal had the lowest EAAI (0.48).

Discussion

Corn and wheat bran had the highest ADC_{EAA} and ADC_{NEAA} among energetic ingredients, in addition to the highest mean EAAI. In fish, corn digestibility depends on the digestibility capacity of each species (Halver and Hardy, 2002). Buzollo et al. (2018) evaluated the digestibility of crude protein, ether extract, and energy of some ingredients used in tambaqui diets and reported that corn had the highest $ADC_{protein}$ (94.5%) of all ingredients tested. Guimarães et al. (2014), for tambaqui, and Abimorad et al. (2008), for pacu (*Piaractus mesopotamicus*) juveniles, also found high $ADC_{protein}$ of corn (87.5 and 85.8%, respectively). Even though protein digestibility of corn by tambaqui and pacu is high, the comparison of amino acid profiles of ingredients and white muscle shows that corn protein quality was lower for tambaqui (EAAI: 0.84) than for pacu (EAAI: 1.03; Abimorad et al., 2008).

Other studies also reported lower protein and amino acid digestibility in wheat bran than in corn: Furuya et al. (2001) for Nile tilapia (*Oreochromis niloticus*), Abimorad et al. (2008) for pacu, and Wilson et al. (1981) for channel catfish (*Ictalurus punctatus*). According to Furuya et al. (2001), this reduced digestibility may be due to the shorter transit time of wheat bran in the gastrointestinal tract and its high content of crude fiber and non-starch polysaccharides. In fact, some of these polysaccharides, including pentosans and beta-glucans in triticale, may act as digestibility reducers, increasing intestinal viscosity and impairing enzymatic action (Furlan et al., 1997). Nevertheless, based on the high ADC values observed for tambaqui in this study, the digestibility of wheat bran and corn was not affected by crude fiber or polysaccharide content.

In this study, ADC of amino acids of broken rice were on average 16 and 13% lower than those of corn and wheat bran, respectively. These results are in agreement with our previous study (Buzollo et al., 2018), in which we observed low values of $ADC_{protein}$ (71.21%) of broken rice for tambaqui. These low values may be related to the high levels of trypsin inhibitors in broken rice (Butolo, 2002). A similar $ADC_{protein}$ of broken rice (81%) was reported by Abimorad and Carneiro (2004) for pacu. However, even lower $ADC_{protein}$ values were reported for other carnivorous species: 43% for *Pseudoplatystoma corruscans* (Gonçalves and Carneiro, 2003) and 71% for hybrid striped bass (*Morone saxatilis* × *M. chrysops*) (Sullivan and Reigh, 1995). In fact, the enzymatic profile of carnivorous species does not support the use of starchy foods such as broken rice (Lundstedt et al., 2004). Nevertheless, higher $ADC_{protein}$ values of broken rice than the $ADC_{protein}$ of corn found in this study for tambaqui were reported for *Rhamdia quelen* (86%; Oliveira Filho and Fracalossi, 2006) and Nile tilapia (96%; Gonçalves et al., 2007), but these values may reflect methodological differences in fecal collection across studies.

Sorghum is the preferred substitute for corn due to its higher crude protein content and lower concentration of ether extract, lysine, and methionine in its composition (Antunes et al., 2007). In the current study, sorghum had larger quantities of phenylalanine, isoleucine, leucine, lysine, threonine, tryptophan, and valine than corn. Nevertheless, the ADC and EAAI of sorghum were low, indicating that for tambaqui, protein quality was significantly lower in sorghum than in the other energetic ingredients tested. Similar results were reported by Buzollo et al. (2018) for tambaqui and Pezzato et al. (2002), who found lower $ADC_{protein}$ in sorghum than in corn for Nile tilapia. The low nutrient digestibility of sorghum may be due to tannins, which are an antinutritional factor found in many sorghum varieties (Rostagno, 1986).

In general, protein digestibility of energetic ingredients by tambaqui was high. Considering the large contribution of energetic ingredients in commercial diets, we conclude that they contribute significantly to meet the amino acid requirements of the species.

Corn gluten meal and soybean meal had the highest ADC of all proteic ingredients tested. High ADC_{protein} values of corn gluten meal have also been reported for tambaqui 98.09% (Buzollo et al., 2018) and other omnivorous and carnivorous species: 96% for Nile tilapia (Pezzato et al., 2002), 93.6% for largemouth bass (*Micropterus salmoides*; Portz and Cyrino, 2004), 92.3% for haddock (*Melanogrammus aeglefinus*; Tibbetts et al., 2004), 94.4% for cobia (*Rachycentron canadum*; Zhou et al., 2004), 95% for catfish (Oliveira Filho and Fracalossi, 2006), and 95.6% for pacu (Abimorad et al., 2008). In our study, corn gluten meal showed an imbalance in essential amino acid composition (EAAI: 0.82). The CSI for some amino acids such as leucine (2.21) and lysine (0.20) differed from this profile, and a similar imbalance in the same amino acids detected by CSI was also reported for pacu in a study by our research group (Abimorad et al., 2008). Thus, chemical scoring of AA in our study showed that, despite its high digestibility, availability of some amino acids in corn gluten meal is limited, which may hinder its use as the primary protein source in animal diets, further increasing its inclusion cost.

Soybean meal was the best protein source for tambaqui. This ingredient had the highest EAAI (1.02) and a balanced amino acid profile with chemical scores ranging from 0.60 for lysine to 1.46 for arginine. Similar to corn gluten meal, lysine had the lowest CSI (0.60) in soybean meal. Similar results have also been reported for other species, including channel catfish (Lim et al., 1998), Nile tilapia (Furuya et al., 2001; Köprücü and Özdemir, 2005), rainbow trout (*Oncorhynchus mykiss*; Cheng et al., 2003), largemouth bass (Portz and Cyrino, 2004), Murray cod (*Maccullochella peelii peelii*), Australian shortfin eel (*Anguilla australis*; De Silva et al., 2000), and pacu (Abimorad et al., 2008). Other studies observed reduced growth when using soybean meal as the primary protein source in carnivorous fish diets, which was mainly attributed to antinutritional factors and methionine deficiency (Anderson et al., 1993; Baeverfjord and Krogdahl, 1996; Degani, 1987; García-Gallego et al., 1998). Nevertheless, soybean meal is a potential substitute for protein sources such as fish meal and poultry byproduct meal in tambaqui diets.

Mean ADC values of poultry byproduct meal were significantly higher than those of fish meal. Conversely, Abimorad and Carneiro (2004) found no significant difference in ADC between the two ingredients for pacu. Chemical scores of EAA of poultry byproduct meal were high and showed little variation (0.75-1.58), except for tryptophan (0.31), which was limiting for tambaqui. Moreover, poultry byproduct meal had the third highest EAAI (0.93) of all ingredients tested. However, ADC of byproduct meals such as poultry byproduct meal may vary according to the composition and percentage of ingredients used in their production (Thompson et al., 2008).

In this study, the two fish meal sources tested, one made from tilapia filleting byproducts and produced in Brazil and one of Chilean origin made from salmon byproducts, had high ADC_{AA} for all amino acids. However, tilapia processing residue and salmon meal had only satisfactory amino acid profiles, with EAAI values of 0.96 and 0.80, respectively. In addition, the mean ADC for total amino acids was 5.0% higher in the poultry byproduct meal than in the processed tilapia residue. Crude protein and ash content indicate that poultry byproduct meal was a superior protein source for tambaqui over the processed tilapia residue: even though crude protein content was similar (poultry byproduct meal: 65.8%, processed tilapia residue: 60.2%), mineral matter content was higher in the processed tilapia residue (25.2%) than in the poultry byproduct meal (16.3%), indicating that a larger amount of bone was used in the processed tilapia residue production, resulting in an inferior ingredient.

Wheat gluten meal is an excellent protein source, but despite its high ADC values (mean: 84.5%), it had the lowest EAAI (0.48) as a result of the large variation in CSI and the low CSI of lysine (0.12) and tryptophan (0.06). Few studies have evaluated the digestibility of wheat gluten meal in fish (Buzollo et al., 2018; Allan et al., 2000; Robaina et al., 1999; Storebakken et al., 2000; Sugiura et al., 1998). Allan et al. (2000) also reported high ADC_{AA} of wheat gluten meal (100%) for Australian silver perch

(*Bydianus bydianus*). However, none of the studies evaluated the CSI and EAAI of wheat gluten meal, and thus failed to determine the actual protein quality of wheat gluten meal for the species evaluated.

Similar to wheat gluten meal, feather meal and cottonseed meal also had high ADC, but an unbalanced amino acid profile, resulting in low chemical scores for tryptophan (feather meal: 0.06, cottonseed meal: 0.18) and low EAAI values (feather meal: 0.66, cottonseed meal: 0.61). Pezzato et al. (2002) compared the mean ADC_{protein} of proteic ingredients for Nile tilapia and reported that feather meal had the lowest ADC (29.1%), which was lower than the value observed for tambaqui. Feather meal hydrolysis was not as efficient at the time of that study, which may explain the low ADC found by Pezzato et al. (2002). Cottonseed meal had no harmful effects on tambaqui juveniles, despite the presence of gossypol, an antinutritional factor in cottonseed meal that can reduce its digestibility and affect biochemical processes by inhibiting enzyme activity (Beaudoin, 1985).

Mean ADC_{AA} values of alcohol yeast for tambaqui were low in this study. In our previous study (Buzollo et al., 2018), we also observed that alcohol yeast was not classified as a good source of protein and energy for juvenile tambaqui. Similar results were reported by Storebakken et al. (1998) for Atlantic salmon (*Salmo solar*), and the authors attributed the low digestibility of the alcohol yeast to the low digestibility of certain amino acids in its composition. In fact, essential amino acids of alcohol yeast such as arginine, threonine, and valine had low digestibility (<54%) by tambaqui juveniles. This low digestibility may be explained by the high inclusion level of alcohol yeast in test diets (300 g/kg), which has been generally lower in fish diets (Koch et al., 2015; Meurer et al., 2000; Sheikhzadeh et al., 2012). Moreover, the amino acid balance of alcohol yeast was suitable for the species, with little variation in chemical score and EAAI values (0.70).

This is the first study to combine apparent digestibility coefficients and chemical scores to evaluate a large number of ingredients used in fish diets for tambaqui. Our findings may improve least-cost diet formulations and enable effective substitution of ingredients that meet the limiting amino acid requirements of the species. Moreover, our findings may provide the basis for future studies on the digestible amino acid requirements for tambaqui.

Conclusions

Amino acids of proteic and energetic ingredients are well utilized by juvenile tambaqui. Corn and wheat bran have the highest mean ADC for total amino acids among energetic ingredients (95 and 92%, respectively), whereas corn gluten meal and soybean meal have the highest ADC for total amino acids among proteic ingredients (97.6 and 96.6, respectively).

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: T.M.T. Nascimento, E.G. Abimorad and D.J. Carneiro. Data curation: T.M.T. Nascimento, H. Buzollo and D.J. Carneiro. Formal analysis: T.M.T. Nascimento, H. Buzollo and D.J. Carneiro. Funding acquisition: T.M.T. Nascimento and D.J. Carneiro. Investigation: H. Buzollo, L.C.G. Sandre, L.M. Neira and D.J. Carneiro. Methodology: T.M.T. Nascimento, E.G. Abimorad and D.J. Carneiro. Project administration: T.M.T. Nascimento and D.J. Carneiro. Supervision: E.G. Abimorad and D.J. Carneiro. Writing-original draft: T.M.T. Nascimento, H. Buzollo, L.C.G. Sandre, L.M. Neira, E.G. Abimorad and D.J. Carneiro. Writing-review & editing: T.M.T. Nascimento, H. Buzollo and D.J. Carneiro.

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