R. Bras. Zootec., 54:e20240125, 2025
https://doi.org/10.37496/rbz5420240125

Aquaculture
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

PCA of performance, gastrointestinal proteins, and digestive enzymes of tambaqui (*Colossoma macropomum*) fed lysine

Ariany Rabello da Silva Liebl[1]*, **Wilson Massamitu Furuya[2]**, **Márcia Regina Fragoso Machado Bussons[3]**, **Wallice Luiz Paxiúba Duncan[4]**, **Thayson Pinto Lima[5]**, **Maiko Willas Soares Ribeiro[6]**, **Flávia Dayane Félix Farias[6]**, **Márcia Simões dos Santos[6]**, **Ellan Rodrigo Monteiro Paes Fernandes[6]**, **Antônia Jaqueline Vitor de Paiva[7]**, **Paulo Henrique Rocha Aride[1]**, **Adriano Teixeira de Oliveira[1]**

[1] Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Núcleo de Estudos de Invertebrados e Vertebrados da Amazônia, Campus Manaus Centro, Manaus, AM, Brasil.
[2] Universidade Estadual de Ponta Grossa, Departamento de Zootecnia, Ponta Grossa, PR, Brasil.
[3] FishTech Projetos em Piscicultura e Representações Ltda, Rio de Janeiro, RJ, Brasil.
[4] Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Departamento de Morfologia, Manaus, AM, Brasil.
[5] Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Biologia de Água Doce e Pesca Interior, Manaus, AM, Brasil.
[6] Universidade Federal do Amazonas, Programa de Pós-Graduação em Ciência Animal e Recursos Pesqueiros, Manaus, AM, Brasil.
[7] Universidade Federal do Amazonas, Programa de Pós-Graduação em Ciências do Ambiente e Sustentabilidade na Amazônia, Manaus, AM, Brasil.

***Corresponding author:**
ny.rabello@gmail.com

Received: October 28, 2024
Accepted: February 17, 2025

How to cite: Liebl, A. R. S.; Furuya, W. M.; Bussons, M. R. F. M.; Duncan, W. L. P.; Lima, T. P.; Ribeiro, M. W. S.; Farias, F. D. F.; Santos, M. S.; Fernandes, E. R. M. P.; Paiva, A. J. V.; Aride, P. H. R. and Oliveira, A. T. 2025. PCA of performance, gastrointestinal proteins, and digestive enzymes of tambaqui (*Colossoma macropomum*) fed lysine. Revista Brasileira de Zootecnia 54:e20240125.
https://doi.org/10.37496/rbz5420240125

Editors:
Leandro Cesar de Godoy
Fernando Yugo Yamamoto

ABSTRACT - The current study aimed to identify the variables that best explain performance using Principal Component Analysis (PCA), in addition to detecting gastrointestinal proteins, amylase, and lipase in juvenile tambaqui (*Colossoma macropomum*) fed diets with lysine. The diets contained 6.60, 9.72, 12.84, 15.96, 19.08, and 22.20 g.kg^{-1} of total lysine and were supplied to the fish (n = 180; 10 fish per tank) for 90 days. The data that were inserted into the productive performance equations were collected during the experimental period to perform the PCA. At the end of the 90 days, the fish were anesthetized, and gastrointestinal tissues were collected for analysis of protein concentration and enzymology. As a result, PCA showed that PC1 and PC2 (components 1 and 2) explain 80.40% of the performance. In PC1, the highlighted variables were feed and protein efficiencies and final weight, contributing 19.59, 17.31, and 17.12%, respectively. In PC2, the variables that contributed most were dietary intake (14.06%) and weight gain (13.42%). The previously mentioned variables showed high projections in the regression analysis ($P<0.001$). Gastrointestinal proteins varied; however, it was not possible to detect through post-hoc tests which diets influenced them ($P>0.05$). Enzyme activities did not vary ($P>0.05$). Feed efficiency is the variable with the most significant contribution to PC1, and diets containing up to 22.20 g.kg^{-1} of lysine do not influence gastrointestinal proteins nor amylase and lipase enzymes in juvenile tambaqui.

Keywords: amino acids, amylase, fish farming, lipase, nutrition, performance

1. Introduction

According to "The State of World Fisheries and Aquaculture" by the Food and Agriculture Organization of the United Nations, 94 million tons of aquatic animals were produced worldwide in 2022 (FAO, 2024). In 2023, fish production in Brazil totaled 887,029 tons, of which 263,479 tons corresponded to the contribution of native fish species (Peixe BR, 2024). The data presented by the Associação Brasileira da Piscicultura (Peixe BR) for the year 2023 show that there was a 3.1% increase in national fish production compared with the previous year and expose the need to make the most of the productive potential of native fish (Peixe BR, 2024).

In fish production, nutrition accounts for around 70% of investments, and its correct practice requires balanced diets with nutrients of high biological value that meet the requirements in the energy and protein fraction (proteins and amino acids) (Nelson and Cox, 2018). Lysine is an amino acid classified as essential for the physiological activities of fish, such as protein synthesis and deposition of muscle fibers (Huang et al., 2022). Dietary proteins are the main sources of amino acids for production animals (Wilson, 2002). These macronutrients are cleaved by digestive enzymes, and their presence and activity interfere with organic availability, absorption, and assimilation by fish (Fabrikov et al., 2020).

Some studies establish the requirement of lysine to optimize tambaqui performance (Silva et al., 2018; Marchão et al., 2020; Liebl et al., 2022; Martins et al., 2024). For a more accurate conclusion, these studies present different evaluation metrics, such as weight gain, feed conversion, and feed efficiency. However, it is important to understand which variable best summarizes tambaqui performance in relation to lysine. In this sense, Principal Component Analysis (PCA) is a tool that identifies a reduced set of variables projected by the original ones with minimal loss of information. It can elucidate which evaluated variables contribute most to productive performance (Kherif and Latypova, 2020).

Information on productive performance is closely related to the physiology of fish nutrition, including enzyme analyses (Almeida et al., 2006; Liebl et al., 2021). Thus, the study of digestive enzymes makes it possible to understand the adaptations of fish species to diets and to promote strategies to prevent proteins and amino acids from being diverted to meet energy demands (Konnert et al., 2022). For the energy fraction of the diet, the enzymes amylase and lipase act by hydrolyzing, respectively, carbohydrates and lipids, and are generally present in omnivorous fish (Moraes and Almeida, 2020).

Diets formulated considering the omnivorous eating habit and the growth phase, not considering the species' specificities, are still used for native Amazonian fish such as the tambaqui (*Colossoma macropomum* Cuvier, 1818). Tambaqui is highly qualified for fish farming and is traditionally appreciated by the market, mainly in the North of Brazil (Buzollo et al., 2019; Lima et al., 2020). Furthermore, it tends to present reasonable animal performance rates when under correct production (Aride et al., 2016, 2020; Nascimento et al., 2020; Bussons et al., 2021; Polese et al., 2022; Silva Júnior et al., 2022). The objective of the current study was to identify, through PCA, the most significant performance variables and analyze the concentration of gastrointestinal proteins and the activity of amylase and lipase enzymes in juvenile tambaqui fed diets with lysine levels.

2. Material and methods

2.1. Experimental conditions

2.1.1. Animal ethics

The experiment followed the regulations in force at the National Council for the Control of Animal Experimentation, with approval from the ethics committee on the use of animals (protocol number 005/2016) of the Universidade Federal do Amazonas (UFAM).

2.1.2. Fish acclimation and water quality

One hundred and eighty juvenile tambaqui (initial average biometry: 33.54±1.9 g and 11.72±0.36 cm) were randomly distributed and acclimatized in round polyethylene tanks (310 L; n = 10 per tank). The fish were kept under constant oxygenation in the Laboratório de Produção de Organismos Aquáticos at the Universidade Nilton Lins, Manaus, AM, Brazil.

Water quality was maintained using a biological filter and daily siphoning of the tanks (replacement water: 20%). The water temperature, dissolved oxygen content, and pH (26.25±0.06 °C, 4.95±0.32 mg.L^{-1}, and 5.74±0.07, respectively), were measured using a multiparameter device (Horiba® G-50, Kyoto, Japan). The nitrite content was measured using colorimetric kits (Alfakit®, Florianópolis, Brazil) and was maintained between 0.00 and 1.0 mg.L^{-1}. All parameters were monitored to ensure water quality remained within recommendations for welfare and tambaqui production (Mattos et al., 2021; Rebouças et al., 2014).

2.1.3. Experimental diets

For 90 days, the fish were fed diets (27% crude protein) formulated with L-lysine (Ajinomoto®, Chuo, Japan) levels of 0.00, 4.00, 8.00, 12.00, 16.00, and 20.00 g.kg^{-1}, which correspond to 6.60, 9.72, 12.84, 15.96, 19.08, and 22.20 g.kg^{-1} of total lysine. The ingredients of each diet (Table 1) were extruded at the

Table 1 - Calculated and analyzed composition (as fed basis) of the experimental diets with increasing lysine levels for juvenile tambaqui (*C. macropomum*)

Item	Dietary lysine (g.kg^{-1})					
	6.60	9.72	12.84	15.96	19.08	22.20
Ingredient[1] (g.kg^{-1})						
Corn	356.20	360.20	364.20	368.20	372.20	376.20
Corn gluten 60	230.00	230.00	230.00	230.00	230.00	230.00
Rice flour	120.00	120.00	120.00	120.00	120.00	120.00
Wheat bran	120.00	120.00	120.00	120.00	120.00	120.00
Fish meal	60.00	60.00	60.00	60.00	60.00	60.00
Dicalcium phosphate	20.00	20.00	20.00	20.00	20.00	20.00
Soy oil	10.00	10.00	10.00	10.00	10.00	10.00
Ashes and vitamin supplement[3]	7.00	7.00	7.00	7.00	7.00	7.00
Calcitic limestone	5.00	5.00	5.00	5.00	5.00	5.00
Sodium bicarbonate	2.00	2.00	2.00	2.00	2.00	2.00
Antifungal	2.00	2.00	2.00	2.00	2.00	2.00
Butyl hydroxy toluene (BHT)	0.20	0.20	0.20	0.20	0.20	0.20
Mix of crystalline amino acids[4]	17.60	17.60	17.60	17.60	17.60	17.60
L-glutamic acid	50.00	42.00	34.00	26.00	18.00	10.00
L-lysine	0.00	4.00	8.00	12.00	16.00	20.00
Calculated and analyzed composition						
Crude protein[2] (%)	26.62	27.16	26.40	26.97	26.83	27.67
Total lipids[1] (%)	4.00	4.00	4.00	4.00	4.00	4.00
Gross energy (kcal.kg^{-1})	3601.0	3613.6	3626.2	3638.8	3651.4	3664.0
Moisture[2] (%)	5.51	4.76	4.28	5.69	6.28	5.52
Total minerals[2] (%)	5.08	4.99	5.04	5.13	5.14	4.91
Calcium[1] (%)	1.10	1.10	1.10	1.10	1.10	1.10
Available phosphorus[1] (%)	0.70	0.70	0.70	0.70	0.70	0.70

[1] Composition calculated according to Furuya et al. (2006) and Rostagno et al. (2011).
[2] Composition analyzed (AOAC, 2005).
[3] Composition per kg of product: selenium, 75.00 mg; copper, 2,000.00 mg; choline chloride, 125.00 g; manganese, 3750.00 mg; zinc, 20.00 g; iron, 15.00; iodine, 125.00 mg; niacin, 7,800.00 mg; folic acid, 750.00 mg; pantothenic acid, 3,750.00 mg; biotin, 125.00 mg; vitamin C, 53.00 g; iodine, 125.00 g; vitamin A, 2,000.000.00 IU; vitamin D3, 500,000.00 IU; vitamin E, 15,000.00 IU; vitamin K3, 1,000.00 mg; vitamin B1, 2,500.00 mg; vitamin B2, 2,500.00 mg; vitamin B6, 2,000.00 mg; vitamin B12, 5,000.00 mg.
[4] DL-methionine, 2.50 g; L-arginine, 2.50 g; L-histidine, 2.50 g; L-isoleucine, 2.50 g; L-phenylalanine, 2.50 g; L-threonine, 2.50 g; L-tryptophan, 1.60 g; L-valine, 1.00 g.

Laboratório de Aquicultura of the Instituto Nacional de Pesquisas da Amazônia (INPA) using a 2.5-mm single screw. Initially, they were ground in a knife mill (Tecnal®, Piracicaba, Brazil; 0.5 mm) and added to crystalline amino acids. The mixture was homogenized and moistened with 20% water and dried in a forced-ventilation oven (55 °C; 24 h). Subsequently, the prepared diet was offered to the fish until apparent satiety, at a frequency of four times a day (08:00, 11:00, 14:00, and 17:00 h).

2.2. Data collection and analysis

2.2.1. Productive performance

During the entire experimental period, the diets were weighed before being offered. Subsequently, the unconsumed fraction was removed, dried in an oven, and weighed on a digital scale (accuracy of 0.001 g; Gehaka®, São Paulo, Brazil). The fish were biometrically measured for weight (g) using a digital scale (accuracy of 0.001 g; Gehaka®, São Paulo, Brazil) (Figure 1).

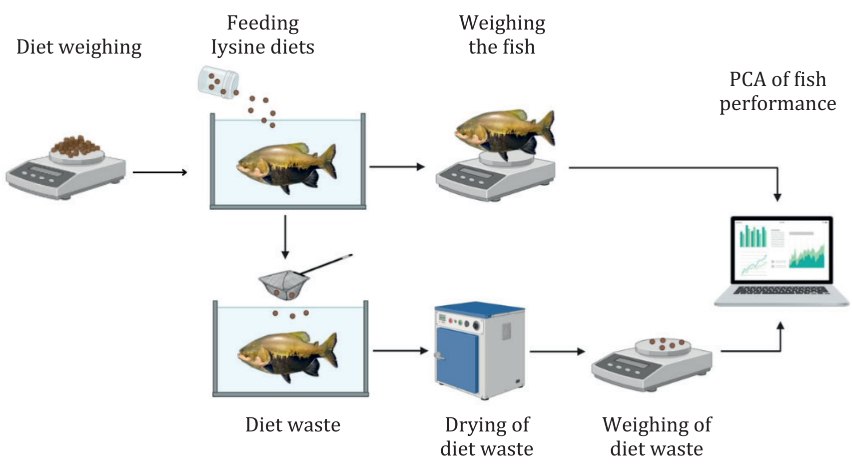

Figure 1 - Infographic of data collection and processing in Principal Component Analysis (PCA) of the productive performance of juvenile tambaqui (*C. macropomum*) fed diets with increasing lysine levels.

The performance data used in PCA (Table 2) were calculated according to Liebl et al. (2022) with the following equations:

Final weight (FW; g) = average final weight of the fish × number of fish^{-1};

Body weight gain (BWG; %) = 100 × {[final weight (g) − initial weight (g)] × initial weight (g)$^{-1}$};

Feed conversion (FCR; kg.kg^{-1}) = dry feed fed (kg) × body weight gain (kg)$^{-1}$;

Lysine intake (LI; %) = {[dry lysine intake (g) × final fish weight (g)$^{-1}$] days fed^{-1}} × 100;

Lysine efficiency (LE; %) = [body weight gain (g) × lysine intake (g)$^{-1}$] × 100;

Protein efficiency (PE; %) = [body weight gain (g) × protein intake (g)$^{-1}$] × 100;

Feed efficiency (FE; %) = [body weight gain (g) × dry feed intake (g)$^{-1}$] × 100;

Feed intake (FI; %) = {[dry feed intake (g) × final fish weight (g)$^{-1}$] days fed^{-1}} × 100.

Table 2 - Average performance values and nutritional rates of juvenile tambaqui (*C. macropomum*) fed diets with increasing lysine levels

Growth performance	Dietary lysine (g.kg^{-1})					
	6.60	9.72	12.84	15.96	19.08	22.20
Initial weight (g)	31.00±2.57	32.2±2.33	32.96±2.29	33.90±2.40	34.43±0.89	38.80±2.89
Final weight (g)	58.09±3.14	68.02±6.42	67.84±2.98	80.69±4.68	70.79±5.93	79.86±7.43
Body weight gain (%)	87.39±11.13	111.24±10.48	105.79±7.45	138.01±31.96	105.59±22.10	105.84±27.22
Lysine intake (g.fish^{-1})	0.12±0.00	0.16±0.01	0.21±0.00	0.22±0.01	0.30±0.02	0.31±0.02
Feed intake (g.fish^{-1})	1.09±0.00	1.02±0.10	0.99±0.04	0.93±0.03	0.98±0.04	0.98±0.00
FCR (kg.kg^{-1})	2.12±0.12	1.76±0.14	1.74±0.10	1.45±0.11	1.73±0.81	1.74±0.20
LE (%)	7.15±0.43	5.88±0.46	4.49±0.23	4.31±0.35	3.02±0.43	2.60±0.32
PE (%)	1.77±0.12	2.12±0.28	2.18±0.12	2.52±0.16	2.15±0.17	2.01±0.25
FE (%)	47.15±2.85	57.03±3.48	57.11±4.48	68.75±5.59	57.03±6.92	57.59±8.22

FCR - feed conversion ratio; PE - protein efficiency; FE - feed efficiency; LE - lysine retention efficiency.

2.2.2. Gastrointestinal proteins and digestive enzymes

Tissues of the gastrointestinal system (stomach, pyloric cecum, and intestine) were extracted for protein quantification (g.dL^{-1}) and verification of amylase (U.mg^{-1}) and lipase (U.mg^{-1}) enzyme activity (Figure 2).

The extraction was performed on randomly selected, anesthetized fish kept on feed restriction for 24 h (benzocaine 100 mg.L^{-1}). The fish were euthanized with a high-dose anesthetic (benzocaine 250 mg.L^{-1}), followed by medullary section (Leary et al., 2020). They were sectioned on the surface at −4 °C, with an incision in the dorsoventral region to remove the gastrointestinal tissues. The extracted intestine was sectioned into three equal portions (anterior, middle, and posterior). The collected samples were placed in liquid nitrogen and kept at −20 °C until analysis (Figure 2).

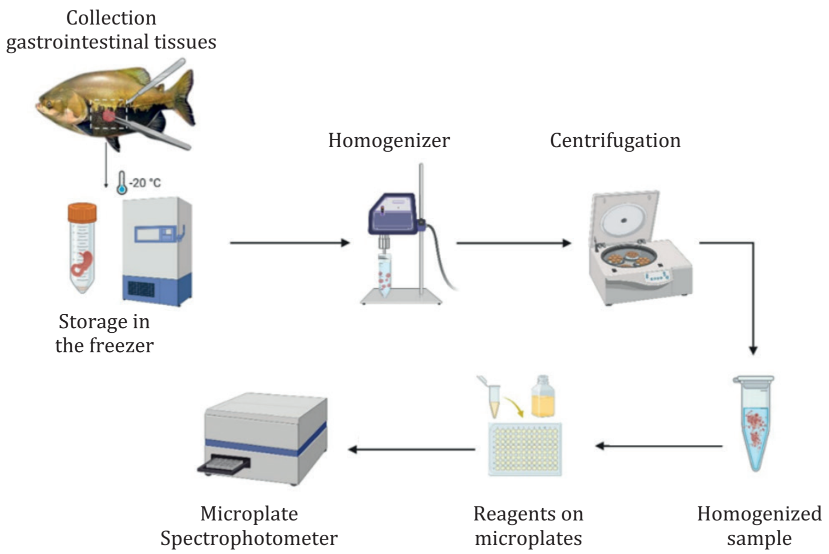

Figure 2 - Infographic of the collection and processing of samples used to analyze the concentration of gastrointestinal proteins and the enzymes amylase and lipase in juvenile tambaqui (*C. macropomum*) fed diets with increasing lysine levels.

The samples (n = 54 per tissue; nine replicas per diet) were homogenized (Potter-Elvehjem) with 1 mL of ultrapure water in a glass tube submerged in ice. The homogenate was centrifuged (Nova®, NI 1800, Piracicaba, Brazil) at 10,000 rpm for 10 min, and the supernatant was placed in microtubes (200 µL).

Protein quantification in tissues was performed using the Bradford method (1976) with a commercial colorimetric kit (Bio-Rad®, Hercules, USA). The analysis took place in 96-well microplates with an absorbance reading of 595 nm, following the manufacturer's recommendations.

2.2.3. Lipase digestive enzyme activity

Lipase activity was determined (Albro et al., 1985) using 20 µL of extracted supernatants reacting with substrate (50 µL) 4-nitrophenyl myristate (10 mM) in Tris-HCl (200 mM), taurodeoxycholate (2 mM), Triton X-100 (0.4%), and gum arabic (0.1%). After 60 min, the reaction was stopped with calcium ($CaCl_2$; 10 mM), sodium (NaCl; 10 mM), and Tris-HCl chlorides at pH 8.0 (130 µL). Reading was performed at an absorbance of 405 nm. Data were calculated using information on tissue protein concentration and expressed as the amount of hydrolyzed substrate in 1 µmol.mg^{-1} of protein.minute^{-1} (U.mg^{-1} of protein).

2.2.4. Activity of the digestive enzyme amylase

In determining amylase activity, the method used was adapted from Bernfeld (1955) for a chemical reaction of 130 µL of buffer solution with 100 mM sodium acetate (pH 7.6), 20 mM $CaCl_2$, 10 mM NaCl, and 5% starch, with 20 µL of sample supernatant and 50 µL of Lugol (1% iodine and 2% potassium iodide). Amylase absorbance was read after incubation for 60 min at 20 °C in a spectrophotometer at 554 nm and was expressed as a function of protein concentrations and maltose calibration curve as µmol of maltose.mg^{-1} protein.minute^{-1} (U.mg^{-1} of protein).

2.2.5. Statistical analysis

The experiment with experimental diets was organized in a completely randomized design, containing six treatments and three replications. Before the other analyses, the normality (Shapiro-Wilk test) and homogeneity (Bartlett test) of data were checked. The results of the quantification of gastrointestinal proteins and digestive enzyme activity were subjected, respectively, to the Analysis of Variance (ANOVA) and the Kruskal-Wallis test ($P<0.05$). When variation in means was found, Tukey and Bonferroni post hoc tests were used ($P<0.5$).

The performance indices were summarized in a PCA and constituted the dependent variables in the multiple regression analyses. The independent variables were adjusted to the model for the first three principal components (PC). This method was used to extract the values that explained most of the data variation, helping to reduce the multicollinearity of the data that can affect the analysis of conventional regressions. Thus, the following multiple regression model was used:

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \varepsilon$$

to

$$Y = \alpha 0 + \alpha 1PC1 + \alpha 2PC2 + \cdots + \alpha kPCk + \varepsilon,$$

in which Y = dependent or response variable (animal performance); PC1, PC2, and PC3 = independent variables (principal component values), being the linear combination of the original variables; α0 = intercept of the model, which are the regression coefficients related to the principal components; α1 and α2 = regression coefficient explaining how much each principal component contributes to the prediction of Y; and ε = residual, explaining the variation in Y that cannot be explained by the independent variables.

All statistical analyses were performed using the statistical software R® (r-project, Auckland, New Zealand), version 4.4.1.

3. Results

3.1. Principal Component Analysis of performance

The PCA showed that the sum of the first two components (PC1 and PC2; Dimensions 1 and 2) explains between 80.31 (Table 3) and 80.40% (scree plot; Figure 3). The sum of the other components (Dimensions 3 to 8) explains the remaining percentage (Figure 3, Table 3).

The prominent variables in all components are observed in regions far from the center of the graph (Figure 4), with each component being the linear combination of the original variables. In component 1, the variables that stood out the most were final weight, protein efficiency, and feed efficiency, while in component 2, they were lysine intake, lysine efficiency, and weight gain (Table 4).

Table 3 - The percentage of the contribution of each component is formed by the set of performance variables of juvenile tambaqui (*C. macropomum*) in the Principal Component Analysis

Components	Variable (%)		
	Eigenvalue	Variance percent	Cumulative variance percent
1	4.40	55.06	55.06
2	2.02	25.25	80.31
3	0.75	9.41	89.73
4	0.58	7.335	97.06
5	0.15	1.959	99.02
6	0.05	0.625	99.65
7	0.02	0.28	99.93
8	0.00	0.061	100.00

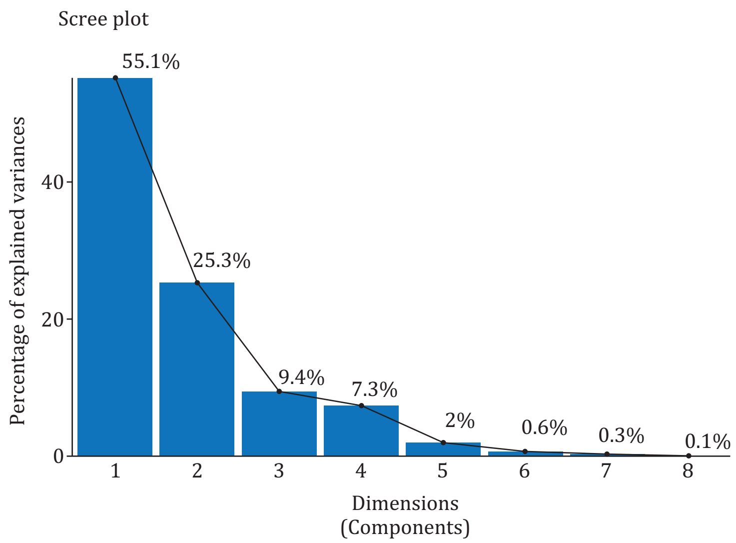

Figure 3 - Scree plot (eigenvalues) with the percentage of variance explained for each component formed by the performance variables of juvenile tambaqui (*C. macropomum*) within the Principal Component Analysis.

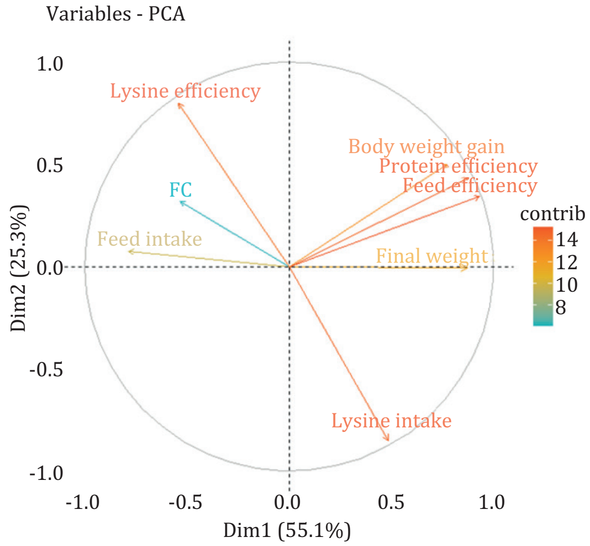

Figure 4 - Mode of each performance variable of juvenile tambaqui (*C. macropomum*) within dimensions 1 and 2 of the Principal Component Analysis (PCA).

Table 4 - Contribution percentage of each variable to the performance of juvenile tambaqui (*C. macropomum*) within the dimensions of the Principal Component Analysis

Variable (%)	Component				
	1	2	3	4	5
Final weight	17.12	0.00	17.13	4.62	56.29
Body weight gain	13.42	12.25	0.85	22.05	10.83
Feed conversion	6.50	5.02	73.30	8.48	6.37
Lysine intake	5.30	35.77	1.18	0.11	14.93
Feed intake	14.06	0.26	0.90	61.51	1.36
Lysine efficiency	6.66	31.61	5.68	1.37	1.22
Protein efficiency	17.31	9.21	0.92	1.30	3.04
Feed efficiency	19.59	5.83	0.00	0.51	5.92

The PCA graph of individuals (Figure 5) presented a few individuals with low observations (highlighted in shades of blue, closer to the center or origin of the graph) and intermediate observations (highlighted in shades of yellow). High-quality individuals are in greater quantity (highlighted in red) and located further from the center of the graph.

The regression analysis for each performance variable of tambaqui juveniles was carried out based on the first three PC (PC1, PC2, and PC3). Principal component 1 showed high projections (eigenvectors or loads) for the variables final weight, weight gain, protein efficiency, and feed efficiency ($P<0.001$). In PC2, the projections were for lysine intake and efficiency ($P<0.001$). In PC3, the final weight variable (estimate: −4.00; t value: −3.94) showed an inversely significant relationship with feed conversion (estimate: −0.41; t value: −11.36) and other variables ($P<0.001$).

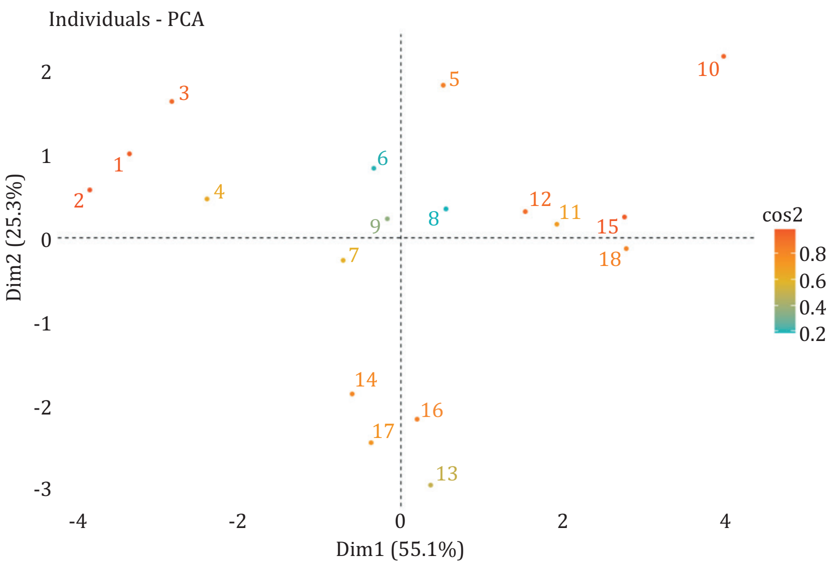

Figure 5 - Distribution of individual values (observations) of the components formed by the performance variables of juvenile tambaqui (*C. macropomum*) within dimensions 1 and 2 of the Principal Component Analysis (PCA).

3.2. Protein quantification

Protein concentrations in the stomach, pyloric cecum, and anterior and posterior portions of the intestine did not vary when lysine was added to the diet (P<0.01; Table 5). Different lysine levels in the diet influenced the middle portion of the intestine (P<0.01). However, according to Tukey (P>0.05) and Bonferroni (P>0.5) post hoc tests, it was not possible to identify at which level the influence occurred.

Table 5 - Protein concentration in the tissues of the stomach, pyloric caeca, proximal intestine, middle intestine, and distal intestine of juvenile tambaqui (*C. macropomum*) fed diets with increasing lysine levels

Protein ($g.dL^{-1}$)	Dietary lysine ($g.kg^{-1}$)						P-value
	6.60	9.72	12.84	15.96	19.08	22.20	
Stomach	18.74±0.98	21.29±4.51	23.88±4.43	19.21±1.30	19.30±1.71	19.85±2.50	0.53
Pyloric caeca	15.22±1.50	17.39±1.51	17.10±0.40	17.95±0.25	16.71±0.46	16.73±1.36	0.26
Proximal intestine	13.50±0.80	14.36±0.89	15.32±1.69	15.18±0.23	15.17±1.05	15.54±0.19	0.32
Middle intestine	13.87±0.68	13.69±0.35	14.45±0.76	15.23±0.22	15.20±0.38	15.10±0.52	0.03*
Distal intestine	13.12±0.50	13.25±0.91	13.46±0.62	13.58±0.50	13.79±0.60	14.33±0.68	0.56

* Means with significant difference according to Analysis of variance (P<0.05).

3.3. Digestive enzymes: Amylase and lipase activity

No changes were observed between the average activities of amylase (Table 6) and lipase (Table 7) and in the tissues of the stomach, pyloric ceca, and intestine (anterior, middle, and posterior) of tambaqui juveniles fed diets containing lysine (P>0.05).

Table 6 - Amylase enzyme activity in the tissues of the stomach, pyloric caeca, proximal intestine, middle intestine, and distal intestine of juvenile tambaqui (*C. macropomum*) fed diets with increasing lysine levels

Amylase ($U.mg^{-1}$ of protein)	Dietary lysine ($g.kg^{-1}$)						P-value
	6.60	9.72	12.84	15.96	19.08	22.20	
Stomach	308.07±105.24	233.75±14.98	246.93±80.29	300.23±144.05	192.73±11.68	215.82±12.03	0.39
Pyloric caeca	133.64±45.60	128.70±49.81	145.29±70.35	91.14±22.17	155.26±54.13	117.45±50.79	0.74
Proximal intestine	276.57±29.92	215.02±29.43	265.52±113.30	184.92±26.67	194.83±13.06	199.05±36.11	0.36
Middle intestine	198.55±41.28	144.78±20.55	183.23±82.51	129.58±19.06	144.34±35.57	134.13±40.93	0.69
Distal intestine	284.48±22.44	229.96±64.93	323.08±95.42	187.74±29.39	211.15±26.55	208.16±64.88	0.41

All means were analyzed with Kruskal–Wallis test (P<0.05).

Table 7 - Lipase enzyme activity in the tissues of the stomach, pyloric ceca, proximal intestine, middle intestine, and distal intestine of juvenile tambaqui (*C. macropomum*) fed diets with increasing lysine levels

Lipase ($U.mg^{-1}$ of protein)	Dietary lysine ($g.kg^{-1}$)						P-value
	6.60	9.72	12.84	15.96	19.08	22.20	
Stomach	5.35±2.10	2.78±0.31	2.88±0.43	2.79±0.36	3.32±0.68	4.38±1.14	0.19
Pyloric caeca	2.19±0.35	2.79±0.53	2.42±0.43	2.19±0.59	2.31±0.82	5.96±4.06	0.33
Proximal intestine	1.69±1.06	1.55±0.87	1.87±0.39	2.29±0.74	2.12±0.75	5.85±5.36	0.85
Middle intestine	4.84±2.03	4.72±0.89	7.06±1.68	5.64±2.12	4.61±1.31	8.99±2.95	0.26
Distal intestine	0.86±0.82	1.94±0.89	1.46±0.91	1.30±0.77	1.28±0.65	4.85±5.80	0.89

All means were analyzed with Kruskal–Wallis test (P<0.05).

4. Discussion

4.1. Principal Component Analysis productive performance

Principal Component Analysis is based on original data and there are no simple statistical tests to assist in decision-making about the components (Oliveira et al., 2017; Iodice D'Enza et al., 2022). According to Kherif and Latypova (2020), the responses obtained in the percentage of cumulative variance tend to guide this stage and can be set at a minimum of 80.00%. The present study showed that the first two components together (scree plot; Figure 3) are sufficient to explain approximately 80.00% of the productive performance of tambaqui juveniles fed up to 22.20 $g.kg^{-1}$ of total lysine. Thus, the decision about the significant components of the current study can be inferred through the percentage of cumulative variance recorded in PC2 (80.31%; Table 4).

Of the variables that make up PC1 (Table 3), feed efficiency, protein efficiency, final weight, and weight gain were highly projected in the linear regression, being good indicators of the influence of lysine on tambaqui performance. However, feed efficiency contributed the most to the explanation of the data in the PCA (19.59%). Increased feed efficiency values reflect an excellent diet (Zlaugotne et al., 2022). High-quality diets favor reducing intake with a concomitant increase in fish growth and improvement in other performance variables (Zlaugotne et al., 2022). Thus, the high contribution of feed efficiency in the current study shows that a substantial fraction of the nutrients in diets balanced with lysine levels were used by tambaqui juveniles to maximize productive performance.

Liebl et al. (2022), analyzing juvenile tambaqui fed diet with lysine levels, found that the feed efficiency rate increased to the estimated level of 15.96 $g.kg^{-1}$ of lysine, with the formation of a plateau afterward. The level seen by the authors corroborated the lysine level that showed the best feed conversion (1.45 $kg.kg^{-1}$) in the same study, reaffirming the contribution of this variable to performance responses. According to Bomfim et al. (2010), the maximum estimated value for feed efficiency is reached when the fish can express its full potential for protein deposition.

In the current study, lysine efficiency, weight gain, and lysine intake are part of PC2, which explains around 25.25% (Table 4) of the tambaqui performance responses. In the graphical figure on the mode of the variables (Figure 4), it is observed that vectors represent the efficiency of lysine and weight gain and form an obtuse angle, indicating a negative correlation. This information corroborates studies that show the tendency of lysine efficiency (relationship between weight gain and dietary and lysine intake) to reduce proportionally to the increase in lysine levels in the diet (Hua and Bureau, 2012; Hua et al., 2019). The fact indicates that additions of lysine to tambaqui diets generate a minor contribution of the amino acid to the fish's weight gain. The behavior of amino acid efficiency in the fish body is a response to nutritional requirements and catabolism during the growth and maintenance of the animal's body (Hua et al., 2019).

Although in the current study the first two components presented observations with high-quality representation (Figure 5), the third component (Table 3) was equally analyzed. The final weight variable, part of PC3, showed an inverse behavior about feed conversion (Figure 4). Thus, the final weight increases as feed conversion decreases according to the model used in the linear regression analysis. Feed conversion (quotient between dietary intake and weight gain) is essential in fish production and varies according to characteristics inherent to the species, nutrients, and diet composition, among others (Fry et al., 2018; Stone et al., 2024). However, PCA placed this variable in the component that explains a smaller amount of information (9.4%; Table 3).

Feed efficiency (quotient between weight gain and dietary intake) is the most prominent variable, and the most impactful component for the PCA may be related to the structuring of the data within each variable and the responses they generate. Feed conversion translates to the diet necessary for weight gain, while feed efficiency explains how much weight gain was influenced by diet intake. Thus, feed conversion is essential for fish production as it meets the economic aspect of production (expenses on diet). Otherwise, feed efficiency can generate a robust and effective response when the objective is to understand the contribution of diet quality to fish performance, as proposed in the current study. It should be noted that the higher the diet quality, the lower the quantity offered. Thus, the response generated by feed efficiency will also have an economic impact, being crucial to formulate and produce cost-effective diets (Ayub et al., 2023).

4.2. Quantification of gastrointestinal proteins

Diets with an imbalance in lysine tend to interfere with the use of all nutrients by the animal organism (Ahmed and Ahmad, 2021). This occurs because lysine is the most limiting amino acid in ingredients used as a protein source for fish (Ebeneezar et al., 2019). In the current study, protein concentrations were influenced by diet with manifestation in the middle intestine ($P<0.05$) of juvenile tambaqui. However, it was impossible to identify at which lysine level the variation occurred. The issue may be related to a slight variation between the data, making it difficult to detect the tests used accurately.

Tambaqui can modulate the action of its main diets to the composition of the diets when necessary, allowing better use of compounds from the amino acid fraction, lipids, and carbohydrates (Almeida et al., 2006). Furthermore, the diet provided to the juvenile tambaqui in the present study was composed mainly of bioavailable amino acids, ready to be integrated.

Fish do not absorb intact protein (Wilson, 2002). In general, intact protein is cleaved into peptides (di- and tripeptides) and bioavailable amino acids, such as L-lysine (Bakke et al., 2010; Kombat et al., 2024). The process that releases L-lysine occurs after hydrolysis by the enzyme trypsin, allowing the amino acid to be absorbed by intestinal cells (NRC, 2011; Nelson and Cox, 2018). According to Rotta (2003), the middle intestine is specialized in the absorption of bioavailable proteins and amino acids, which explains why the influence of total protein from diets rich in amino acids was significant, specifically in the middle intestine of juvenile tambaqui.

4.3. Digestive enzymes: Amylase and lipase activity

The lack of influence (P>0.05) of diets on amylase and lipase activity in the stomach, intestine, and pyloric ceca shows that the diets were balanced and equally met the energy demands of tambaqui. Concomitantly, lysine concentrations did not interfere with the metabolism of amylase and lipase in juvenile tambaqui. For striped catfish (*Pangasius hypophthalmus*) analyzed by Liaqat et al. (2024), intestinal amylase and lipase were higher (P<0.05) in fish fed different combinations of lysine, methionine, and tryptophan. The authors also recorded a decline in enzyme activities in fish fed diets without amino acid supplementation, showing that the correct balance of amino acids tends to favor the action of the digestive enzymes amylase and lipase, in addition to proteases.

Although there was no difference (P>0.05) in the current study with juvenile tambaqui, amylase was recorded for all treatments and in all tissues analyzed (stomach, intestine, and pyloric ceca). Corrêa et al. (2007) conducted a study with diets containing starch levels (30, 40, and 50%) for juvenile tambaqui, and the fish responded with an increase in amylase in treatments with higher levels, which was more significant (P<0.05) in the pyloric ceca. Similarly, Almeida et al. (2006) assumed the pyloric ceca of tambaqui fed different diets (protein: 205 to 350 g.kg^{-1}; lipids: 49 to 145 g.kg^{-1}) as the fraction of the gastrointestinal tract with the highest amylase activity (P<0.05). The pyloric ceca act in the hydrolysis of starch, and this amylase activity tends to increase with an increase in dietary lipids, concomitant with a reduction in amino acids or proteins (Almeida et al., 2006). Thus, the lack of significant differences between treatments for pyloric cecum in the current study may be associated with the fact that the diets were prepared with different lysine levels (6.60 to 22.20 g.kg^{-1}), but with equivalent lipid concentrations.

For lipid hydrolysis, the lipase enzyme in teleosts can begin its activity in the stomach and continue through the gastrointestinal tract (Costa and Baldisserotto, 2021). However, triglyceride hydrolysis occurs mainly in the pyloric cecum and in the initial portion of the intestine (Nolasco-Soria, 2021). Analyzing lipase in juvenile tambaqui, Almeida et al. (2006) found greater activity in the stomach, with a reduction observed in the same proportion as the increase in lipid concentration in the diet. In the present study, the highest lipase concentrations were observed, with no difference between them (P>0.05), in the middle intestine of tambaqui (181.75 to 539.24 U.mg^{-1} of protein). However, lower and active concentrations of the lipolytic enzyme were present throughout the gastrointestinal tract. Based on the above, it can be stated that, since in the current study there was no oscillation in the concentrations of lipids added to the diets and the concentrations of lysine used did not interfere in this aspect, the lipase activity did not vary.

5. Conclusions

According to the PCA, the set of variables in component 1 (PC1: feed efficiency, protein efficiency, final weight, weight gain, and diet intake) added to component 2 (PC2: weight gain and lysine intake) are sufficient to express 80.4% of the performance of juvenile tambaqui fed diet with lysine levels. These variables together can be characterized as good indicators of the performance/lysine relationship analyzed in this study. However, feed efficiency was the variable with the most expressive contribution to PC1, which also showed high projections by linear regression analysis. It was impossible to identify the influence of diet on the concentration of gastrointestinal proteins, and the activities of amylase and lipase enzymes were not influenced by the presence of up to 22.20 g.kg^{-1} of dietary lysine for juvenile tambaqui.

Data availability

Data supporting the conclusions of the current study are available from the corresponding author upon request.

Author contributions

Conceptualization: Liebl, A. R. S.; Furuya, W. M.; Bussons, M. R. F. M. and Oliveira, A. T. **Data curation:** Furuya, W. M.; Bussons, M. R. F. M.; Duncan, W. L. P. and Oliveira, A. T. **Formal analysis:** Liebl, A. R. S.; Duncan, W. L. P.; Lima, T P. and Ribeiro, M. W. S. **Funding acquisition:** Liebl, A. R. S.; Paiva, A. J. V. and Aride, P. H. R. **Investigation:** Liebl, A. R. S.; Farias, F. D. F.; Santos, M. S. and Paiva, A. J. V. **Methodology:** Liebl, A. R. S.; Ribeiro, M. W. S.; Farias, F. D. F.; Fernandes, E. R. M. P. and Aride, P. H. R. **Project administration:** Liebl, A. R. S. **Supervision:** Oliveira, A. T. **Writing – original draft:** Liebl, A. R. S.; Lima, T P. and Oliveira, A. T. **Writing – review & editing:** Liebl, A. R. S.; Duncan, W. L. P.; Santos, M. S. and Fernandes, E. R. M. P.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful for the financial support from the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM/Amazonas, Brazil) through Call for Proposals 013/2021 — Programa de Desenvolvimento Científico e Tecnológico Regional (PDCTR/AM), with the project "Consumo e produção responsáveis: Potencial de subprodutos da pesca e aquicultura na Amazônia inseridos na nutrição e alimentação humana e animal" (SEI grant no.: 01300.011). Thanks are extended to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship granted (313567/2022-0) to the first author in relation to the aforementioned project, allowing the continuity of the work developed during the doctorate. Likewise, the authors would like to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support provided during the first analyses of this research, and Núcleo de Estudos de Invertebrados e Vertebrados da Amazônia (NEIVA) for the intellectual support and human resources necessary to carry out the research. The authors also thank the Fundação de Apoio ao Ensino, Pesquisa, Extensão e Interiorização (FAEPI) of the Instituto Federal de Educação, Ciência e Tecnologia do Amazonas (IFAM) for the funding that made the publication of this manuscript possible.

References

Ahmed, I. and Ahmad, I. 2021. Dietary lysine modulates growth performance, haemato-biochemical indices, non-specific immune response, intestinal enzymatic activities and antioxidant properties of rainbow trout, *Oncorhynchus mykiss* fingerlings. Aquaculture Nutrition 27:124-139. https://doi.org/10.1111/anu.13409

Albro, P. W.; Hall, R. D.; Corbett, J. T. and Schroeder, J. 1985. Activation of nonspecific lipase (EC 3.1.1.-) by bile salts. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 835:477-490. https://doi.org/10.1016/0005-2760(85)90117-1

Almeida, L. C.; Lundstedt, L. M. and Moraes, G. 2006. Digestive enzyme responses of tambaqui (*Colossoma macropomum*) fed on different levels of protein and lipid. Aquaculture Nutrition 12:443-450. https://doi.org/10.1111/j.1365-2095.2006.00446.x

AOAC - Association of Official Analytical Chemists. 2005. Official methods of analysis of the AOAC International. AOAC International, Gaithersburg.

Aride, P. H. R.; Oliveira, A. T.; Oliveira, A. M.; Ferreira, M. S.; Baptista, R. B.; Santos, S. M. and Pantoja-Lima, J. 2016. Growth and hematological responses of tambaqui fed different amounts of cassava (*Manihot esculenta*). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 68:1697-1704. https://doi.org/10.1590/1678-4162-8704

Aride, P. H. R.; Oliveira, A. M.; Ferreira, M. S.; Liebl, A. R. S.; Comassetto, L. E.; Ladislau, D. S.; Bassul, L. A.; Silva, B. R.; Mattos, D. C.; Lavander, H. D.; Souza, A. B.; Polese, M. F.; Ribeiro, M. W. S.; Castro, P. D. S. and Oliveira, A. T. 2020. Growth and hematological responses of tambaqui, *Colossoma macropomum* fed different levels of rice, *Oryza* spp. Brazilian Journal of Biology 81:962-968. https://doi.org/10.1590/1519-6984.232560

Ayub, A.; Rasool, F.; Khan, N.; Qaisrani, S. N.; Parveen, S.; Anjum, K. M.; Fatima, M.; Matiullah; Mahmood, S. and Zulfiqar, T. 2023. Limiting amino acids supplementation in low crude protein diets and their impacts on growth performance and carcass composition in *Labeo rohita* (rohu) adult fish. Brazilian Journal of Biology 83:e249422. https://doi.org/10.1590/1519-6984.249422

Bakke, A. M.; Glover, C. and Krogdahl, A. 2010. Feeding, digestion and absorption of nutrients. p.57-110. In: Fish physiology: The multifunctional gut of fish. Grosell, M.; Farrell, A. P. and Brauner, C. J., eds. Academic Press. https://doi.org/10.1016/S1546-5098(10)03002-5

Bernfeld, P. 1955. Amylases, *α* and β. Methods in Enzymology 1:149-158. https://doi.org/10.1016/0076-6879(55)01021-5

Bomfim, M. A. D.; Lanna, E. A. T.; Donzele, J. L.; Quadros, M.; Ribeiro, F. B. and Souza, M. P. 2010. Níveis de lisina, com base no conceito de proteína ideal, em rações para alevinos de tilápia-do-nilo. Revista Brasileira de Zootecnia 39:1-8. https://doi.org/10.1590/S1516-35982010000100001

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Bussons, I. N. B.; Souza, E. S.; Aride, P. H. R.; Duncan, W. L. P.; Pantoja-Lima, J.; Furuya, W. M.; Oliveira, A. T.; Bussons, M. R. F. M. and Faggio, C. 2021. Growth performance, hematological responses and economic indexes of *Colossoma macropomum* (Cuvier, 1818) fed graded levels of glycerol. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 249:109122. https://doi.org/10.1016/j.cbpc.2021.109122

Buzollo, H.; Sandre, L. C. G.; Neira, L. M.; Nascimento, T. M. T.; Jomori, R. K. and Carneiro, D. J. 2019. Digestible protein requirements and muscle growth in juvenile tambaqui (*Colossoma macropomum*). Aquaculture Nutrition 25:669-679. https://doi.org/10.1111/anu.12888

Corrêa, C. F.; Aguiar, L. H.; Lundstedt, L. M. and Moraes, G. 2007. Responses of digestive enzymes of tambaqui (*Colossoma macropomum*) to dietary cornstarch changes and metabolic inferences. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 147:857-862. https://doi.org/10.1016/j.cbpa.2006.12.045

Costa, S. T. and Baldisserotto, B. 2021. Structure and physiology of the gastrointestinal tract. p.50-66. In: Biology and aquaculture of tilapia. López-Olmeda, J. F.; Sánchez-Vásquez, F. J. and Fortes-Silva, R., eds. CRC Press, Boca Raton.

Ebeneezar, S.; Vijayagopal, P.; Srivastava, P. P.; Gupta, S.; Sikendrakumar; Varghese, T.; Prabu, D. L.; Chandrasekar, S.; Varghese, E.; Sayooj, P.; Tejpal, C. S. and Wilson, L. 2019. Dietary lysine requirement of juvenile Silver pompano, *Trachinotus blochii* (Lacepede, 1801). Aquaculture 511:734234. https://doi.org/10.1016/j.aquaculture.2019.734234

Fabrikov, D.; Sánchez-Muros, M. J.; Barroso, F. G.; Tomás-Almenar, C.; Melenchón, F.; Hidalgo, M. C.; Morales, A. E.; Rodriguez-Rodriguez, M. and Montes-Lopez, J. 2020. Comparative study of growth performance and amino acid catabolism in *Oncorhynchus mykiss*, *Tinca tinca* and *Sparus aurata* and the catabolic changes in response to insect meal inclusion in the diet. Aquaculture 529:735731. https://doi.org/10.1016/j.aquaculture.2020.735731

FAO - Food and Agriculture Organization of United Nations. 2024. The State of World Fisheries and Aquaculture 2024. Blue transformation in action. FAO, Rome. 264p. https://doi.org/10.4060/cd0683en

Fry, J. P.; Mailloux, N. A.; Love, D. C.; Milli, M. C. and Cao, L. 2018. Feed conversion efficiency in aquaculture: do we measure it correctly? Environmental Research Letters 13:024017. https://doi.org/10.1088/1748-9326/aaa273

Furuya, W. M.; Santos, V. G.; Silva, L. C. R.; Furuya, V. R. B. and Sakaguti, E. S. 2006. Exigências de lisina digestível para juvenis de tilápia-do-nilo. Revista Brasileira de Zootecnia 35:937-942. https://doi.org/10.1590/S1516-35982006000400001

Hua, K.; Suwendi, E. and Bureau, D. P. 2019. Effect of body weight on lysine utilization efficiency in Nile tilapia (*Oreochromis niloticus*). Aquaculture 505:47-53. https://doi.org/10.1016/j.aquaculture.2019.02.030

Hua, K. and Bureau, D. P. 2012. Exploring the possibility of quantifying the effects of plant protein ingredients in fish feeds using meta-analysis and nutritional model simulation-based approaches. Aquaculture 356:284-301. https://doi.org/10.1016/j.aquaculture.2012.05.003

Huang, X.; Song, X.; Wang, X.; Zhou, H.; Liu, C.; Mai, K. and He, G. 2022. Dietary lysine level affects digestive enzyme, amino acid transport and hepatic intermediary metabolism in turbot (*Scophthalmus maximus*). Fish Physiology and Biochemistry 48:1091-1103. https://doi.org/10.1007/s10695-022-01098-w

Iodice D'Enza, A.; Markos, A., and Palumbo, F. 2022. Chunk-wise regularised PCA-based imputation of missing data. Statistical Methods and Applications 31:365-386. https://doi.org/10.1007/s10260-021-00575-5

Kherif, F. and Latypova, A. 2020. Principal component analysis. p.209-225. In: Machine learning. Mechelli, A. and Vieira, S., eds. Academic Press. https://doi.org/10.1016/B978-0-12-815739-8.00012-2

Kombat, E. O.; Abakari, G.; Salifu, A. R.; Alhassan, E. H. and Zhao, J. L. 2024. Proteolytic enzyme activity and mRNA expressions of amino acid and peptide transporter genes in tissues of Nile tilapia (*Oreochromis niloticus*) exposed to different salinities. Aquaculture, Fish and Fisheries 4:e70024. https://doi.org/10.1002/aff2.70024

Konnert, G. D. P.; Gerrits, W. J. J.; Gussekloo, S. W. S. and Schrama, J. W. 2022. Balancing protein and energy in Nile tilapia feeds: A meta-analysis. Reviews in Aquaculture 14:1757-1778. https://doi.org/10.1111/raq.12671

Leary, S.; Underwood, W.; Anthony, R.; Cartner, S.; Grandin, T.; Greenacre, C.; Gwaltney-Brant, S.; McCrackin, M. A.; Meyer, R.; Miller, D.; Shearer, J.; Turner, T. and Yanong, R. 2020. AVMA guidelines for the euthanasia of animals: 2020 edition. American Veterinary Medical Association, Schaumburg, IL.

Liaqat, R.; Fatima, S.; Komal, W.; Minahal, Q. and Hussain, A. S. 2024. Dietary supplementation of methionine, lysine, and tryptophan as possible modulators of growth, immune response, and disease resistance in striped catfish (*Pangasius hypophthalmus*). Plos One 19:e0301205. https://doi.org/10.1371/journal.pone.0301205

Liebl, A. R. S.; Bussons, M. R. F. M.; Pinto, E. A. S.; Aride, P. H. R. and Oliveira, A. T. 2021. Exigência de aminoácidos nas dietas: uma necessidade para peixes amazônicos. p.146-158. In: Aquicultura na Amazônia: Estudos técnico-científicos e difusão de tecnologias. Mattos, B. O.; Pantoja-Lima, J.; Oliveira, A. T. and Aride, P. H. R., orgs. Atena, Ponta Grossa.

Liebl, A. R. S.; Cáo, M. A.; Nascimento, M. S.; Castro, P. D. S.; Duncan, W. L. P.; Pantoja-Lima, J.; Aride, P. H. R.; Bussons, M. R. F. M.; Furuya, W. M.; Faggio, C. and Oliveira, A. T. 2022. Dietary lysine requirements of *Colossoma macropomum* (Cuvier, 1818) based on growth performance, hepatic and intestinal morphohistology and hematology. Veterinary Research Communications 46:9-25. https://doi.org/10.1007/s11259-021-09872-6

Lima, C. A. S.; Bussons, M. R. F. M.; Oliveira, A. T.; Aride, P. H. R.; O'Sullivan, F. L. A. and Pantoja-Lima, J. 2020. Socioeconomic and profitability analysis of tambaqui *Colossoma macropomum* fish farming in the state of Amazonas, Brazil. Aquaculture Economics & Management 24:406-421. https://doi.org/10.1080/13657305.2020.1765895

Marchão, R. S.; Ribeiro, F. B.; Siqueira, J. C.; Bomfim, M. A. D.; Silva, J. C; Sousa, T. J. R.; Nascimento, D. C. N. and Sousa, M. C. 2020. Digestible lysine requirement for Tambaqui (*Colossoma macropomum*) juveniles using the diet dilution technique. Aquaculture Reports 18:100482. https://doi.org/10.1016/j.aqrep.2020.100482

Martins, G. P.; Mazini, B. S.; Campos, M. A.; Oliveira, D. S.; Bueno, G. W.; Camargo, T. R. and Guimarães, I. G. 2024. Adequate levels of dietary digestible lysine improve growth, feed utilization and carcass composition but do not affect muscle morphology of tambaqui (*Colossoma macropomum*) juveniles. Aquaculture Reports 38:102334. https://doi.org/10.1016/j.aqrep.2024.102334

Mattos, B. O.; Pantoja-Lima, J.; Oliveira, A. T. and Aride, P. H. R. 2021. Aquicultura na Amazônia: Estudos técnico-científicos e difusão de tecnologias. Atena, Ponta Grossa. https://doi.org/10.22533/at.ed.042211503

Moraes, G. and Almeida, L. C. 2020. Nutrition and functional aspects of digestion in fish. p.251-271. In: Biology and physiology of freshwater neotropical fish. Academic Press. https://doi.org/10.1016/B978-0-12-815872-2.00011-7

Nascimento, M. S.; Mattos, B. O.; Bussons, M. R. F. M.; Oliveira, A. T.; Liebl, A. R. S.; Carvalho, T. B. 2020. Supplementation of citric acid in plant protein-based diets for juvenile tambaqui, *Colossoma macropomum*. Journal of the World Aquaculture Society 52:231-243. https://doi.org/10.1111/jwas.12735

NRC - National Research Council. 2011. Nutrient requirements of fish and shrimp. National Academies Press, Washington, DC.

Nelson, D. L. and Cox, M. M. 2018. Princípios de bioquímica de Lehninger. 7.ed. Artmed Editora.

Nolasco-Soria, H. 2021. Amylase quantification in aquaculture fish studies: A revision of most used procedures and presentation of a new practical protocol for its assessment. Aquaculture 538:736536. https://doi.org/10.1016/j.aquaculture.2021.736536

Oliveira, A. T.; Araújo, M. L. G.; Lemos, J. R. G.; Santos, M. Q. C.; Pantoja-Lima, J.; Aride, P. H. R.; Tavares-Dias, M. and Marcon, J. L. 2017. Ecophysiological interactions and water-related physicochemical parameters among freshwater stingrays. Brazilian Journal of Biology 77:616-621. https://doi.org/10.1590/1519-6984.01816

Peixe BR. 2024. Anuário 2024 Peixe BR da Piscicultura. Associação Brasileira da Piscicultura, São Paulo. Available at: <https://www.peixebr.com.br/anuario-2024>. Accessed on: Aug. 01, 2024.

Polese, M. F.; Polese, M. F.; Radael, M. C.; Mendonca, P. P.; Motta, J. H. S.; Azevedo, R. V.; Souza, A. B.; Shimoda, E.; Lavander, H. D.; Aride, P. H. R.; Oliveira, A. T. and Vidal Junior, M. V. 2022. Zootechnical indices and digestibility in juveniles of tambaqui *Colossoma macropomum* fed a diet containing particulate maize. Brazilian Journal of Biology 82:e232612. https://doi.org/10.1590/1519-6984.232612

Rebouças, P. M.; Lima, L. R.; Dias I. F. and Barbosa Filho, J. A. D. 2014. Influência da oscilação térmica na água da piscicultura. Journal of Animal Behaviour and Biometeorology 2:35-42.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. D.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Brazilian tables for poultry and swine: Composition of feedstuffs and nutritional requirements. 3rd ed. UFV, DZO, Viçosa, MG. 252p.

Rotta, M. A. 2003. Aspectos gerais da fisiologia e estrutura do sistema digestivo dos peixes relacionados à piscicultura. (Documentos, 53). Embrapa Pantanal, Corumbá. 48p.

Silva, J. C.; Bomfim, M. A. D.; Lanna, E. A. T.; Ribeiro, F. B.; Siqueira, J. C., Sousa, T. J. R.; Marchão, R. S. and Nascimento, D. C. N. 2018. Lysine requirement for tambaqui juveniles. Semina: Ciências Agrárias 39:2157-2168. https://doi.org/10.5433/1679-0359.2018v39n5p2157

Silva Júnior, G.; Copatti, C. E.; Costa, L.; Souza, G.; Bonfá, H. C. and Melo, J. F. 2022. Triploidy induction in tambaqui (*Colossoma macropomum*) using thermal shock: fertilization, survival and growth performance from early larval to the juvenile stage. Journal of Applied Aquaculture 34:989-1004. https://doi.org/10.1080/10454438.2021.1914800

Stone, N. M.; Engle, C. R.; Kumar, G.; Li, M. H.; Hegde, S.; Roy, L. A.; Kelly, A. M.; Dorman, L. and Recsetar, M. S. 2024. Factors affecting feed conversion ratios in US commercial catfish production ponds. Journal of the World Aquaculture Society 55:e13053. https://doi.org/10.1111/jwas.13053

Wilson, R. P. 2002. Amino acids and proteins. p.144-179. In: Fish nutrition. 3rd. ed. Hardy, R.W., ed. Academic Press, Orlando.

Zlaugotne, B.; Pubule, J. and Blumberga, D. 2022. Advantages and disadvantages of using more sustainable ingredients in fish feed. Heliyon 8:e10527. https://doi.org/10.1016/j.heliyon.2022.e10527