Effect of Diets With Different Lysine Levels on the Hemograms of Juvenile Tambaqui Colosssoma Macropomum (CUVIER, 1818)

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Abstract

Blood transports lysine and other nutrients derived from the diet and ensures good health and greater productivity for the fish. Therefore, this study aimed to verify the hematological behavior of juvenile tambaqui *Colossoma macropomum* nourished with levels of 9.72, 12.84, 15.96, 19.08 and 22.20 $g.Kg^{-1}$ of total lysine, corresponding, respectively, to the supplementation levels with L-lysine: 0.00, 4.00, 8.00, 12.00, 16.00 and 20.00 $g.Kg^{-1}$ of L-lysine. The blood of the fish was collected in the initial and final periods by puncture of the caudal vein. The variables were validated by ANOVA and Tukey's test ($p< 0.05$). No differences were found for hemoglobin, hematocrit, CHCM, leukocytes of the eosinophil and monocyte types, and thrombocytes ($p> 0.05$). The changes observed in total leukocytes were not attributed to diets ($p= 0.00$). Means of CMV, HCM, lymphocytes and neutrophils, varied over the initial period, but not in regard to different lysine levels ($p< 0.05$). Thus, it is concluded that the varying lysine levels of the diets did not compromise the hematological parameters analyzed.

Introduction

Hematological analysis in monitoring the fish physiology is a consolidated methodology, with simple sampling, and promotes reliable results regarding the animal's health (Aride et al. 2016, 2018, 2020; Castro et al. 2020; Lima et al. 2020; Nascimento et al. 2020; Oliveira et al. 2016, 2017; Pantoja-Lima et al. 2020; Burgos et al. 2019).

Multidisciplinary hematology as prognostic device in environmental and xenobiotic stress-induced response in fish (Fazio et al. 2013; Vajargah et al. 2019; Aliko et al. 2018). It can also indicate when it is necessary to provide interventions that solve or mitigate any unfavorable condition during cultivation (Pereira et al. 2016). Blood count analyzes the levels of erythrocytes (erythrogram), leukocytes (leukogram), and thrombocytes (thrombogram) (Sula et al. 2020; Prado et al. 2016) and becomes more efficient when associated with the analysis of the chemical composition of the blood.

The components of blood are distributed in the following proportions: 3% is made up of hormones and enzymes, 7% is proteins, and 90% is water (Ranzani-Paiva et al. 2013), in the aqueous tissue, erythrocytes, leukocytes, and thrombocytes (Oliveira et al. 2016). Since the cellular concentration has the functions of protecting the biological system, eliminating residues from metabolism, and transporting gases and nutrients, it can be used to measure responses to exogenous functional changes, such as temperature, pollutants and stress, and endogenous changes, such as hormonal action, weight, length variations and nutritional conjunction resulting from the elements absorbed in the diet (Aride et al. 2016, 2018, 2020; Castro et al. 2020; Oliveira et al. 2016, 2017, Seriani et al. 2011; Ranzani-Paiva et al. 2013).

The need for specialized diets to enhance the productive gains in fish farming has evolved proportionally with the increase in fish production (Nascimento et al. 2020). L-lysine is a typified crystalline amino acid for protein synthesis in tissues, which conditions greater growth, weight gain and other zootechnical performance indices (Richter et al. 2020). It also acts in the production of L-carnitine which contributes to lipid oxidation and helps the immune system by preventing degeneration response mechanisms (Grisdale-Helland et al. 2011; Furuya et al. 2013; Hua et al. 2019). Fish farming requires the fish's health to be protected by the body's defenses in order to make better use of the diet (Aride et al. 2016, 2018, 2020; Nascimento et al. 2020; Hamed et al 2020; Van Doan et al. 2019; Hossein et al. 2020; Gholampour et al. 2020; Rashidian et al. 2019, 2020)

Active in the protection of vital processes against infectious disorders and stress, leukocytes are mediators of the innate immune system, and can present different types of granulocytic and agranulocytic cells according to the species of fish investigated (Ngugi et al. 2015; Lemos et al. 2018; Oliveira et al. 2016). Thrombocytes are small cells, ovoid, elliptical or fusiform, which act in the blood clotting process (Oliveira et al. 2016). Although they are not leukocyte cells, thrombocytes are inserted as an active blood component in the organic defense of freshwater fish (Ishikawa et al. 2008; Oliveira et al. 2016).

Erythrocytes in the blood of teleost's represent on average 45% of the total blood volume, have a central nucleus and structures with oval to elliptical conformations, in addition to homogeneous cytoplasm containing vacuoles from the depreciation of cellular organelles, where hemoglobin is located (Ranzani-Paiva et al. 2013). Hemoglobin is usually configured by 96% globins and 4% heme, a prosthetic group formed by iron and porphyrin groups that gives protein its red color (Prado et al. 2016). This amino acid macromolecule influences the color, size and morphology of erythrocytes, is specialized in gas displacement, has a serum concentration which is dependent on the sex, age and nutritional status of the fish. Its alterations may characterize anemias and other dysfunctions (Tavares-Dias et al. 2009; Drumond et al. 2010; Prado et al. 2016; Signor et al. 2017). For these reasons, we may confirm that the FBC is applicable both in studies regarding the health of the fish and in the analysis of nutritional responses (Tavares-Dias et al. 2009; Signor et al. 2017; Aride et al. 2018).

The requirements for lysine and other nutrients for native fish species already established in Brazilian fish farming such as tambaqui *Colosssoma macropomun* (Cuvier, 1818) are still unclear (Chagas and Val 2003). Tambaqui is highly recommended for fish farming, has an omnivorous eating habit, ease in adapting to the farming systems used and, in a commercial sense, is well-appreciated in northern Brazil (Araújo-Lima and Gomes 2005; Rodrigues et al. 2014). Given the above, the objective of this study was to investigate the influence on the blood parameters when feeding juvenile tambaqui diets with different L-lysine levels.

Materials And Methods

Ethical animal use

The experiment was developed in accordance with the rules of ethical principles for animal experimentation approved by the National Council for the Control of Animal Experimentation (CONCEA), subject to approval by the Ethics Commission on the Use of Animals (ECUA) of the Federal University of Amazonas under approval No. 005/2016 and ECUA of the Nilton Lins University under approval No. 003/2017. All experiments were conducted according to local and ARRIVE guidelines (Persie du Sert et al. 2020).

Initial considerations: acquisition, location and acclimation of fish

The experiment with L-lysine diets was carried out at the Aquatic Organisms Production Laboratory at Nilton Lins University, Manaus - AM, using juvenile tambaqui *Colossoma macropomun* with average initial biometric data of 33.54 ± 1.9 g and 11.72 ± 0.36 cm, from the Balbina Fish Farming Station (Aquaculture Training, Technology and Production Center), Presidente Figueiredo – Amazonas state, Brazil. The fish (N= 180) were randomly stored in polyethylene tanks (10 fish/tank) (310 L^{-1}), with constant oxygenation and a biological filter. The siphoning and replacement of water in the tanks (20%), the dissolved oxygen levels (mg. L^{-1}), temperature (°C), pH and nitrite (mg. L^{-1}) were were measured daily with a multiparametric device (Horiba® G-50, Kyoto, Japan). The fish received a commercial diet (32% crude protein) during the acclimatization period.

The water temperature, dissolved oxygen content, and pH were respectively 26.25 ± 0.06 °C, 4.95 ± 0.32 mg.L^{-1}, and 5.74 ± 0.07, while the nitrite content remained between 0.00 and 1.0 mg. L^{-1}. All parameters monitored to ensure that the quality of the water in the tanks remained within the recommendations for production and well-being of tambaqui *C. macropomum*, in order to eliminate possible interference on the information obtained regarding nutrition with different lysine levels and the respective parameters evaluated (Schimittou 1993; Araujo-Lima and Gomes 2005; Aride et al. 2007; Rebouças et al. 2014).

Experimental diets

During the 90-day experimental period, the fish were fed with diets (27% crude protein) formulated with L-lysine (Ajinomoto®, Chuo, Japan) at levels 0.00, 4.00, 8.00, 12.00, 16.00 and 20.00 g.Kg^{-1} (Table 1) and processed at the Aquaculture Laboratory of the National Institute of Amazonian Research - INPA, Manaus - Amazonas.

The diets were composed of maize (356.20 to 376.20 g) and maize gluten 60 (230.00 g), rice grains (broken) (120.00 g), wheat bran (120.00 g), powdered dried fish (60.00 g), dicalcium phosphate (20.00 g), soybean oil (10.00 g), mineral and vitamin supplement (7.00 g), calcitic limestone (5.00 g) and sodium bicarbonate (2.00 g), in addition to antifungal and antioxidant BHT (Butyl-hydroxy-toluene).

After being ground in a knife mill (Tecnal®, Piracicaba, Brazil), the ingredients were mixed with the crystalline amino acids (Table 1), homogenized with the addition of water (20%) at a temperature of 50 °C. The mixture was extruded, dried in a forced ventilation oven (55 °C for 24 hours), and supplied four times a day (08:00, 11:00, 14:00 and 17:00 hours), until the apparent satiety of the juvenile tambaqui *C. macropomum*.

Sample collection, biometrics and blood parameters

At the beginning and at the end of 90 days of the experiment, nine fish in total (N= 9) were captured with nets (n= 9 fish per diet; N= 54) after fasting for 24 hours. The juvenile tambaqui collected were anesthetized with benzocaine (100 mg.L^{-1}), for blood collection (1 mL per fish) by puncturing the caudal vessel, mediated by 1 mL syringes, 13 x 4.00 mm needles, containing 15 µL of the anticoagulant Heparin sodium 5,000 IU (150 IU.mL^{-1} of blood) diluted in 0.65% saline solution (1:50) (Pádua et al. 2012; Oliveira et al. 2012).

The samples, preserved at -4 °C throughout the collection process, were used in the analysis of the components of the complete blood parameters (erythrogram, leukogram and thrombogram). Fish were measured biometrically for weight (g) and length (cm) using a digital scale (accurate to 0.001g; Gehaka®, São Paulo, Brazil) and an ichthyometer.

The hemoglobin concentration (g.dL^{-1}) was verified by the cyanomethemoglobin method (Collier 1944) with the addition of homogenized blood (20 µL) in Drabkin's solution (5 mL), centrifugation and reading at 540 nm (Ranzani-Paiva et al. 2013).

To count the total erythrocytes or RBC (10⁶.µL^{-1}) blood samples fixed in formaldehyde-citrate solution (1: 200) were analyzed in a Neubauer chamber under an optical microscope (Leica®, DM 500, Wetzlar, Germany) (Aride et al. 2018). The percentage of hematocrit (%) was determined by the micro hematocrit method (Goldenfarb et al. 1971), with centrifugation of heparinized micro capillary tubes (12,000 rpm/5 minutes) (Lemos et al. 2018; Aride et al. 2018). When verifying hematimetric indices, the mean corpuscular volume (MCV; fL), mean corpuscular hemoglobin (MCH; pg) and mean corpuscular hemoglobin concentration (MCHC; g.dL^{-1}) were calculated, as described by Wintrobe (1934).

To count total leukocytes, differentiated leukocytes and thrombocytes, three slides were made with blood extensions for each treatment (N= 63), stained panchromically with MGGW (May-Grunwald, Giemmsa, Wright and methanol) (Tavares-Dias and Moraes 2003). A total of 200 leukocytes, differentiated into lymphocytes, neutrophils, monocytes and eosinophils, were counted in percentage calculations according to Ranzani-Paiva et al. (2013).

The indirect method was used to check total leukocytes, observing 2000 erythrocytes with concomitant identification of the amount of leukocytes and thrombocytes in homogeneous fields of blood extension (Ranzani-Paiva et al. 2013). The information obtained was related to the erythrocyte count in a Neubauer chamber (µL of blood) and inserted in the formulas, according to Tavares-Dias and Mataqueiro (2004).

Statistical analysis

The experiment consisted of a completely randomized design (DIC), with seven treatments (6 elaborated diets + initial commercial diet) in triplicate. The data were verified by Analysis of Variance (ANOVA) ($p< 0.05$), Tukey's multiple comparison test using the statistical software R® (r-project, Auckland, New Zealand), version 3.5.3.

Results

The final weight of the juvenile tambaqui *C. macropomun* was more expressive in fish fed with 15.96 g. Kg^{-1} and 22.20 g. Kg^{-1} of lysine, and showed a difference between all other treatments ($p= 0.01$). The greatest lengths were recorded in fish fed with 15.96 and 22.20 g. Kg^{-1} of lysine, with a difference only for fish from the initial period ($p= 0.01$; Table 2).

The varying levels of lysine added to the diet did not change the mean hemoglobin ($p= 0.430$), hematocrit ($p= 0.430$) and MCHC ($p= 0.950$) concentrations. The influence of the diet was observed on the amount of erythrocytes in fish fed with 9.72 g. kg^{-1} of lysine ($p <0.05$), since these fish presented a greater amount of erythrocytes, with variation in relation to the initial diet (Table 3). The MCV and MCH indices for the fish in the initial period expressed variation in the mean values in relation to the treatments with lysine, the exception occurred in the MCH of the level of 12,84 $g.kg^{-1}$ ($p <0.05$) (Table 3).

No difference was found between the averages of thrombocytes in juvenile tambaqui (Table 4). The average of the total leukocytes from the levels of 6.60 $g.kg^{-1}$ and 15.96 $g.kg^{-1}$ of lysine differed from the averages from levels 9.72, 19.08 and 22.20 $g.kg^{-1}$ of lysine ($p= 0.02$) (Table 4).

In the analysis of differential white blood cell counts, lymphocytes, eosinophils, monocytes and neutrophils were found. There were no statistical changes caused by the addition of lysine to the diet in eosinophils and monocytes (Table 5). The percentage of lymphocytes in fish in the final period decreased in relation to the values found in juvenile tambaqui fed diets in the initial period ($p= 0.00$). Diets with lysine levels above 12.84 $g.kg^{-1}$ generated an increase in the neutrophils of the fish ($p= 0.00$; Table 5).

Discussion

The length and final weight of the tambaqui increased proportionally to the addition of lysine in the diets up to the level of 15.96 $g.kg^{-1}$ of lysine, where a mean growth of around 119% was registered in relation to the initial weight. This behavior was also detected by Costa et al. (2020) for other parameters of animal performance such as weight gain and apparent feed conversion, of tambaqui fed with different levels of lysine.

Biometric data on the influence of the crystalline amino acid can be better understood when related to information on the fish's physiological and metabolic variables. Zhou et al. (2007) and Ruchimat et al. (1997) related the performance responses, respectively, of cobia or bijupirá *Rachycentron canadum* (Linnaeus 1766) and yellowtail *Seriola quinqueradiata* (Temminck and Schlegel 1845), to the hematological condition of the fish, and did not identifying losses at the ideal level of L-lysine in fish health. However, the excess of L-lysine can be catabolized and inserted in the energy metabolism, providing lipid reserves in the visceral region of the animal's body (NRC 2011) and insufficient amounts limit the synthesis of L- carnitine and lipid transport into hepatocytes (Furuya et al. 2013).

According to Tavares et al. (2009), the amount of erythrocytes expected for tambaqui *C. macropomum* weighing between 1.63 to 369.50 g and length from 26 to 45.6 cm is between 1.25 and 2.96 (10^{6} µL^{-1}), with a reference range comprising values from 1.62 to 3.38 (10^{6} µL^{-1}). Although the response observed in the erythrocytes of juvenile tambaqui on diets with 9.72 $g.kg^{-1}$ of lysine ($p= 0.02$) approaches the maximum detected for the species by Tavares et al. (2009), cannot be considered polycythemia, which results from the elevation of plasma osmolality, blood chemical components and stressful situations (Clauss et al. 2008). This functional disorder is more easily validated when the hematocrit percentage exceeds 45%, which did not occur for fish treated with 9.72 g of $lysine.kg^{-1}$ (hematocrit= 32, 72%), likewise, for fish from other treatments analyzed (Clauss et al. 2008; Prado et al. 2016).

In hematological studies with juveniles of cobia *R. canadum* on diets with L-lysine, contrary to what was observed for tambaqui, the hematocrit means differed (mean hematocrit: 30.97%), however, they were similar to the percentage verified for the Amazonian species (average hematocrit: 31.65%), which did not occur with erythrocytes (RBC: 3.53 to 3.86 × 10^{9}.ml^{-1}) (Zhou et al. 2007). The absence of a difference between the hemoglobin concentrations also corroborated what was detected in this study.

The mean corpuscular volume (MCV) expresses the link between hematocrit and the abundance of erythrocytes, denoting the amount of red series cells present in the animal's blood, and can be altered by conditions such as diet, age, and cell maturity of fish of different species or of the same species (Hrubec et al. 2001; Ranzani-Paiva et al. 2013). The experimental tambaqui differed in relation to MCV ($p= 0.00$) in the fish from the initial period (commercial diet) and the fish fed with different lysine levels verified in the final period.

The MCV of fish under diets with lysine showed minimum and maximum values of, respectively, 113.35 and 145.98 fL. Such values fall in the MCV reference range (112.7-192.69) for tambaqui as described in the literature (Tavares et al. 2009). However, the MCV of fish in the initial period was higher than the expected maximum volume of 192.69 fL, generating uneven cells and configuring erythrocyte anisocytosis, which initially suggests physiological disorders, such as anemia, that may originate in nutrition. However, the anemic prognosis was ruled out because high concentrations of erythrocytes in immature forms were not found in the blood of the analyzed fish and the biometric data were not affected (Satake et al. 2009).

The maturation of fish erythrocytes occurs naturally in the circulating medium, however, when anemic, the unripe erythrocytes have a greater concentration, making it difficult to interpret the parameters of the erythrogram (Satake et al. 2009). Furthermore, the possibility of nutritional deficiency in the initial period was not expressed in fish in the final period, given that, if real, it would be circumvented by diets containing L-lysine. The amino acid tends to bind with other compounds such as minerals and micro minerals, and increases the villi of the intestinal mucosa, optimizing the transport and absorption of other nutrients necessary for animal maintenance and production.

Information on mean corpuscular hemoglobin (MCH), even when calculated using hemoglobin data, is obtained as a function of the average value of fish erythrocytes. This elucidates the behavior of data aligned with the pattern observed in the MCV, where the analysis of fish from the initial period (MCH: 81.65 pg) was higher than fish fed with lysine in the final period (mean final MCH: 45.54 ± 5.69 pg).

Tavares-Dias and Sandrin (1998) verified MCH of tambaqui weighing between 500 and 700 g and identified amplitude variation of between 32.2 and 51.3 pg and a mean value of 41.40 pg, which is close to the MCH average found in this work for fish fed with L-lysine. The high levels of hemoglobin observed in the blood of juvenile fish indicates a nutritional demand that was not met in the initial period, but was appears to have been solved by the addition of L-lysine in the diets, according to the MCH of the final period.

The interpretation of the MCH and MCV values requires careful analysis because the calculation base (total erythrocytes) may present a margin of error that influences the final response (Tavares-Dias and Sandrin 1998). According to Tavares-Dias and Sandrin (1998), the RBC that expresses greater precision is the MCHC (mean MCHC: 36.34 ± 1.06 ($g.DL^{-1}$), which in the current study did not fluctuate between the means analyzed ($p > 0.05$).

In the observation of neutrophils and lymphocytes, as well as eosinophils and monocytes for fish that received experimental diets, it is clear that the diets with lysine did not alter the different circulating leukocytes. When verifying the different types of leukocytes for all treatments (initial diet + diets with lysine), fish do not fed the experimental diets showed a higher percentage of lymphocytes and a lower percentage of neutrophils. The increase in the percentage of neutrophils, or neutrophilia, followed by a reduction in the amount of lymphocytes, or lymphopenia, suggests a stress condition that would be proven if a reduction in animal performance was detected (Urbinati et al. 2015). Such condition was not observed, since biometric analyses showed an increase in the weight and length of juvenile tambaqui up to the highest productive level due to the L-lysine addition, which shows the absence of impediment to the manifestation of the tested amino acid on the performance of the fish.

Evaluating the changes that occurred between the means of each differentiated leukocyte, it was observed that the neutrophils from treatments with diets containing lysine above 12.84 g. kg^{-1} exceeded that of fish which received 9.72 $g.kg^{-1}$ and those which did not receive lysine (treatments with lysine 9.72 $g.kg^{-1}$ and initial diet). The highest percentage was recorded at the lysine level of 15.96 g. kg^{-1}. Tavares-Dias et al. (1999) analyzed lymphocytes in tambaqui blood (average live weight: 584.6 g; diet with 28% crude protein - CP) and registered 1566.2 ± 754 µL of neutrophils for a total of 2663.3 ± 1288 µL leukocytes, which represents about 58.80% of neutrophils. This percentage is close to the neutrophil values found in the highest levels of lysine in our study with tambaqui.

In the studies by Tavares-Dias et al. (1999) with tambaqui, neutrophils were the most abundant circulating leukocytes. In teleosts, such cells raise the fish's hypersensitivity to adaptations to the environment (Tavares-Dias et al. 1999), which is the same as was found in this study. In general, the highest percentage of leukocytes in the blood of Brazilian freshwater teleosts corresponds to lymphocytes (LF) (Tavares-Dias et al. 1999), as detected for the Amazonian pirarucu *Arapaima gigas* (Schinz 1822) by Drumond et al. (2010) (LF: 89.9 ± 6.3% for fry and 55.6 ± 9.9% for juveniles). However, for parasitized tambaqui juveniles (live weight: 35.53 ± 2.77 g and 62.71 ± 2.75 g), fed with a commercial diet (CP: 36%) and evaluated for the effect of different biofilters under different densities, Lima et al. (2019) recorded higher concentrations of lymphocytes (65.4 to 84.9 x 10^3 μL^{-1}).

The absence of a pattern expressed by the means of the total leukocytes does not allow a clear association with the effect of the diet. The same de-patterning was observed by Zhou et al. (2007) for total leukocytes of cobias (*R. canadum*) under nutrition with different levels of lysine (4.90 ± 0.20 to 6.87 ± 0.21 × $10^6.mL^{-1}$). Zhou et al. (2007) stated that such differences were probably caused by nutritional stress and not by inefficient action by different concentrations of the amino acid. The average of the values analyzed in this work with tambaqui (30401 ± 12560 µL) did not differ from the average verified by Pádua et al. (2013) for tambaqui (*C. macropomum*; average weight: 357.5 ± 94.5 g) used as a control in studies on the effect of anesthetics on hematological parameters (Total leukocytes: 32.2 ± 6.1 × 10^3 μL^{-1} or 32200 µL), and for juvenile pirarucu (*A. gigas*; total leukocytes: 25091 ± 6121 µL) farmed in a semi-intensive system in the Amazon.

Leukocytes have recognized importance in the non-specific immunity of fish organisms, and act in inflammatory processes of the tissues, and are thus, indicators of the animal's health (Ishikawa et al. 2008; Faggio et al. 2015, 2014a, 2014b; Fazio et al. 2015).

Difficulties in differentiating leukocytes and thrombocytes in the Neubauer chamber (direct method) led to the use of the indirect methodology used in this work (Ishikawa et al. 2008). The thrombocyte concentrations for the juveniles also analyzed (mean: 10412.20 ± 4729.97 µL) remained within or

close to the range of 2,000-78,900 µL (except treatments 9.72 and 19.08 g. Kg^{-1}) described by Tavares-Dias et al. (2009). However, in relation to the values of thrombocytes found for tambaqui by Padua et al. (2013) in untreated fish and under treatment with clove and benzocaine oils (39.2 ± 15.6; 37.8 ± 12.5; 57.8 ± 8.1 × 10^3 μL^{-1}, respectively), concentration found for juveniles was lower. This condition is repeated with thrombocytes from other species of fish grown in Brazil such as gray jundiá *Rhamdia quelen* (Quoy and Gaimard 1824), Nile tilapia *Oreochromis niloticus* (Linnaeus 1758), piracanjuba *Brycon orbignyanus* (Valenciennes 1850) and pacu *Piaractus mesopotamicus* (Holmberg 1887), characterized by Dal'bó et al. (2015) (respectively: 48.6 ± 16.8; 35.9 ± 14.4; 37.9 ± 7.8; and 30, 0 ± 7.8 × 10^3 μL^{-1}).

Biotic and abiotic factors can interfere with thrombocyte concentrations (Tavares-Dias et al. 2009). Despite participating in coagulation, the clinical translation of the functions and behavior of fish thrombocytes have not yet been fully elucidated. However, it is known that some neotropical fish, such as traíra *Hoplias malabaricus* (Bloch 1794), can express a reversible reduction of thrombocytes, or thrombopenia, through prolonged fasting (Rios et al. 2005; Clauss et al. 2008). Considering that the tambaqui analyzed had fasted for 24 hours, the occurrence of thrombopenia due to food restriction is a possibility that, in order to be confirmed, require further hematological analyses under fasting, followed by re-feeding.

The study concluded that different levels of lysine added to the diet did not influence the parameters verified in the blood count of juvenile tambaqui. Thus, lysine does not promote physiological changes, and can be used routinely without problems of well-being in tambaqui *C. macropomum*.

Declarations

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Authors contributions

ARSL, MSN, PDSC conceived the study. WLPD, PHRA, MRFMB, ATO designed the study; ARSL, MSN, PDSC undertook laboratorial analyses. ARSL, PHRA, CF, ATO drafted the paper with contributions from all other authors. All authors read and approved the final manuscript.

Data availability

The datasets in this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethics approval and consent to participate

The experiment was developed in accordance with the rules of ethical principles for animal experimentation approved by the National Council for the Control of Animal Experimentation (CONCEA), subject to approval by the Ethics Commission on the Use of Animals (ECUA) of the Federal University of Amazonas under approval No. 005/2016 and ECUA of the Nilton Lins University under approval No. 003/2017. All experiments were conducted according to local and ARRIVE guidelines.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1. Amino acid components of experimental diets elaborated with lysine levels for juveniles of tambaqui *Colossoma macropomum*.

Composition of amino acids [1] (g.Kg^{-1})	Lysine (g.Kg^{-1})					
	6.60	9.72	12.84	15.96	19.08	22.20
L-lysine	0.00	4.00	8.00	12.00	16.00	20.00
L-glutamic acid	50.00	42.00	34.00	26.00	18.00	10.00
Dl- methionine	2.50	2.50	2.50	2.50	2.50	2.50
L- tryptophan	1.60	1.60	1.60	1.60	1.60	1.60
L-valine	1.00	1.00	1.00	1.00	1.00	1.00
L-arginine	2.50	2.50	2.50	2.50	2.50	2.50
L-histidine	2.50	2.50	2.50	2.50	2.50	2.50
L-isoleucine	2.50	2.50	2.50	2.50	2.50	2.50
L- phenylalanine	2.50	2.50	2.50	2.50	2.50	2.50
L- threonine	2.50	2.50	2.50	2.50	2.50	2.50

[1]Composition formulated according to Furuya et al. (2006) and Rostagno et al. (2011).

Table 2. Biometrics of juveniles tambaqui *Colossoma macropomum* fed diets containing increasing levels of lysine.

Biometric variable	Lysine (g.Kg^{-1})								
	6.60	9.72	12.84	15.96	19.08	22.20	[1]SD	[2]VC (%)	[3]P
Starting weight (g)	31.00	32.20	32.97	33.90	34.43	38.80	2.47	1.44	0.07
Final weight (g)	58.09^{a}	68.02^{a}	67.84^{a}	80.69^{b}	70.79^{a}	79.86^{b}	7.72	2.15	0.01*
Final length (cm)	14.34^{a}	15.17^{ab}	15.18^{ab}	16.57^{b}	16.02^{ab}	16.30^{b}	1.65	1.51	0.01*

P: Probability of significance; *: Significant difference.

[1]Standard deviation; [2] Variation coefficient; [3]Analysis of variance (p <0.05); Means followed by the same lowercase letter do not differ statistically from each other by the Tukey test (p <0.05).

Table 3. Effect of the inclusion of lysine in the diet on the erythrogram of juveniles of tambaqui *Colossoma macropomum*.

		Lysine (g.Kg^{-1})								
Erythrogram	Initial diet	6.60	9.72	12.84	15.96	19.08	22.20	[1]SD	[2]VC (%)	[3]P
Hemoglobin (g dL^{-1})	12.20	10.98	12.03	10.59	11.58	11.42	11.71	0.52	0.75	0.43
Hematocrit (%)	34.58	30.50	32.72	30.11	30.39	31.78	31.50	1.47	0.76	0.43
RBC (10⁶ µL^{-1})	1.49^{a}	2.189^{ab}	2.88^{b}	2.48^{ab}	2.08^{ab}	2.74^{ab}	2.65^{ab}	0.44	2.92	0.02*
MCV (fL)	231.71^{b}	139.29^{a}	113.35^{a}	121.21^{a}	145.98^{a}	115.83^{a}	118.71^{a}	38.81	4.93	0.00*
MCH (pg)	81.75^{b}	50.12^{a}	41.66^{a}	42.64^{ab}	55.62^{a}	41.617^{a}	44.14^{a}	13.42	4.67	0.00*
MCHC (g. dL^{-1})	35.28	35.99	36.76	35.18	38.10	35.93	37.19	0.98	0.44	0.95

[1]Standard deviation; [2]Variation coefficient; [3]Analysis of variance (p <0.05); P: Probability of significance; *: Significant difference. Means followed by the same lowercase letter do not differ statistically by the Tukey test (p <0.05).

Table 4. Total leukocytes and thrombocyte of juveniles tambaqui *Colossoma macropomum* under diets containing increasing levels of lysine.

		Lysine (g.Kg^{-1})								
Variable (µL^{-1})	Initial diet	6.60	9.72	12.84	15.96	19.08	22.20	**¹SD**	**²VC (%)**	**³P**
Total leukocytes	24,110.17^{ab}	13,575.42^{a}	43,220.67^{b}	31,797.33^{ab}	13,551.33^{a}	43,267.00^{b}	43,285.33^{b}	12,560.82	8.83	0.02*
Thrombocyte	9,404.16	2,189.58	1,561.00	8,280.55	17,651.11	11,186.67	8,563.33	4,729.97	8.27	0.362

¹Standard deviation; ²Variation coefficient; ³Analysis of variance (p <0.05); P: Probability of significance; *: Significant difference. Means followed by the same lowercase letter do not differ statistically by the Tukey test (p <0.05).

Tabela 5. Differential leukocyte count of tambaqui *Colossomema macropomum* juveniles under diets containing increasing levels of lysine.

	Lisina (g.Kg^{-1})									
Leukogram (%)	Inicial	6.60	9.72	12.84	15.96	19.08	22.20	**¹SD**	**²VC (%)**	**³P**
Lymphocytes	50.75^{b}	31.44^{a}	34.88^{a}	27.67^{a}	27.33^{a}	35.33^{a}	22.88^{a}	8.36	4.48	0.00*
Eosinophils	1.83	1.83	2.78	0.78	1.72	0.78	2.56	0.72	6.71	0.93
Monocytes	16.25	17.33	14.78	12.00	11.61	10.22	12.56	2.43	3.01	0.26
Neutrophils	31.16^{b}	49.77^{ab}	47.55^{ab}	59.55^{a}	59.33^{a}	53.66^{a}	62.00^{a}	9.80	2.91	0.00*

¹Standard deviation; ²Variation coefficient; ³Analysis of variance (p <0.05); P: Probability of significance; *: Significant difference. Means followed by the same lowercase letter do not differ statistically by the Tukey test (p <0.05).