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Amazonic fruit flour Camu - camu (*Myrciaria dubia*) in diets for Nile tilapia

Farinha do fruto amazônico camu – Camu (*Myrciaria dubia*) em dietas para tilápia do Nilo

Camu de harina de frutas Amazonicas - Camu (*Myrciaria dubia*) en dietas para tilapia del Nilo

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Abstract
This study aimed to evaluate the effects of different levels of camu-u fruit (Myrciaria dubia) flour in diets on zootechnical performance, body composition and physiological and histological responses of juvenile tilpia. It was used 500 juvenile tilapia, distributed in 20 tanks (1000L), in a completely randomized design with four treatments and five replications, fed three times a day until apparent satiation, for 48 days, with diets containing increasing levels of camu flour. -camu (0, 10, 20 and 30%). The increase in the inclusion of camu-camu in the diet resulted in a reduction in final weight, total length and specific growth rate, and an increase in apparent feed conversion, reduction in total and daily weight gain, fillet yield and carcass yield with and without head. Fish survival was not affected. Higher final weight, total length, file yield and specific growth rate obtained for fish fed a diet without camu-camu. The result is increased crude protein and lower body ether extract content. There was an increase in blood glucose concentration. There were no differences in the length, relationship between length and length and area of the villi of juveniles. For the length of the intestinal villi was statistically significant differences (P<0.05). It is concluded that camu improved

the body composition of the fingerlings, the well-being of the fish and, despite not being able to survive, it improved the productive performance of juvenile Nile tilapia. New studies can be done with levels from 0 to 10%.
Keywords: Nutrition; Oreochromis niloticus; Bromatology; Alternative ingredient.

Resumo

Este trabalho objetivou avaliar os efeitos de diferentes níveis da farinha do fruto camu-camu (Myrciaria dubia) em dietas, sobre o desempenho zootécnico, composição corporal e respostas fisiológicas e histológicas de juvenis de tilápia. Utilizou-se 500 juvenis de tilápias, distribuídas em 20 tanques (1000L), em delineamento inteiramente casualizado com quatro tratamentos e cinco repetições, alimentados três vezes ao dia até a saciedade aparente, durante 48 dias, com dietas contendo níveis crescentes de farinha de camu-camu (0, 10, 20 e 30%). O aumento da inclusão de camu-camu na dieta resultou em redução no peso final, comprimento total e taxa de crescimento específico, e aumento na conversão alimentar aparente, redução do ganho de peso total e diário, rendimento de filé e rendimento de carcaça com e sem cabeça. A sobrevivência dos peixes não foi influenciada. Maior peso final, comprimento total, rendimento de filé e taxa de crescimento específico foram obtidos para peixes alimentados com ração sem camu-camu. O fruto promoveu aumento na proteína bruta e menor teor de extrato etéreo corporal. Houve diminuição da concentração de glicose sanguínea. Não apresentaram diferenças significativas na largura, relação entre comprimento e largura e área dos vilos dos juvenis. Para o comprimento das vilosidades intestinais foi observada diferenças estatísticas significativas (P<0,05). Conclui-se que camu-camu melhorou a composição corporal dos alevinos, promoveu o bem-estar dos peixes e apesar de não afetar a sobrevivência teve um efeito negativo sobre o desempenho produtivo de juvenis de tilápia do Nilo. Novos estudos podem ser feitos com níveis de 0 a 10%.espécie.
Palavras-chave: Nutrição; Oreochromis niloticus; Bromatologia; Ingrediente alternativo.

Resumen

Este estudio tuvo como objetivo evaluar los efectos de diferentes niveles de harina de frutos de camu-camu (Myrciaria dubia) en dietas sobre el rendimiento zootécnico, la composición corporal y las respuestas fisiológicas e histológicas de tilapias juveniles. Se utilizaron 500 juveniles de tilapia, distribuidos en 20 tanques (1000L), en un diseño completamente al azar con cuatro tratamientos y cinco repeticiones, alimentados tres veces al día hasta saciedad aparente, durante 48 días, con dietas que contenían niveles crecientes de harina de camu. (0, 10, 20 y 30%). O aumento da inclusão de camu-camu na dieta resultou em redução no peso final, comprimento total e taxa de crescimento específico, e aumento na conversão alimentar aparente, redução do ganho de peso total e diário, rendimento de filé e rendimento de carcaça com e sin cabeza. La supervivencia de los peces no se vio afectada. Se obtuvo mayor peso final, longitud total, rendimiento de filete y tasa de crecimiento específico para peces alimentados con dieta sin camu-camu. La fruta promovió un aumento en la proteína cruda y un menor contenido de extracto de éter corporal. Hubo una disminución en la concentración de glucosa en sangre. No presentaron diferencias significativas en el ancho, relación entre largo y ancho y área de las vellosidades de los juveniles. Para la longitud de las vellosidades intestinales se observaron diferencias estadísticamente significativas (P<0,05). Se concluyó que el camu-camu mejoró la composición corporal de los alevines, promovió el bienestar de los peces y, a pesar de no afectar la supervivencia, tuvo un efecto negativo en el comportamiento productivo de los juveniles de tilapia del Nilo. Se pueden hacer nuevos estudios con niveles del 0 al 10%.
Palabras clave: Nutrición; Oreochromis niloticus; Bromatología; Ingrediente alternativo.

1. Introduction

Tilapiculture grows every year in Brazil (Vicente et al.,2014) figuring as the main species of fish in the composition of national aquaculture production, representing 63,5% of fish farming with 534.005 tons in 2021, placing the country among the four largest world tilapia producers (Peixe Br, 2018). This is due, among other things, to the species tolerating different storage density conditions and variations in water quality parameters (Lemos et al., 2018). These characteristics, linked to other factors such as the growing consumption of fish and the reduction of fish from fishing extraction, contribute to placing tilapia among the most produced fish in the world, with an expected 5.88 million tons in 2018 (Peixe Br, 2018). This reinforces that fish production and trade contribute significantly to global agricultural production. Thus, aquaculture plays a complementary and important role in the growing demand for fish and other products (such as animal feed and fish oil) at the global commercial level, in addition to increasing income, nutrition and health among small producers and consumers (Thilsted et al., 2016).

Camu-camu (Myrciaria dubia) is an Amazonian fruit much appreciated in the northern region of Brazil, being a partial component of the natural diet of aquatic organisms in these areas, especially certain species of fish (Vilachica, 1996;, Gressler et al, 2006) and even used as food for fish in extensive systems, it has high levels of vitamin C, ranging from 800 to 6,100 mg / 100 g of pulp (Yuytama, 2011; Sánchez et al., 2020), contains carotenoids, flavonoids, anthocyanins and antioxidant properties (Chirinos et al, 2010, Yuyama, 2011a, Neves et al, 2017 ;Sánchez et al., 2020), responsible for prolonging the life of erythrocytes and play an essential role in cellular respiration (Nayak et al., 2007), improving the absorption of nutrients from the diet and preventing the action of free radicals on lipids and protein amino acids (Aride et al., 2010). Studies describe contradictory results regarding the addition of fruits, seeds and extracts of Amazonian plants in diets for native fish. The inclusion of 15% of camu-camu in diets of juvenile pirapitinga (Piaractus brachypomus) had a negative impact on the palatability and use of the diet, resulting in slower growth (Palacios et al, 2006). On the other hand, the replacement of 10% and 30% of camu-camu in natura in the diet of juveniles of pitrapitinga (Piaractus brachypomus) did not influence the growth during the juvenile phase (Santos et al, 2022). However, the inclusion of 15% and 30% camu-camu in the diet of tambaqui juveniles (Colossoma macropomum) resulted in better weight gain and better swimming performance (Aride et al., 2018). Likewise, the addition in juvenile diets. Likewise, supplementation of camu-camu (500mg / kg of feed) and Uncaria tomentosa (300mg / kg of feed) improved the immune response, growth and contributed to the well-being of Nile tilapia (Oreochromis niloticus) (Agnaga et al., 2015; Agnaga et al., 2016).

Considering that Nile tilapia is the most produced fish species in the country, it is important to evaluate a native fruit as a potential food. Therefore, the objective of this study was to evaluate the effects of using different levels of fruit flour in diets to replace corn on zootechnical performance, body composition, physiological response and histology of the intestine of juvenile Nile tilapia.

2. Methodology

The present study was carried out at the Laboratory of Systems for the Production and Reproduction of Aquatic Organisms (LAPERP), Federal University of Paraná (UFPR) Palotina Sector, during 48 days of the productive performance test. This study was approved by the Ethical Committee for Experimentation and Research Animal of the Federal University of Paraná, Palotina Sector, Paraná, Brazil (Protocol No. 07/2017).

The fresh fruit was purchased at the Evaristo Castro da Silva Fair in the municipality of Tabatinga / AM, later they were washed, frozen and sent to UFPR Palotina Sector where a forced ventilation oven was dried for 72 hours at a temperature of 40 ° C. Subsequently, it was crushed in a mill with a 1.0 mm sieve, then presenting itself as a fine brown powder.

Fish with an average weight of 10 ± 0.2 and a standard length of 6 ± 02 were received and acclimatized to the facilities for a period of 20 days, during which they were fed with commercial feed. Subsequently, they were distributed in 20 circular boxes of 1000L of useful volume, in a completely randomized design with four treatments and five repetitions, and each box of 1,000 L received 25 juveniles, which constituted an experimental unit. The experiment was conducted for a period of 48 days, using 500 juveniles of tilapia of the GIFT lineage, with average weight and length of 17 ± 0.6 g and 8.23 ± 0.59 cm, respectively.

The twenty experimental boxes, make of a water recirculation system whose daily renewal was about eight times its volume, in this system there is still a circular tank of PVC netting of 3000L of useful volume where the mechanical and biological filtration of the water occurs through crushed stones, netting, sand. This water from the filters is pumped (3,000L / H pump) after treatment, back to the experimental tanks.

Four isoproteic diets (32% crude protein) and isoenergetic diets (4,600 kcal of digestible energy. Kg-1) were tested with the inclusion of increasing levels of camu-camu. The four levels of percentage replacement of corn by camu-camu (0.0;

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10; 20; 30%) were considered treatments (Table 1).

For the processing of the experimental rations, the ingredients were ground separately in a hammer crusher with a 0.7 mm sieve. Subsequently, they were mixed and moistened with water at 50 ºC for pelletization in granules of 4.0 mm. The rations were dried in an oven at 40 ºC for 72 hours. The ready-made rations were packed in identified plastic bags and stored in a freezer until use. Processing was carried out at the Animal Nutrition Laboratory of the Palotina Sector at UFPR.

The fish were fed three times a day (8 am, 12 pm and 5 pm), until apparent satiety. The diets were analyzed for crude protein, crude fiber, dry matter, ether extract, ash and humidity, according to the methodology Silva e Queiroz (2002).

Table 1: Experimental diets with increasing levels of camu-camu for the performance test of Nile tilapia juveniles.

Ingredients (%)	**Camu-camu (%)**			
	0	**10**	**20**	**30**
Soybean meal	54,07	54,51	54,94	55,38
Camu-camu	0	10	20	30
Corn	31,31	20,87	10,44	0
Wheat offal	8	8	8	8
Dicalcium phosphate	2,87	2,87	2,87	2,87
Soy oil	2	2	2	2
Premix[1]	1	1	1	1
Salt	0,50	0,5	0,5	0,5
DL – methionine	0,23	0,23	0,23	0,23
BHT[2]	0,02	0,02	0,02	0,02
Proximate composition and analyzed gross energy (based on dry matter)				
Moisture (%)	10,81	11,57	6,08	6,35
Dry matter (%)	89,19	88,43	93,92	93,65
Crude protein (%)	32,44	32,12	32,77	33,1
Ethereal Extract (%)	4,57	4,87	4,9	4,91
Crude fiber (%)	4,01	4,34	4,81	5,31
Mineral residue (%)	8,43	8,54	8,89	9,06
Gross energy (kcal\kg)	4.774,0	4.759,0	4.517,0	4.502,0

[1] Mineral and vitamin supplement, guarantee levels per kilogram of the product (Supreme): Vit. A, 1,200,000UI; Vit. D3, 200,000UI; Vit. E, 12,000mg; Vit. K3, 2,400mg; Vit. B1, 4,800mg; Vit. B2, 4,800mg; Vit. B6, 4,000mg; Vit. B12, 4,800mg; B.C. Folic, 1,200mg; Pantothenate Ca, 12,000mg; Vit. C, 48,000mg; Biotin, 48mg; Hill, 65,000mg; Niacin, 24,000mg; Iron, 10,000mg; Copper, 6,000mg; Manganese, 4,000mg; Zinc, 6,000mg; Iodine, 20mg; Cobalt, 2mg; Selenium, 20mg. 2Butyl hydroxytoluene. Source: Authors (2018).

A The water temperature and dissolved oxygen were checked daily at 7:00 am and 5:00 pm, with the aid of a portable oximeter (ALFAKIT AT 315), while the pH was measured using a digital bench pontiometer (TECNOPON mPA 210). The total ammonia contents were determined weekly, according to the methodology Koroleff (1976), the nitrite Baumgarten (1999), and the nitrate by colorimetry, the total alkalinity and the total hardness were determined through volumetry at the University's Water Quality and Limnology Laboratory - Palotina Sector. The physical and chemical variables of the water did not differ from each other ($P > 0.05$). The averages of the physical and chemical variables of the water were: nitrite (0.113 ±

0.063 mg L-1), ammonia (0.029 ± 0.011 mg L-1), alkalinity (85.52 ± 30.53 mg L-1 CaCO3), hardness (87.69 ± 34.83 mg L-1 CaCO3), temperature in the morning (25.76 ± 0.71 ºC) and in the afternoon (26.7 ± 0.68 ºC), dissolved oxygen in the morning (5,73 ± 0.35 mg L-1) and in the afternoon (5.32 ± 0.70 mg L-1) and pH (8.17 ± 0.31). The values found are within the range indicated as suitable for fish farming (Sipauba-Tavares, 1994; Proença & Bittencourt ,1994; Boyd, 1990).

At the end of the experiment, the animals were fasted for 24 h to empty the gastrointestinal tract and after this period, four fish from each experimental unit were immersed in a water solution with clove oil in a concentration of 100 mg L-1 to anesthetize the animals (Taylors & Roberts, 1999), aliquots of blood were removed from them. Subsequently, the rest of the fish were euthanized by immersing them in a clove oil solution (300 mg / L), and all the fish were used to evaluate the zootechnical performance.

Of the total, five fish from each box were used to determine the proximate composition and four for blood analysis. Individual measurements of the zootechnical performance of all fish were performed according to (NRC,2011): total weight (g), weight gain (g), standard length (cm), specific growth rate (g-cm-1) (TCE = Ln final weight - Ln initial weight x100 / Days of experiment), apparent feed consumption (CRA = apparent feed consumption (g) / number of fish), apparent feed conversion (CAA = feed consumption (g) / weight gain in the period), in addition to determining survival (S (%) = final quantity of animals / initial quantity of animals x 100), weight gain (GP = final weight (g) - initial weight (g)), weight gain daily (GPD = Weight gain (g) / Experiment time (day)) and specific growth rate. 12 fish from each box were used for evaluations of carcass yield with and without head (%), fillet yield (%) and 3 fish from each box to assess the hepatosomatic index (%) (IHS = Liver weight (g) / Weight of fish (g) x 100).

For the determination of body chemical composition, at the beginning of the experimental period three batches containing 35 fish were weighed and measured and were subsequently euthanized and frozen. Likewise, at the end of the experimental period, five fish, from each experimental unit, chosen at random, were weighed, measured, euthanized and frozen, to assess body composition, according to the methodology Silva and Queiroz (2002). The fish were ground in a meat grinder until a homogeneous sample was obtained. Subsequently, they were evaluated for: moisture (%), non-nitrogen extract (ENN), ether extract (%), crude protein (%) and mineral matter (%) of each experimental unit, according to the proposed methodology Silva and Queiroz (2002). Bromatological analyzes were performed at the Animal Nutrition Laboratory of the Zootechnics Department at UFPR Palotina Sector.

Hematology was performed at the end of the experiment, to verify the influence of the diet on the concentration of blood parameters. Four fish from each experimental unit were captured from the boxes with the help of nets, these were immersed in water solution with clove oil Taylors and Roberts (1999), in concentrations to anesthetize (50 ± 10 mg L-1). The blood was collected, with a 1 mL disposable syringe, from the caudal vein, and quickly transferred to an ependorff micro tube without anticoagulant and to an ependorff micro tube with 10% citrate. The blood collected was centrifuged at 3000 rpm for 5 minutes to extract plasma for biochemical evaluation. The samples were stored under refrigeration (4ºC), avoiding hemolysis. After centrifugation, the serum was used for the analysis of glucose, total cholesterol, triacylglycerols and total proteins. All analyzes of blood parameters were performed using Biotest® blood analysis kits.

For histological evaluation, fragments of the intestine (proximal part) were collected from three fish, fixed in 10% formaldehyde, for 12 hours and then preserved in 70% alcohol. They were dehydrated in an ascending series of alcohol, diaphanized in xylol, and included in paraffin, to obtain semi-series histological sections. The microtomy was performed, obtaining histological sections of 5μm with the aid of a disposable razor in an automatic microtome (LEICA, RM-2155) and histological sections were stained using the hematoxylin-eosin (HE) and PAS method. The photodocumentation was performed on the Zeiss Primo Star photomicroscope in a 40X objective for hepatopancreas and 10X for the intestine, using a computerized image system (Image Pro Plus - Version 5.2-Cybernetic Media). The morphometry of the villi, the height of the intestinal villi, width, length and area were performed based on the measurements of 20 villi per fish.

For statistical analysis, the assumptions were tested by performing data normality, using the Shapiro-Wilk test, and homoscedasticity (equality of variance), using the Levenne test. The data were then subjected to analysis of variance at the level of 5% probability (ANOVA) and in case of differences it was applied to the analysis of Tukey's test and regression. Data analysis was performed using the STATISTICA 7.0 software.

3. Results and Discussion

The effects of increasing levels (0%, 10%, 20% and 30%) of inclusion of camu-camu on the performance of Nile tilapia fingerlings in the 48-day experimental period are shown in Table 2. The results found for survival and hepatosomatic index did not show a significant effect of the inclusion of camu-camu in the diet (p> 0.05). On the other hand, there was a significant effect (P <0.0001) of the inclusion of this fruit on the final weight, weight gain, daily weight gain, total length, feed consumption, apparent feed conversion, fillet yield and specific growth rate (TCE), as well as, for the carcass yield with head (RC) (P <0.04) and without head (RS) (P <0.05).

The mortality (0-4% per tank and 0.8% per treatment) of juvenile Nile tilapia in the present study was considered low and is not associated with diets, as the fish's survival was not influenced (p> 0, 05) by the presence of camu-camu in the diet, and the same was observed in juvenile pirapitinga fed diets containing 15% wheat flour (control) or an identical level of substitution of three plants native to South America: camu camu, buriti (Mauritia flexuosa), or Peruvian maca (Lepidium meyenii) (Aride et al., 2018). Also, there were no differences in IHS between treatments, whose average value was 2.53%, therefore, it can be inferred that the fish that received the diets with camu-camu were in the same nutritional conditions as the fish in the control treatment, and that the fruit did not present a large number of toxic compounds that could lead to a metabolic overload of the liver, which would increase the size of the organ.

For most of the performance variables evaluated, it should be noted that the inclusion of increasing levels of camu-camu in the Nile tilapia diet, resulted in a worsening of productive performance. For the final weight, total length, feed intake, TBI and carcass yield with head with the increased level of inclusion of camu-camu in the diets, a decreasing linear effect (P <0.05) was verified, on the other hand, an increasing linear effect (P <0.05) was observed for apparent feed conversion of Nile tilapia fingerlings. In other words, as there was an increase in the levels of inclusion (%) of camu-camu in the diets, there was a reduction in feed intake by fish and in the values of final weight, total length, TBI and carcass yield with head, in addition to an apparent worsening in feed conversion.

Although the Nile tilapia juveniles apparently did not reject (intentionally regurgitated) the rations throughout the experimental period, there was a linear reduction in feed consumption, when increasing levels of camu-camu were added, which resulted in decreased growth and a negative effect of on the productive performance of juveniles. This reduction in dietary consumption with the inclusion of camu-camu may have been caused by a possible reduction in palatability and the presence of antinutritional factors in the diet, as already suggested in a study with pirapitinga juveniles who received a 15% camu-camu diet (Palacios et al., 2006). In the previous study, they also found a reduction in feed consumption by fish fed with camu-camu at the beginning of the experimental period, and for that reason, they carried out a neutralization of the diet to pH 7.0, considering that this rejection was due to the acidity that the camu-camu caused in the diet, however this procedure, even resulting in better acceptance of the diet, did not result in better productive performance of the fish (Palacios et al., 2006).

The worsening of AAC and reduction in final weight, weight gain and TBI observed in the present study was also reported for a study with pirapitinga fed with 15% camu-camu in their diet (Palacios et al., 2006). On the other hand, positive results were verified with the incorporation of camu-camu in diets for tambaqui in relation to productive performance, with an increase in weight gain with 15% inclusion, and a better final weight and total length with 30% supplementation with camu-camu. These differences in results may indicate a better adaptation of the tambaqui species, since it is native to the region where a large amount of camu-camu is produced, and in this way, it manages to make better use of the fruit's nutrients.

Carcass yield parameters are very important, even in the early growth stage, especially in work related to the testing of feed ingredients. Consequently, the effects of these ingredients can be linked to the deposition of muscle or less deposition of body and visceral fat, important characteristics to be evaluated at any stage of growth (Meurer et al., 2009). The addition of the fruit in the diets resulted in lower carcass yields in Nile tilapia juveniles. These results are lower than that found in Nile tilapia, which were from 89.13 to 90.09% of carcass with head and 61.67 to 64.43% without head in the same creation phase (Meurer el al., 2009). These differences in results may indicate a better adaptation of the tambaqui species, since it is native to the region where a large amount of camu-camu is produced, and in this way, it manages to make better use of the fruit's nutrients. Carcass yield parameters are very important, even in the early growth stage, especially in work related to the testing of feed ingredients. Consequently, the effects of these ingredients can be linked to the deposition of muscle or less deposition of body and visceral fat, important characteristics to be evaluated at any stage of growth (Meurer et al., 2009; Santos et al 2022). The addition of the fruit in the diets resulted in lower carcass yields in Nile tilapia juveniles. These results are lower than that found in Nile tilapia, which were from 89.13 to 90.09% of carcass with head and 61.67 to 64.43% without head in the same creation phase (Meurer et al., 2009).

Table 2: Performance of Nile tilapia juveniles fed increasing levels of camu-camu in the feed for 48 days.

	Levels of camu-camu in the diet (%)					
Variables	**0**	**10**	**20**	**30**	**SE** $_{ave}$	**P value**
Final weight (g)	65,46[a]	48,45b	42,29c	33,26d	2,79	0,0001
Weight gain (g)	48,60[a]	32,60b	24,04c	16,49c	2,79	0,0001
Daily weight gain	1,01[a]	0,68b	0,50c	0,34d	0,06	0,0001
Total length (cm)	12,59[a]	11,29b	10,86b	9,98c	0,23	0,0001
Feed consumption (g/fish)	47,62[a]	39,49b	38,75bc	33,37c	1,35	0,0001
Feed conversion[1]	0,99[a]	1,25a	1,61b	2,04c	0,10	0,0001
Filet yield (%)	26,67[a]	24,01b	23,81b	22,88b	0,38	0,0001
Carcass yield with head (%)	85,35[a]	84,05ab	84,07ab	83,11b	0,30	0,034
Carcass yield without head (%)	54,23[a]	52,46ab	51,82b	52,21ab	0,34	0,047
Specific growth rate (%)	2,82[a]	2,20b	1,79c	1,43d	0,12	0,0001
Survival (%)[ns]	100,00	99,20	99,20	99,20	0,33	0,77
IHS (%)	2,15	2,82	2,53	2,60	0,08	0,0082

[ns] Non-significant averages at 5% of probability (ANOVA). (%) SE $_{ave}$ (%) = Standard Error for the Average. Source: Authors (2018).

Inclusion of 10% in fish showed lower results than fish in the control treatment, but higher than the other treatments ($p <0.0001$) for the variables of final weight, weight gain, daily weight gain, total length and growth rate specific, in addition to lower value in feed conversion when compared to these treatments. The highest yield of carcass with head was obtained with the inclusion of 0% camu-camu in the feed. For Nile tilapia juveniles fed with increasing levels of camu-camu in the diet, a quadratic effect was observed on weight gain and daily weight gain with the maximum point of the quadratic regression obtained at the level of 3.86% and 1.5% camu-camu. A quadratic effect on the fillet yield and headless carcass yield of the fry was also seen with the increase in the levels of camu-camu in the diet, with the minimum points obtained being at the inclusion levels of 28.57% and 21 .25% camu-camu, respectively.

Although lower weigth gain is not an expected result when we talk about farm animals, when we talk about animals with a high fat mass or obese and with metabolic problems, this goal is highly desired. Still that camu-camu has the ability to prevent obesity induced by diet and improve the metabolic syndrome, due to having in its composition a mixture of nutrients and phytochemicals such as flavonoids, ellagic acid, elagitanins, proanthocyanidins, anthocyanins, in addition to the vitamin C (Anhê et al., 2018). High levels of inclusion (45% of camu-camu) in the diet caused a reduction in weight gain and growth in tambaqui (Colossoma macropomum) associated with the actions of possible antinutritional factors and loss of essential nutrients present in the diet replaced by camu-camu (Aride et al., 2018). Therefore, the results for camu-camu concentrations above 30% suggest a saturation of its intrinsic properties in the diet at this level, reducing nutritional assimilation and productive performance (Aride et al., 2018). For Nile tilapia, possible saturation at a slightly lower level of inclusion was found, between 20 and 25%, considering the variables above reported.

In the bromatological body composition of the fish, no effects of inclusion of camu-camu ($P > 0.05$) were observed in the total humidity and in the ashes of the fingerlings (Table 3). There was an increase ($P < 0.05$) in the deposition of body protein in Nile tilapia fingerlings fed diets with increasing levels of camu-camu, while the opposite effect was observed in the content of ether extract (Table 3), which was reduced ($P < 0.05$) with increasing levels of camu-camu. An increasing quadratic effect of total body protein was observed with increasing levels of camu-camu in the diet, and the maximum value was determined with 20.56% of camu-camu in the diet. Whereas, with the increased inclusion of camu-camu in the diet resulted in a decreasing quadratic effect for ethereal body extract, being found that with 22.22% of dubious Myrciaria in the diet the lowest value of crude body fat is obtained. The crude protein was lower in the treatment with 0% camu-camu in the diet and this differed from the treatments with 10%, 20% and 30% camu-camu. There was a decrease in the amount (%) of ether extract according to the increase in the quantity (%) of camu-camu in the feed, with significant differences in the levels 10%, 20% and 30% when compared to the control treatment (0%) of camu-camu in the feed ($p < 0.01$).

Table 3: Bromatological composition of carcasses of Nile tilapia juveniles fed diets containing increasing levels of camu-camu in the diet (based on dry matter).

	Diets (% camu-camu)					
Variables (%)	0	10	20	30	**SE** ave	*p*
Humidity	79,20	81,01	81,82	79,61	0,57	0,353
Dry matter	20,80	18,99	18,18	20,39	0,57	0,353
Gross protein	59,72b	63,10a	64,50a	63,41a	0,46	0,0001
Ether extract	15,61[a]	9,23b	9,12b	8,57b	0,78	0,0001
Mineral matter	19,28	21,32	20,32	21,78	0,1	0,116

SE ave (%) = Standard Error for the Average. Source: Authors (2018).

Despite the negative effect of camu-camu on the productive performance of Nile tilapia, the improvement in the body's chemical composition of fingerlings, with an increase in crude protein levels and a reduction in the values of ether extract indicate that the inclusion of camu-camu in the diet resulted in better absorption and utilization of some nutrients in the diet. The evidence of this better nutritional utilization was observed more specifically for dietary protein, which was used for body deposition and muscle formation, not being used as a source of energy for growth, as described (Palacios et al., 2006; Aride et al., 2018), with pirapitinga and tambaqui, respectively. On the other hand, it was confirmed that dietary and body lipids were the nutrients directed to be used as a source of energy for juveniles, resulting in body fat reduction, but they were not sufficient to generate growth of the animals.

The data from the present study corroborate the results found in which they also observed that camu-camu in the diet resulted in higher body protein levels in pirapitinga, however, these authors also found an increase in the body ash contents of these animals, which was not observed in the present study with Nile tilapia (Palacios et al., 2006).

There were no statistical differences in blood parameters of total cholesterol (mg dL-1), total proteins (mg dL-1) and total triglycerides (mg dL-1) of Nile tilapia juveniles fed diets with increasing levels of 0.10 %, 20% and 30% camu-camu (Table 4). However, significant differences were found (p<00.05) in the plasma glucose concentrations where a quadratic effect is observed, where, according to the regression analysis, the level of camu-camu in the diet that resulted in a lower glucose level in blood was 24.65%. Camu-camu did not change the values of total cholesterol, triacylglycerol and total blood proteins of fish, results that were consistent with the results of studies with tambaqui fed diets containing camu-camu (Aride et al., 2018). Therefore, it can be said that the diets used in the present test were able to provide at least the minimum necessary nutrients for fish hematopoiesis.

Table 4: Averages ± standard error of blood parameters of Nile tilapia, Oreochromis niloticus, fed with increasing levels (%) of camu-camu in the diet.

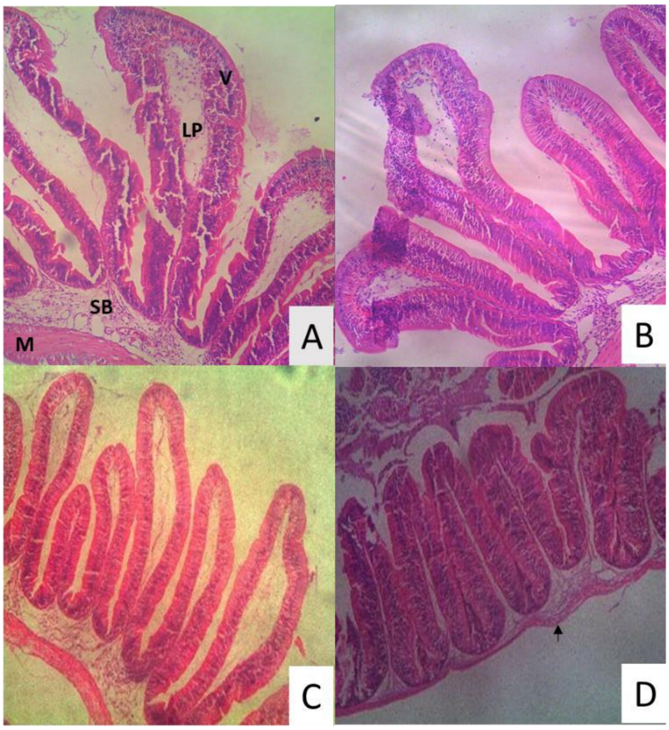
Variables (mg/dL)	Diets (% replacement of corn by camu-camu)					
	0	10	20	30	EPM	p Value
Total cholesterol	68	60	62,2	69,5	1,97	0,273^{ns}
Triacylglycerols	90,6	100,6	110,1	100,4	5,17	0,655^{ns}
Total proteins	2,16	2,22	2,2	2,29	0,04	0,837^{ns}
Glucose	94,40^{a}	68,30ab	69,90ab	63,00b	4,45	0,043*

Average values followed by different letter, on the same line, indicate a significant difference using the Tukey test (P<0,05). ns – not significant. * – significant. Source: Authors (2018).

Camu-camu has the ability to improve glucose tolerance and insulin sensitivity (Okamura et al., 2007), a result that we were able to confirm for Nile tilapia fingerlings, in which we observed a reduction in glucose levels in animals fed with increasing levels of camu - went on the diet. On the other hand, we can correlate this reduction in blood glucose with the presence of vitamin C in the fruit, whereas vitamin C reduces the glycemia of Nile tilapia (Moon,2001). Nile tilapia, like most teleost fish, is considered a glucose-intolerant organism. This is a clinical term used for mammals in the diagnosis of insulin-dependent or type 1 diabetes mellitus, and is related to the inability of an organism to rapidly metabolize high blood glucose levels, that is, to transport it into cells, resulting in hyperglycemia persistent (Wright et al., 1998; Wright et al., 2000; Gargiulo et al., 1998). These authors also suggested that glucose intolerance (IAG), and consequently hyperglycemia in Nile tilapia, is related to low insulin sensitivity, which is a result of low peripheral glucose use. Wright and Yang (1998) suggested that Nile tilapia IAG occurs due to the absence of glucose transporter (GLUT) - 4 in peripheral tissues, in addition to the low distribution of GLUT-1 in the tissues of these fish, both insulin dependent glucose transporters, the latter being responsible for the basal glucose transport in mammals.

The morphology of the proximal intestine region of Nile tilapia fed with increasing levels of camu-camu showed a typical structure of a tubular organ with mucous layer composed of epithelium and lamina propria, submucosa with loose connective tissue, muscle layer (smooth muscle) and layer outermost serous (Figure 1). The mucous layer presented villi with an irregular foliate aspect in terms of height and width, and these structures are lined with epithelium composed of columnar cells called enterocytes and goblet cells producing mucus, with no invagination of the epithelium, which is called crypt (Figure 1).

Figure 1: Photomicrograph of the proximal segment of the intestine of Nile tilapia junevis. (A) Intestinal wall of fish fed with 0% camu-camu, highlighting the villi (V), lamina propria (LP), submucous tunic (SB), muscular tunic (M). (B) Intestinal wall of fish fed with 10% camu-camu. (C) Intestinal wall of fish fed with 20% camu-camu. (D) Intestinal wall of fish fed with 30% camu-camu, highlighting the serous tunic (arrow). HE staining. 10x increase.



Authors (2018).

For the morphology of the proximal intestine region of Nile tilapia juveniles fed with increasing levels of camu-camu, the layer structure of the intestinal wall is within the normal range for tilapia (Fracassetti el al., 2013), and the appearance of the intestinal mucosa and epithelium and lamina propria are similar to those described for Nile tilapia (Gonçalves et al., 2014). However, for the levels of 10 and 30% of inclusion of camu-camu, a lower integrity of the intestinal villi can be observed, which suggests that camu-camu may present a negative interaction with the mucosa, however with the inclusion of 20% of camu-camu the integrity of the intestinal mucosa was not affected, the villi were intact and digitiform. A similar result was observed in studies with pirapitinga, the authors found no problems in the integrity of the intestinal mucosa with supplementation of 15% camu-camu in the diet of the fish (Palacios et al., 2006).

This observation can be confirmed by the morphometry of the intestinal mucosa of juvenile Nile tilapia fed with increasing levels of camu-camu. The level of supplementation of camu-camu of 20% was higher than supplementation of 10 and 30%, this increase in the height of the intestinal villi may have occurred due to the development of the mucosa, which is characterized by the increase of cells present in the epithelium through turnover, and thus improve the ability to maintain digestive function. Camu-camu has phenolic compounds such as ellagic acid, that can act in the intestine being absorbed by enterocytes and used to improve the health of the intestinal mucosa through its anti-inflammatory effect and also by its use in the microflora through the selection of probiotic bacteria (Gonçaves et al., 2014).

For the analysis of the intestinal morphometry of Nile tilapia juveniles fed diets containing increasing levels of camu-camu, villus width, relation between villus length and width and villus area did not differ with the inclusion of the fruit in the diets. For the length of the intestinal villi differences were observed ($P < 0.05$), the treatment with 20% camu-camu showed the highest values of the length of the villi, differing significantly (P <0.05) from the treatments 10 and 30%, not differing from treatment with 0% camu-camu in the diet (Table 5).

Table 5: Morphometry of intestinal villi of juvenile Nile tilapia fed with increasing levels of camu-camu in the diet for 48 days.

Variables	Ration% of camu-camu in the diet					
	0	**10**	**20**	**30**	**SE** $_{ave}$	**P value**
Villus width (LV) (μm)^{ns}	251,04	225,78	255,76	213,67	6,02	0,17575
Villus length (CV) (μm)	737,49^{ab}	648,86^{b}	832,23^{a}	638,01^{b}	13,69	0,00360
Relation CV/LV ^{ns}	0,34	0,35	0,31	0,33	0,009	
Villus area (μm^2) ^{ns}	184829,90	146542,42	212755,55	138300,77	12578,1	0,00751

^{ns} Non-significant averages at 5% probability (ANOVA) Tukey. SE $_{ave}$ = Standard Error for the Average. Source: Authors (2018).

This increase in villi may have been caused by an improvement in the integrity of the mucosa and a decrease in colonization of the epithelium by bacteria. For levels of 10 and 30% of inclusion of camu-camu, the length of the villi may have occurred due to impaired mucosal integrity or the turnover of the intestinal epithelial cells. This same result was observed for tilapia, with the use of the prebiotic mananoligassaccharides (1.5% inclusion), tilapia fed with the prebiotic showed an increase in villus length, but higher levels of inclusion reduced the villus length (Genc et al., 2007).

It can be observed in the present study a worsening in the productive performance of Nile tilapia fed with up to 30% of camu-camu in the diet, but on the other hand good results are observed with regard to body composition, where there is a reduction in the levels of fat and increase of the corporal protein, besides a reduction in the glycemia of the fish that received camu-camu. The best level of camu-camu that resulted in lower body fat content, higher body protein deposition and lower blood glucose level was 22.22%, 20.56% and 24.65%, respectively.

Considering the results in our study, we observed that further studies are needed on potential antinutrients present in this fruit and their respective negative effects on the palatability and absorption of nutrients in the tract of the digestive system of fish. However, the improvement in the body and metabolic conditions of Nile tilapia was confirmed in the present study, and demonstrate the potential that the bioactive compounds that make up camu-camu have in promoting health, reducing weight gain, body fat and blood glucose, but also promoting improvement in protein mass gain, which can be used as functional foods or even nutraceutical foods.

4. Final Considerations

The inclusion of camu-camu in the diet for Nile tilapia fry increases the deposition of body protein, reduces body fat and blood glucose and by 20% does not worsen the intestinal villi, but despite not affecting survival, results in a reduction weight gain and productive performance of fish.

Despite the levels evaluated negatively affected the productive performance of the animals, but studies evaluating intermediate levels, between 0 to 10%, should be investigated, as well as studies that evaluate their attractiveness-palatability.

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