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INFLUENCE OF A BLEND OF DIETARY ESSENTIAL AMINO ACIDS ON GROWTH, ENZYMATIC ACTIVITY, AND INTESTINAL HISTOLOGY OF BLUE DISCUS

Rudã Fernandes Brandão SANTOS[1]
Mayara Schueroff SIQUEIRA[3]
Ryuller Gama Abreu REIS[4]
Weliton Vilhalba da SILVA[2]
Henrique Momo ZIEMNICZAK[2*]
Claucia Aparecida HONORATO[2]

[1] Universidade Federal de Pernambuco - UFPE, Departamento de Bioquímica. Av. Professor Moraes Rego, s/n, Cidade Universitária, 50.670-420, Recife, PE, Brazil.

[2] Universidade Federal da Grande Dourados – UFGD, Faculdade de Ciências Agrárias - FAC, Laboratório de Aquicultura. Rod. Dourados/Itahum, km 12, Unidade II, 79.804-970, Dourados, MS, Brazil. henrique.momo@hotmail.com (*corresponding author).

[3] Instituto de Meio Ambiente de Mato Grosso do Sul – IMASUL. Rua Des. Leão Neto do Carmo, s/n, 79.037-100, Campo Grande, MS, Brazil.

[4] Universidade Federal do Pará – UFPA. Rod. 316, km 61, 68740-970, Castanhal, PA, Brazil.

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ABSTRACT

Symphysodon aequifasciatus is a fish with a disk-shaped body and bright colors, important characteristics of ornamental fish. We evaluated amino acid supplementation strategies to reduce crude protein in the diet for evaluation of performance, the content of digestive enzymes, liver metabolism, and intestinal histopathology. A total of 180 fish were randomly distributed in 12 separate 50 L glass aquariums, consisting of a completely randomized design with four treatments (DC - Control diet with 34.4% crude protein; DL - Control diet plus 1% of lysine; DEAA - Control diet plus 1% free essential amino acids (threonine, phenylalanine, leucine, valine, arginine, and tryptophan); and DHP - Diet with a high level of crude protein 48.4%), three repetitions, lasting 60 days. The use of DL and DEAA diets resulted in higher intestinal villus height and higher zootechnical performance. The use of DL diet increased alkaline phosphatase and digestive amylase activity. The use of DHP diets promotes severe liver changes due to increased activity of Alanine aminotraserase. Therefore, it was possible to observe that the use of amino acids can supply the nutritional need of blue discus. Supplementation of diets with AAs allows the reduction of dietary protein, which is a strategy for feeding management.

Keywords: aquaculture; amino acids; ornamental fish; protein; *Symphysodon aequifasciatus*.

INFLUÊNCIA DE UM "BLEND" DE AMINOÁCIDOS ESSENCIAIS SOBRE O CRESCIMENTO, ATIVIDADE ENZIMÁTICA E HISTOLOGIA INTESTINAL DE ACARÁ DISCO

RESUMO

Symphysodon aequifasciatus é um peixe com corpo em forma de disco e cores brilhantes, características importantes dos peixes ornamentais. Avaliamos estratégias de suplementação de aminoácidos (AAs) para reduzir a proteína bruta na dieta com relação ao desempenho, atividade das enzimas digestivas, metabolismo hepático e histopatologia intestinal. Um total de 180 peixes foram distribuídos aleatoriamente em 12 aquários de vidro de 50 L, consistindo em um delineamento inteiramente casualizado, com quatro tratamentos (DC – Dieta controle com 34,4% de proteina bruta; DL - Dieta controle acrescida de 1% de lisina; DEAA – Dieta controle acrescida de 1% aminoácidos essenciais livres (treonina, fenilalanina, leucina, valina, arginina e triptofano) e DHP – Dieta com alto nível de proteína bruta 48,4%), e três repetições, com duração de 60 dias. Os resultados demostraram que a utilização das dietas DL e DEAA refletiram em maior altura de vilosidade intestinal e maior desempenho zootécnico. A utilização de dieta DL promoveu aumento da atividade da fosfatase alcalina e amilase digestiva. A utilização de dietas DHP resultou em alterações hepáticas graves, devido a maior atividade da Alanina aminotransferase. Portanto, foi possível observar que a utilização de aminoácidos pode suprir a necessidade nutricional de acará disco. A suplementação de dietas com AAs permite a redução da proteína dietética, o que é uma estratégia para o manejo alimentar.

Palavras-chave: aquicultura; aminoácidos; peixe ornamental; proteínas; *Symphysodon aequifasciatus*.

INTRODUCTION

The discus fish *Symphysodon aequifasciatus* is a domesticated species and has been regarded as the "King of Aquarium Fishes" because of its disk-shaped body and bright colors (Froese and Pauly, 2019). The breeding of *S. aequifasciatus* has been conducted in many parts of the world. With the increase in the *S. aequifasciatus* industry worldwide, there are increasing research efforts regarding their nutritional requirements, such as feed ingredients (Wen et al., 2018), protein requirements (Chong et al., 2008), and supplemental food.

Because of the need to increase productivity in aquaculture systems, there is a growing demand for diets with greater use and efficiency (NRC, 2011). Some studies to determine the protein requirement for ornamental fish species may have overestimated the requirement because they did not consider the digestibility of ingredients (Chong et al., 2008), which may cause the results of productive performance to be nonviable. The protein level of the diet should be closely related to the ingredients used, according to their inclusion and the nutritional value of their amino acids (AAs) (Chong et al., 2008), as well as the balance of energy and other nutrients in the diet (Zuanon et al., 2009; Ribeiro et al., 2008).

However, the reduction of protein levels essentially depends on AA supplementation in the synthetic form (Xu et al., 2017). The results obtained with AAs appear to be closely related to the foods used, their levels of inclusion, and the nutritional value of their AAs (Murashita et al., 2018). Recent findings highlight that AA transporters, as a selective barrier to AAs, play a key role in responding to changes in intracellular and extracellular AAs, providing a crucial link between the availability of AAs and protein anabolism (Ren et al., 2019). The increase in intracellular AAs regulates signaling pathways of cell growth regulators (Xu et al., 2017), resulting in improved productive performance.

To optimize the use of ingredients, fish must be adaptable in terms of their digestive processes, such as their profile, enzyme secretion (Honorato et al., 2010), and absorption and transport of nutrients (Xu et al., 2017). Correlations between nutrient and digestive enzymes have been reported in freshwater tropical fish (Honorato et al., 2014). This information has also contributed to creating suitable diets. A limiting factor in the food conversion of fish is the availability of digestive enzymes in the gastrointestinal tract (Pujante et al., 2017). The effects of macronutrient contents on the production and secretion of digestive enzymes have been widely reported in fish (Ota et al., 2019). Small biomolecules can also affect the production of digestive enzymes (Xiao et al., 2011). Polypeptides and AAs from protein digestion can modulate the production of pancreatic proteases, particularly trypsin (Zambonino Infante and Cahu, 2007).

Diet supplemented with AAs can lead to improvements in the adaptability of the digestive processes, such as the enzymatic profile and secretion, and absorption and transport of nutrients. In this context, a morphological study can reveal the performance of the digestive process, absorption, and metabolism (Magouz et al., 2020), as well as its function as a selective barrier allowing nutrient absorption, eliminating many toxic substances (Liquori et al., 2007), and developing different adaptations in the digestive tract as a function of diet (Dorce et al., 2020). Therefore, histological and enzymatic studies are essential for decision-making regarding the kind of food that should be recommended for fish (Romarheim et al., 2008). There is a considerable set of data on the requirements of nutrients for fishes, adaptations of the digestive processes, and growth performance. There are also several comparisons between strategies to decrease protein levels in diets.

The goal of this study was to evaluate the AA supplementation strategies for the reduction of crude protein (CP) in the diet of the discus fish, in relation to the evaluation of performance, the content of digestive enzymes, hepatic metabolism, and histopathology of the intestine.

MATERIAL AND METHODS

The Animal Use Ethics Committee of the Centro Universitário da Grande Dourados – UNIGRAN, approved the experimental protocols used (Protocol CEUA 004/14). Discus fish specimens were obtained from Cascavel Fish Farm - Cascavel / Paraná, Brazil.

The experiment was conducted for 60 days. One hundred and eighty juvenile discus fish (*S. aequifasciatus*) of the blue lineage, with an initial weight of 2.15 ± 0.15 g, total length 3.81 ± 0.50 cm, were used in a completely randomized design, composed of 4 treatments, 3 repetitions, and 15 fish per experimental unit.

The juveniles went through an acclimatization period of 15 days, kept in 12 glass aquariums, with a volumetric capacity of 50 L, equipped with recirculation systems with filtration, water supply, and bottom changes of 20% per day. The water temperature was maintained at 27.0 ± 0.5ºC, pH 7.2 ± 0.3, and the oxygen dissolved in 5.4 ± 0.42 mg L^{-1}. The photoperiod was maintained in 12 hours of light, through the lighting from mixed lamps, controlled by an automatic timer.

The formulated diets used in the experiment were developed by a national company and were supplied daily in four meals at 8 am, 11 am, 1 pm, and 5 pm, added in small quantities until the apparent satiety, reducing the possibility of leaching.

At the end of the experiment, after 24 h without food, all fish were transferred to a benzocaine bath (100 mg L^{-1}) for 30 s (Chellapan et al., 2013), collected, weighed, and measured. Three fish were collected per aquarium, totaling nine fish per treatment. In each analysis, 12 fish were used, being: nutrient retention efficiency, digestive and metabolic enzyme tests, and histopathological analysis. Samples of body tissues collected were stored at -80°C for further analysis.

Experimental diets

Four commercial extruded diets (Poytara ltda) were used with the following treatments: DC - Control (control diet); DL - Diet control + 1% lysine (control diet plus free lysine); DEAA - Diet Control + 1% free essential amino acids (threonine, phenylalanine, leucine, valine, arginine, and tryptophan); DHP - Diets High Protein (commercial diet, 48% crude protein) (Table 1).

The protein resource used in the diets were fish meal and soybean meal, gelatinized starch as a source of carbohydrate, and soybean

Table 1. Composition of commercial diets and the diets supplemented with amino acids used in the feeding of *Symphysodon aequifasciatus*.

Composition (%)	Diets			
	DC	DL	DAAE	DHP
Comercial diet (%)	100	99	99	100
Lysine	-	1.0	-	-
Arginine	-	-	0.16	-
Histidine	-	-	0.16	-
Tryptophan	-	-	0.16	-
Phenylalanine	-	-	0.16	-
Leucine	-	-	0.16	-
Valine	-	-	0.16	-
Chemical composition (%)				
Dry matter	98.0	96.7	92.5	94.9
Crude protein	34.4	30.2	31.9	48.4
Lipid	3.1	3.7	4.6	7.9
Non-nitrogenous extraction	55.5	58.5	51.8	35.9
Crude fiber	2.5	2.4	2.3	1.5
Mineral matter	2.5	1.9	1.9	2.6
Calcium	3.4	3.0	3.1	3.2
Phosphor	1.8	1.7	1.8	1.7
Gross energy (kcal kg^{-1})	4983.8	5051.0	4795.6	4806.5

Control - DC (control diet); Diet lysine - DL (control diet plus free lysine); DEAA (control diet plus free essential amino acids – arginine, histidine, tryptophan, phenylalanine, leucine and valine); Diets High Protein - DHP (commercial diet with 48% crude protein).

oil as a source of lipids and supplemented with vitamin-mineral, protected vitamin C, betaine, and cauline. Diet composition was analyzed in laboratory of feed analysis of UNIGRAN, following Association of Official Analytical Chemists (AOAC, 1999) methodology.

Experimental design

After 60 days the fish were measured and weighted. The Weight Gain (WG), Length Gain (LG), Specific Growth Rate (SGR), Diet Consumption (DC), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER), Condiction Factor (K) and Uniformity of Weight (U) were calculated using the following equations:

1) $Weight\ Gain\ (g) = (final\ weight - initial\ weight)$;

2) $Length\ Gain\ (cm) = (final\ length - initial\ length)$;

3) $Specific\ Growth\ Rate(\%day^{-1}) = \left(\begin{matrix}100\ x\ [(ln\ final\ weight - \\ ln\ initial\ weight)\end{matrix}\right) / experimental\ days]$;

4) $Diet\ Consumption\ (g) = feed\ intake\ (g)$;

5) $Feed\ Conversion\ Ratio = feed\ intake\ (g) / weight\ gain\ (g)$;

6) $Protein\ Efficiency\ Ratio = weight\ gain\ (g) / crude\ protein\ intake\ (g)$;

7) $Condition\ Factor = \left[final\ weight\ (g) / final\ length\ (cm)^3\right] x\ 100$;

8) $Uniformity\ of\ Weight = \left(\begin{matrix}number\ of\ animals\ in\ the \\ tank / total\ number\ of\ animals \\ with\ weight\ 20\%\ higher\ or\ lower \\ than\ the\ average\ live\ weight\ in\ each \\ experimental\ unit\end{matrix}\right)$.

Nutrients-retention efficiency

At the beginning of the trial, 10 fish were separated and three fish per treatment (nine per tank) were sampled to determine the efficiency of nutrient retention. The fish were dried at 65°C for 16h for determination of dry matter and crude protein (AOAC, 1999), calculated using the following equation:

$$Protein\ Productive\ Value\ (PPV) = (final\ weight\ x\ final\ protein) - (initial\ weight\ x\ initial\ protein) / (dietsconsumption(g)\ x\ percentage\ of\ crude\ protein\ of\ the\ diet)$$

Enzymes assay

After experimental period, 12 fish from each treatment were transferred to a container with benzocaine 100 mg L^{-1}. After anesthetized, the fish were euthanized by medullar section, the digestive tract was excised, over a cold Petri-dish. Tissue homogenates were done over an ice-bath with a Potter-Elvehjem homogenizer into 0.02 M Tris/0.01 M phosphate buffer pH 7.0 mixed with anhydrous glycerol v/v. The homogenates were centrifuged at 11,400 ×g for 3 min and the supernatants (crude homogenate) were used as enzyme source. Were measure nonspecific protease, lipase, amylase and alkaline phosphatase.

The protease activity was performed with 1% casein (Walter, 1984). Protease activity was assayed in Tris-HCl 0.1M (pH 9.0) at 25ºC for 1h, interrupted with 15% TCA. Was performed reading at 280 nm against tyrosinase standard. The specific activity was expressed in the micromole of protein of hydrolyzed substrate (protein U mg^{-1}).

Amylase was tested at 0.2 M citrate/phosphate buffer pH 7.0 with 5% starch solution as substrate and 0.5% NaCl (Bernfeld, 1955) in 0.2 M citrate/phosphate buffer pH 7.0 with 5% starch solution as substrate and 0.5% NaCl at 25°C for 35 min and interrupted by the addition of 5% $ZnSO_4$–$Ba(OH)_2$. Subsequently, it was centrifugated at 1000 × g for 3 minutes and the reaction product was read at 690 nm (Park and Johnson, 1949).

Lipase determination was incubated with 0.4 mM r-nitrophenyl myristate at 24 mM of ammonium bicarbonate (pH 7.8) with Triton X-100 of 0.5% and interrupted with NaOH (10 mM); the reading was taken at 405 nm for 30 minutes. One unit was defined as micromole of substrate hydrolyzed by min and expressed by milligram protein (protein U mg^{-1}) (Albro et al., 1985).

Soluble protein from tissue homogenates was determined according to the method of Bradford (1976), using bovine serum albumin (Sigma) as standard.

The Alkaline Phosphatase was measured using colorimetric methods (Alkaline phosphatase OSR6004), analysis by spectrophotometry (semi-automatic spectrophotometer Bio plus S-200).

Liver tissue (100 mg) was used for the analysis of metabolic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Liver tissues were homogenized separately in sodium phosphate buffer (glycerol v/v in sodium phosphate buffer 20 mM and Tris 10 mM - pH 7.0) with a Potter-Elvehjem type homogenizer. Then, they were centrifuged at 600 x *g* at 4°C for three minutes, and the supernatant was subjected to new centrifugation at 6000 x *g* at 4°C for eight minutes. From this product, the supernatant was collected for enzymatic analysis by spectrophotometry (semi-automatic spectrophotometer Bio plus S-200), at wavelengths suitable for each test (Reitman and Frankel, 1957).

Histopathological analysis of liver and intestine

The histopathology analysis intestine fragments were immersed in Bouin solution for 24 hours and subsequently washed in 70% alcohol. After were dehydrated in graded ethanol solutions (70%, 80%, 90% and 96%), diaphonized and embedding in paraffin with plastic polymer. Microtomy was performed to obtain 5 µm thickness, which was stained by hematoxylin-eosin (HE) and by the histochemical method of periodic acid Schiff hematoxylin (PAS-H) (McManus, 1948).

Qualitative histological analyses were done to observe main treatment effects, after the material morphometry seven slices per treatment were selected and seven sections were photographed. The height of the intestinal villi was measured at its base to apex straight. The intensity of the reaction of the Goblet cells in the intestine, indicated by PAS-H dyeing (McManus, 1948; Ortiz-Delgado et al., 2003).

Statistical procedure

Statistical analyses were performed using R Studio software (version 1.1.423 – © 2009-2018 R Studio, Inc.). Data normality and variance homogeneity of parameters were checked by the Shapiro-Wilk test, using Bioestat software (version 5.0). It was performed an Analysis of variance (ANOVA); and when differences were significant (p <0.05), the means were compared by Tukey test. The results were expressed as means ± standard deviation (SD).

RESULTS

The results showed that the weight gain (WG) and SGR of the DEAA and DHP groups were significantly higher than those of the other treatments. The gain in length (LG) was significantly higher in the AED group than in the other groups. Feeding with the DHP diet presented lower PER compared to other experimental diets (Table 2).

Digestive enzymes

The results of the digestive enzymes are shown in Figure 1. The nonspecific proteases were significantly higher for DHP than that of the other groups, and the DL and DEAA groups did not differ. Digestive amylase was significantly superior to that of DL and DHP. Higher lipase activity was observed for DC. The alkaline phosphatase LD treatment favored its elevation, whereas the DHP treatment caused a decrease.

Metabolic enzymes

Hepatic ALT activity was higher in the DEAA and DHP diets. AST activity was higher for DEAA followed by DHP and DEAA diets without significant differences between them (Figure 2).

Histopathology

Four layers characterized the anterior intestine of the discus fish: the mucous, submucosa, muscular and serous. The mucosa layer was composed of cylindrical epithelium with a brush border and enterocytes interspersed with Goblet cells, with a blade containing intraepithelial lymphocytes. The submucosa was formed by cells, collagen fibers, and blood vessels. The muscular layer was composed of circular smooth muscle fibers and was external to the serosa layer, characterized by loose connective tissue PAS (positive) and pavement cells (Figure 3). It was observed that the villus height (Figure 4) was directly related to the diet used. In the intestine of fish fed DEAA diets, there was an increase in intestinal villi height.

Table 2. Performance of *Symphysodon aequifasciatus* fed different diets.

Variables	DC	DL	DAAE	DHP
Weight gain (g)	1.97±0.83^{b}	1.27±0.49^{b}	2.85±0.49^{a}	2.80±0.69^{a}
Length gain (cm)	0.40±0.03^{c}	0.21±0.01^{c}	0.70±0.01^{a}	0.50±0.02^{b}
Specific growth rate (% day^{-1})	1.04±0.34^{b}	0.71±0.23^{c}	1.37±0.16^{a}	1.35±0.20^{a}
Protein efficiency ratio (g kg^{-1})	1.21±0.60^{a}	1.07±0.51^{a}	1.26±0.31^{a}	0.80±0.19^{b}
Feed conversion	2.87±0.21^{b}	3.57±0.28^{a}	2.53±0.34^{b}	2.69±0.66^{b}
Survival (%)	84.09±9.37	87.50±8.12	79.55±10.41	71.59±9.84
Weight homogeneity	80.60	77.90	78.30	69.80
K	13	18.90	10.40	6.70

The data were evaluated by ANOVA and values are expressed as average ± SD; different letters in the row indicate significant differences (p <0.05). Main effects were compared by Tukey's test. Control - DC (control diet); Diet lysine - DL (control diet plus free lysine); DEAA (control diet plus free essential amino acids – arginine, histidine, tryptophan, phenylalanine, leucine and valine); Diets High Protein - DHP (commercial diet with 48% crude protein).

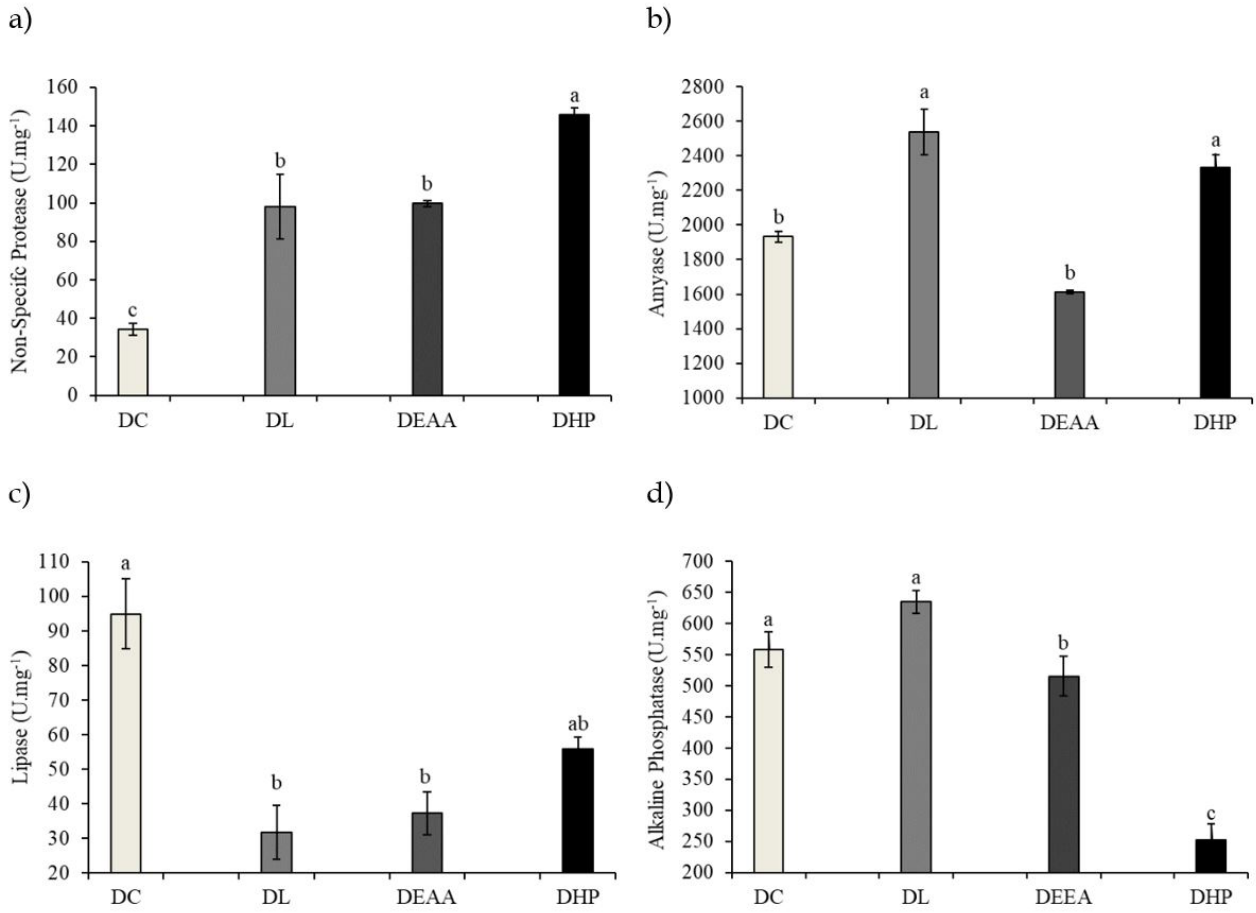

Figure 1. Specific activity in the digestive tract of discus (*Symphysodon aequifasciatus*) in response to diets. a) non-specific protease, b) amylase, c) lipase, d) alkaline phosphatase. Control - DC (control diet); Diet lysine - DL (control diet plus free lysine); DEAA (control diet plus free essential amino acids – arginine, histidine, tryptophan, phenylalanine, leucine and valine); Diets High Protein - DHP (commercial diet with 48% crude protein). Different letters mean statistical differences between treatments (p <0.05). Values are about mean ± SD.

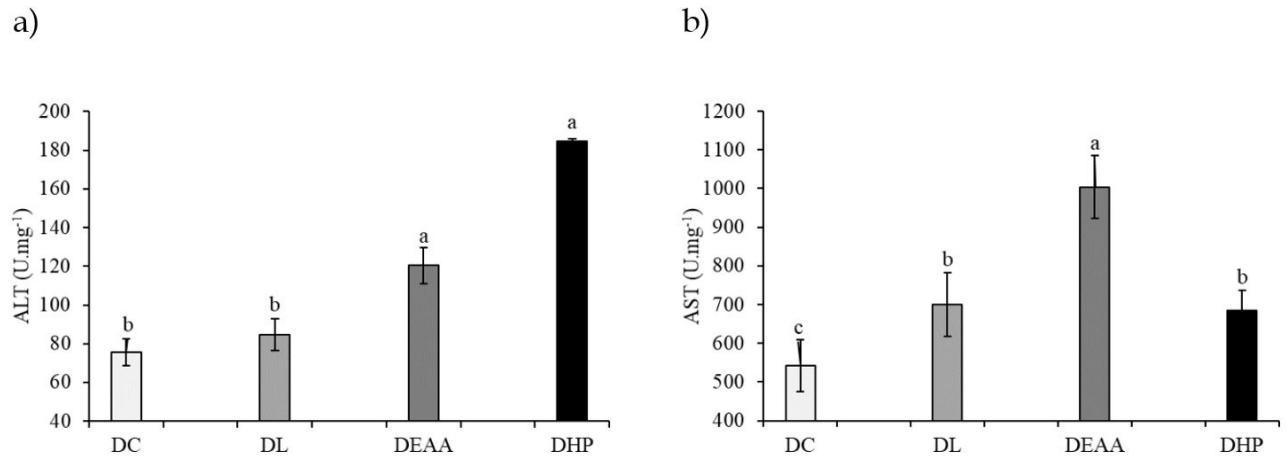

Figure 2. Specific activity (UI mg^{-1} protein) of a) alanine aminotransferase (ALT); b) aspartate aminotransferase (AST), in the liver of discus (*Symphysodon aequifasciatus*) in response to dietary. Control - DC (control diet); Diet lysine - DL (control diet plus free lysine); DEAA (control diet plus free essential amino acids – arginine, histidine, tryptophan, phenylalanine, leucine and valine); Diets High Protein - DHP (commercial diet with 48% crude protein). Different letters mean statistical differences between treatments (p <0.05). Values are about mean ± SD.

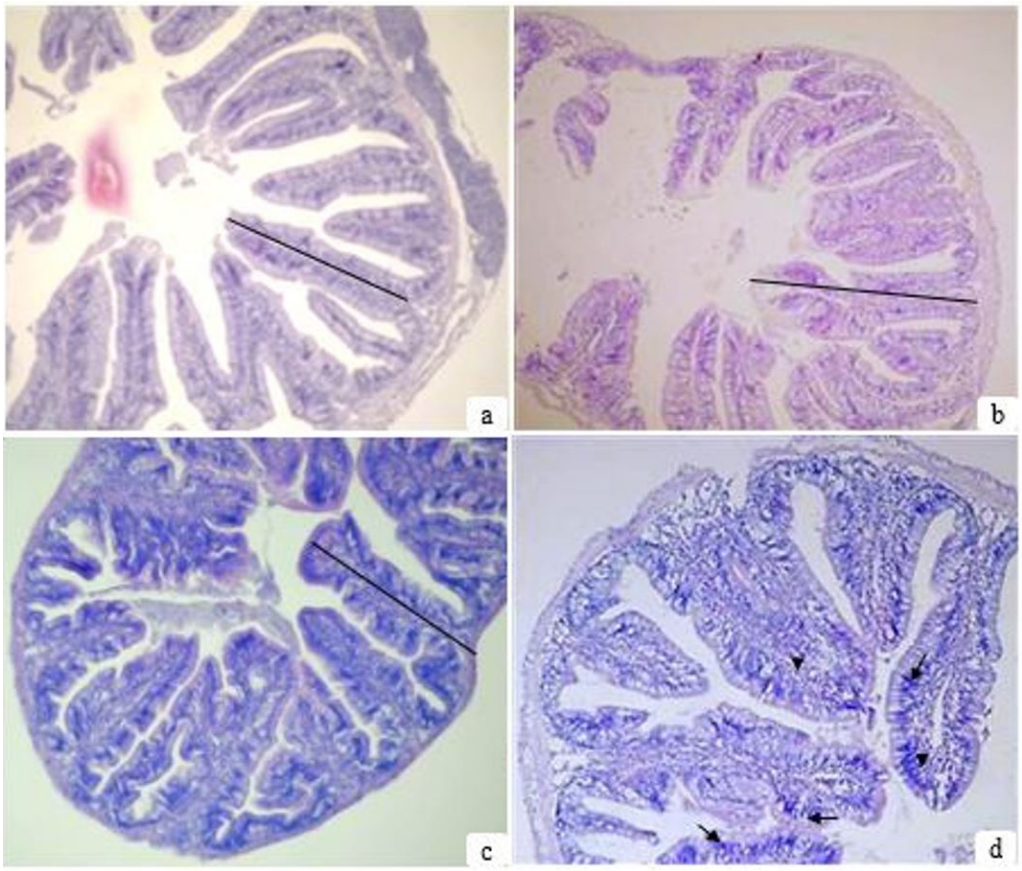

Figure 3. Discus anterior intestine subjected to different diets. Normal morphology of the anterior portion of the intestine with villi and crypts (fine trace) mucosa without injury. Fish displayed mucosa layer composed of cylindrical epithelium with brush border and enterocytes interspersed by goblet cells (arrowhead), with its own lamina containing intraepithelial lymphocytes (arrow). a) Control - DC (control diet); b) Diet lysine - DL (control diet plus free lysine); c) DEAA (control diet plus free essential amino acids – arginine, histidine, tryptophan, phenylalanine, leucine and valine); d) Diets High Protein - DHP (commercial diet with 48% crude protein). HE 10x.

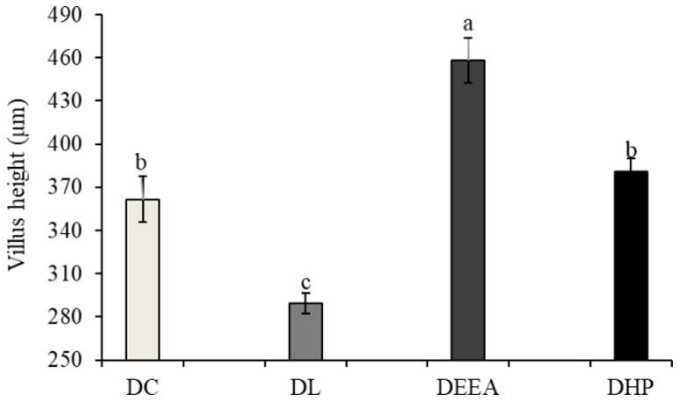

Figure 4. Height of instinct villi according to diets. Control - DC (control diet); Diet lysine - DL (control diet plus free lysine); DEAA (control diet plus free essential amino acids – arginine, histidine, tryptophan, phenylalanine, leucine and valine); Diets High Protein - DHP (commercial diet with 48% crude protein). Different letters mean statistical differences between treatments (p <0.05). Values are about mean ± SD.

DISCUSSION

Amino acid supplementation (DAAE) in the diet resulted in high productivity indexes using a protein level of 31.9%. Considering the economic aspects, due to the better uniformity observed, these fish have a higher market value. This result demonstrated divergent evidence about the high-protein (44.9 ± 5.1%) requirement recommended in the literature (Chong et al., 2002) and the ornamental fish market for discus fish. In recent studies, it has been possible to consider aspects, such as the prioritization of AA balance in the diet formulation (Chong et al., 2002) and the AA profile of the species (Chong et al., 2008).

The results obtained with lysine supplementation in diets with low levels of protein were not sufficient to subsidize the growth of *S. aequifasciatus*, reiterating the importance of the recommendations above. Although lysine is a major limiting AA for fish (NRC, 2011), its use by itself is not effective for this species. Positive results of lysine supplementation in diets that resulted in growth improvement have been reported for *Oreochromis niloticus* (Bomfim et al., 2010) and *Rhamdia voulezi* (Diemer et al., 2014). In this study, it was shown that

dietary protein reduction in *S. aequifasciatus* was only possible by supplementation of a limiting essential AA blend, as reported for rainbow trout, *Oncorhynchus mykiss* (Cheng et al., 2003) and Nile tilapia, *O. niloticus* (Botaro et al., 2007).

The protein requirement for the discus fish is above 45% CP (Chong et al., 2002), but, in its natural environment, discus fish have a diet composed of small crustaceans, mollusks, insects, and large volumes of periphyton, a food item that makes up 70% of the total found in the digestive tract of the species (Crampton, 2008). This indicates the possibilities of improvements in the formulation of diets about the protein level.

In addition, comparing the protein requirements of discus fish with that of angelfish *(Pterophyllum scalare*), a species belonging to the same family, which adopts feeding strategy in its natural environment similar to that of the discus fish (Ribeiro et al., 2008). For angelfish, the 40% CP (Degani, 1993), close to the values observed for the discus fish by Chong et al. (2002). However, studies conducted to maintain equal amounts of AAs with protein variation (Zuanon et al., 2016) resulted in a decrease in the requirement to 30% CP.

It should be emphasized that one of the goals of animal nutrition is to minimize dietary protein levels and increase its utilization efficiency (NRC, 2011). Considering the scarce requirement for high protein (Saavedra et al., 2017) and that AAs are essential for growth, the AA profile of the diet must meet the needs of the fish (Saavedra et al., 2017). It is worth noting that reducing protein in the diet of a species requires a more precise understanding of its nutritional needs (AAs, energy) and mechanisms of nutrient utilization (Ren et al., 2019), regarding morphological and physiological adaptations.

The results of ALT and AST obtained for DHP also indicated that the protein requirement of the species is not very high. The increase in transaminase activity indicated liver damage. Changes in the content of these enzymes are indications of liver damage caused by an inadequate nutritional balance in the diet, such as excess protein (Nunes et al., 2013), and the effect of anti-nutritional factors or source of the nutrient (Ota et al., 2019). The increase in AST and ALT activities are considered to be a response of the organism to stressors and nutritional metabolism (Meng et al., 2018), which deserves attention because, for this species, longevity is required. It is evident that the AA supplementation in the present study also caused elevation of ALT and AST, indicating that the ideal is when AAs form a balanced diet.

The activity of the digestive enzymes also demonstrated the possibility of reducing the CP content of the diet of *S. aequifasciatus*. AF activity in DHP was reduced, which demonstrated that the nutrients could be better used. Alkaline phosphatase (AF) activity corresponded to a higher uptake of nutrients by the digestive tract (Ren et al., 2019). It was observed that the DHP diet did not enable *S. aequifasciatus* to express its digestive plasticity to absorb nutrients that were too high. Ota et al. (2019) suggested that dephosphorylation of nutrients by intestinal AF is necessary to make them permeable to the plasma membrane, indicating an increase in intestinal AF activity during the maximum feeding period.

Considering the possibility that the requirement of *S. aequifasciatus* is lower than that recommended by Chong et al. (2002), it was observed that the amylase increased with the increase of carbohydrates in the diet in DL and with the decrease of carbohydrates in DHP. This demonstrated the substantial plasticity of *S. aequifasciatus* when metabolizing carbohydrates at low or high availability in the diet. The use of carbohydrates as a protein-sparing effect with beneficial effects on hepatic health has been reported for *C. carpio* (Ren et al., 2019). The responsive activity observed for *S. aequifasciatus* reveals the plasticity of this species in the use of carbohydrates, as already demonstrated for fish species, which depends on the complexity of the carbohydrate source (Honorato et al., 2014).

For the proteolytic enzymes, it was observed that there was an increase in the digestive protease activity in the digestive tract of the fish fed with DEAA, concomitant with an improvement in the architecture of the intestinal villi and increase in production of the glycoproteins, indicative of an improvement in nutrient absorption. This result was supported by the study by Morales et al. (2017), which showed greater activity of proteolytic enzymes, in addition to greater absorption in the presence of free amino acids in the feed and swine.

The greater activity of digestive enzymes in the digestive tract results in a greater contribution to the digestive processes, and consequently, the use of food (Murashita et al., 2018). Proteases are enzymes responsible for the digestion of proteins, from which the AAs are converted to the body mass of the fish. Proteins are the main organic constituents of fish, accounting for from 65 to 75% of the total body dry matter and are associated with fish growth and/or yield (Nunes et al., 2013). The stimulation to produce enzymes by the presence of some specific food is associated with both the production and modulation of genetic expression in the hepatopancreas (Honorato et al., 2010). Adequate protein utilization is very important for fish nutrition and strongly depends on the activity of the proteases and the increase in alkaline protease activity (Ota et al., 2019).

CONCLUSIONS

Our study demonstrated a possibility that supplementation with AAs could balance the diet for *S. aequifasciatus*, improving the growth rates, with morphophysiological adaptations beneficial to the use of diet. Supplementation of diets with AAs allows the reduction of dietary protein, which is a strategy for the culture system.

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