



## Evaluation of commercial and experimental grower diets for use in intensive culture of *Symphysodon aequifasciatus*

RUDÃ FERNANDES BRANDÃO SANTOS<sup>1</sup>, JEAN CARLOS DE SOUZA-MELO<sup>2</sup>, RYULLER GAMA ABREU REIS<sup>3</sup>, MAYARA SCHUEROFF SIQUEIRA<sup>4</sup>, HENRIQUE MOMO ZIEMNICZAK<sup>2</sup> & CLAUCIA APARECIDA HONORATO<sup>2\*</sup>

<sup>1</sup> Yepist Produtos animal LTDA, Av. Prof. Luiz Freire, 700, Curado, Recife-PE, CEP: 50740-437.

<sup>2</sup> Federal University of Grande Dourados, Faculty of Agricultural Sciences Unit II: Dourados / Itahum Highway, Km 12 - Unit II | PO Box: 364 | CEP: 79.804-970, Brazil. [ClauCIAahonorato@gmail.com](mailto:ClauCIAahonorato@gmail.com)

<sup>3</sup> Universidade Federal do Pará, rod. BR 316, km 61, cep 68740-970, Casanhal -PA

<sup>4</sup> Av. Desembargador Leão Neto do Carmo, s/n, Parque dos Poderes, Campo Grande

\* Corresponding author: [ClauCIAahonorato@gmail.com](mailto:ClauCIAahonorato@gmail.com)

**Abstract:** The present study investigated the growth performance and impacts on the metabolism, digestive morphology, and hepatic metabolism of blue discus *Symphysodon aequifasciatus* fed a dry commercial diet and a wet diet. The fish were divided into diet groups: i) DBH - beef hamburger; ii) BH + DD - 50% beef hamburger + 50% commercial dry diet; iii) DD - commercial dry diet. All diets was provided ad libitum for 60 days. DBH contained a higher rate of soluble protein, followed by BH + DD. The highest growth and condition factors was observed in fish fed BH + DD followed by DBH. In the enzymatic activity, the fish in the DBH group contained a higher level of alkaline phosphatase, nonspecific protease, and amylase, while the nonspecific hepatotoxic markers did not differ. The intestinal histopathology showed that a diet of BH and BH + DD favored the development of goblet cells and higher intestinal villus. The combination of a wet diet and a commercial dry diet aids the structural maintenance of the intestine, and stimulates the production of alkaline phosphatase and nonspecific protease of the digestive tract. The moisture content associated with greater nutrient solubility encourages better development and overall well-being in this species.

**Key words:** Amazonian ornamental fish, acará disco, digestive system growth; nutrition,

**Avaliação de dietas comerciais e artesanais para uso em sistema de produção intensiva de *Symphysodon aequifasciatus*. Resumo:** Avaliação de dietas comerciais e dieta úmida utilizadas no sistema intensivo de produção de *Symphysodon aequifasciatus*. O presente estudo investigou o desempenho do crescimento e os impactos no metabolismo, morfologia digestiva e metabolismo hepático do acará disco *Symphysodon aequifasciatus* alimentado com uma dieta comercial DU + DC - 50% dieta úmida + 50% dieta seca comercial; iii) DC - dieta seca comercial. Todas as dietas foram fornecidas ad libitum por 60 dias. DC continha uma maior taxa de proteína solúvel, seguido por BH + DD. Os maiores fatores de crescimento e condição foram observados em peixes alimentados com BH + DD seguido por DC. Na atividade enzimática, os peixes do grupo DAP continham maior teor de fosfatase alcalina, protease inespecífica e amilase, enquanto os marcadores hepatotóxicos inespecíficos não diferiram. A histopatologia intestinal mostrou que a dieta de DU e DU + DC favoreceu o desenvolvimento de células caliciformes e vilosidades intestinais altas. A combinação de uma dieta úmida e uma dieta seca comercial auxilia na manutenção estrutural do intestino e estimula a produção de fosfatase alcalina e protease não específica do trato digestivo. O teor de umidade

associado a uma maior solubilidade de nutrientes estimula um melhor desenvolvimento e bem-estar geral nesta espécie.

**Palavras-chave:** Peixe ornamental amazônico, acará disco, crescimento; sistema digestivo, nutrição,

## Introduction

The blue discus fish (*Symphysodon aequifasciatus*, Pellegrin, 1904) is a freshwater cichlid native to the rivers of the Amazon basin in Brazil, Peru, and Colombia (Wattley *et al.* 1991). It is one of the most prized species in fishkeeping, receiving the nickname "king of the aquarium", due to its external anatomy, non-aggressive behavior, and pattern of colors and prints (Wen *et al.* 2018). It is a species of fish with food diversity in its trophic environment. Studies describe that in natural environment high consumption of algae, plant material, small terrestrial invertebrates, organic dentins and perifiton is observed (Crampton, 2008).

Breeders and exporters of wild *S. aequifasciatus* are known to use live and homemade foods that are referred to as the humid beef heart hamburger (Satoh *et al.* 2017, Wen *et al.* 2018). The manufacturing of ornamental fish diets follows a very different path and is focused on the manufacture of extruded foods with low moisture content. Dry foods are known to provide very little attraction to carnivorous fish species (Planas & Cunha 1999, Wen *et al.* 2018).

Some studies have reported the effectiveness of wet diets for farmed fish when in the food transition phase (Salaro *et al.* 2003, Papadakis *et al.* 2008, Przybyla *et al.* 2014), and the viability of using them with dry diets as consumption stimulators (Beerli *et al.* 2009). The use of moist diets with higher levels of soluble protein can be an alternative for feeding ornamental fish. These foods, called a wet diet, can vary in their moisture content, ranging from 74% to 84% crude protein (NRC, 2006). A wet diet constitutes one of the most important sources of protein (Papadakis *et al.* 2008) and stimulates important sensory chemoreceptors increasing food intake rate (Tesser & Portela 2006).

In an attempt to improve the efficiency of their diet, fish have the ability to adapt digestive processes, such as enzymatic profile and secretion, absorption, and transport of nutrients (Ota *et al.* 2019). Morphological investigations have revealed the performance of the digestive, absorptive, and metabolic processes of different animals (Ota *et al.* 2019). Morphological studies have also identified a selective barrier that allows the absorption of

nutrients (Liquori *et al.* 2007) and also revealed the different adaptations of the digestive system to the diet that is essential for guaranteeing the health and well-being of fish (Sørensen *et al.* 2016). Therefore, histological studies associated with the modulation of digestive functions are essential to identify the type of food source to recommend for feeding fish (Evans *et al.* 2005).

The present study investigated the growth performance and impacts on the metabolism, digestive morphology, and hepatic metabolism of *S. aequifasciatus* fed a dry commercial diet and a wet diet.

## Material and Methods

**Animals:** The experiment was conducted at the Piscicultura Casacavel – Cascavel/Paraná, Brazil for 60 days. The experimental fish were acclimated for 15 days. First, 180 fish were individually weighed and measured (initial body weight  $2.15 \pm 0.15$  g, body length  $3.81 \pm 0.50$  cm with four month of age) and randomly allocated into 12 separate 50 L glass aquaria, with 15 individuals per aquarium. The aquaria were further divided into four groups with three replicates per group. During the following 60 d of the experiment, *S. aequifasciatus* were fed *ad libitum* at 08:00, 11:00, 15:00, and 19:00 h. The water temperature was maintained at  $27.0 \pm 0.5$  °C, pH 7.2, and dissolved oxygen  $5.4 \pm 0.42$  mg L<sup>-1</sup>. The aquaria were supplied with a constant flux of water with total renewal approximately 20 times daily. At the end of the experiment, after 24 h without feeding, all fish were transferred to a benzocaine bath (100 mg.L<sup>-1</sup>) for 30 s (Inoue *et al.*, 2003), collected, weighed, and measured. Three fish were collected per aquarium, for nine fish per treatment. The fish were subdivided into nine fish for analysis of nutrient retention efficiency, nine fish for digestive and metabolic enzyme assays, and nine fish for histopathology analysis. Samples of collected body tissues were stored at -80 °C for further analysis. The experiment was approved by the Ethics Committee for Animals Use of Centro Universitário da Grande Dourados (Protocol number 004/14).

**Experimental diets:** The commercial diet selected contained the following ingredients: Açaí, Albumin,

Garlic, Cornstarch, Oats, Dehydrated Cockroach, Beets, Canthaxanthin, Carboxy-Methyl-Cellulose, DL-Methionine, Spinach, Yeast Extract, Flaxseed Bran, Soybean Meal, Algae *Schizochytrium* sp Flour, Artemia Flour, Lula Flour, Fish Flour, Salmon Flour, Passion Fruit Fiber, Gelatin, Dehydrated *Gryllus assimilis*, Dehydrated *Musca domestica*, Soy Protein Isolate, Lycopene, L-Lysine, corn, Linseed Oil, Salmon Oil, Paprika, Beetroot Pulp, Rice Chops, Salt, Spirulina, Tenebrio Molitor, Yucca Extract, Premix Mineral Vitamin and Preservative Additives, Antioxidant, Mycotoxin Adsorber, Probiotic, Prebiotic, and Multi-Enzymatic.

The moist diet used was made by a fish producer, the ingredients included: fishmeal, beef heart, beef liver, soybean meal, wheat bran, gelatin, vitamin supplements, and mineral supplements.

The moisture content of the feed given to the fish varied per group (Table I):

DBH - Diets beef hamburger;

BH+DD - 50% Diets beef hamburger + 50% Commercial diets;

DD- Dry diet - feed had a 7% moisture, which is the standard level in commercial diets.

Diet composition was analyzed as established by Association of Official Analytical Chemists (AOAC, 2000). The moisture content was determined by submitting the samples to 105 °C in an oven until constant mass. The crude protein content was determined by the Kjeldahl method, where the nitrogen content obtained is multiplied by the factor 6.25. The ethereal extract levels were determined using the Soxhlet extraction apparatus and petroleum ether as solvent (e.g., 30 - 60°C) and continuous reflux for 6 hours. The ash concentration was determined by incinerating the sample in a muffle at 600°C until the constant mass is obtained.

**Protein solubility:** diet sample, stirred after five minutes the content of protein measured (Breadford

1976) at 450nm, and 1.0mg mL<sup>-1</sup> of albumin was used as standard (Fig. 1).

**Growth performance:** After 60 days the fish were transferred to a benzocaine bath (100mg L<sup>-1</sup>) for 30 sec (Inoue, 2003), measured and weighted. The following performance parameters were estimated as: 1) Weight Gain (WG) = (final body weight } initial body weight) fish<sup>-1</sup>; 2) Length Gain (LG) = (final body length } initial body length) fish<sup>-1</sup> 3) Specific Growth Rate (SGR) = (100 } [(ln final body weight } ln initial body weight) number of experimental days<sup>-1</sup>]); 4) Diet Consumption (DC) = feed intake (g) day<sup>-1</sup>; 5) Feed Conversion Ratio (FCR) = feed intake (g) weight gain<sup>-1</sup> (g); 6) Protein Efficiency Ratio (PER) = weight gain (g) fish<sup>-1</sup> crude protein intake (g)<sup>-1</sup>. The Condition Factor (K) of the fish was calculated by the allometric method, from the expression  $K = W/L^b$ , where, W represents the total mass, L the standard length of the individuals and b the regression coefficient. To estimate the value of the coefficient b, a single equation of weight-length ratio ( $W = aL^b$ ) was adjusted, from the set of all individuals collected, using the least squares method applied to the data converted in their respective natural logarithms, according to the methodology suggested by (Lima-Junior 2002).

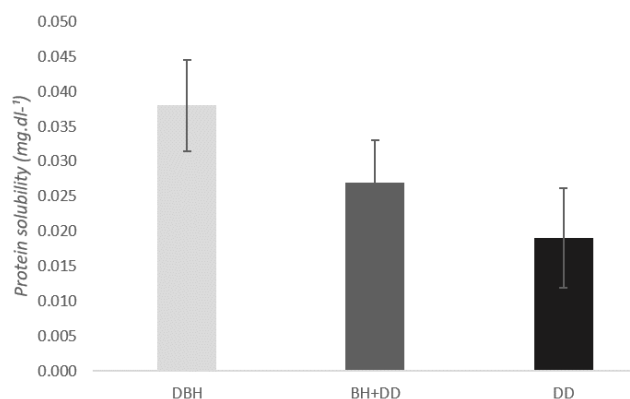
Prior to the growth assay, an initial set of ten fish were sampled to determine the efficiency of nutrients retention. After the trial, nine fish per treatment (three per tank) were sampled to determine the efficiency of nutrient retention. The fish were dried at 65°C for 16h for determination of dry matter and crude protein (AOAC, 2000). Efficiency of nutrient retention was determined as follows: Protein Productive Value (PPV) = [(final body protein } initial body protein } (initial body protein } initial body weight)] 100 total protein intake<sup>-1</sup> and Proportion of Crude Protein in the Weight Gain (PPWG) = (final body protein } initial body protein) 100 weight gain<sup>-1</sup>.

**Table 1.** Chemical composition of the experimental diets utilization for *Symphysodon aequifasciatus*.

| Diets                                 | DBH    | BH+DD  | DD     |
|---------------------------------------|--------|--------|--------|
| Dry matter                            | 78.9   | 84.7   | 96.6   |
| Crude protein                         | 46.8   | 48.4   | 49.6   |
| Lipid                                 | 3.6    | 3.1    | 3.1    |
| Carbohydrate**                        | 27.0   | 24.0   | 21.2   |
| Mineral matter                        | 1.5    | 2.5    | 1.7    |
| Crude energy (Kcal.kg <sup>-1</sup> ) | 4213.6 | 4143.5 | 4122.6 |

\*\*carbohydrate=dry matter-(crude protein-lipid - crude fiber - mineral matter).

DBH - Diets wet (beef hamburger); BH + DD - 50% Diets wet (beef hamburger) + 50% Commercial dry diet; DD - Commercial dry diet.



**Figure 1.** Protein solubility tenhe diets. DBH - Diets wet (beef hamburger); BH + DD - 50% Diets wet (beef hamburger) + 50% Commercial dry diet; DD- diet dry.

**Enzymatic analysis:** After the trial, fifteen fish per treatment (five per tank) were sampled to determine the enzyme. The digestive tract and liver were excised. The extracts to measure the enzymatic activity were produced individually for each fish and it was used 15 samples per treatment.

The intestine homogenates were done into 0.02 M Tris / 0.01 M phosphate buffer pH 7.0 mixed with anhydrous glycerol v/v under ice-bath, with a Potter-Elvehjem homogenizer. The homogenates were centrifuged at  $12.000 \times g$  for 3 minutes and the supernatants (crude homogenates) were used as enzyme source. The digestive enzymes are nonspecific protease, lipase, amylase and alkaline phosphatase.

Non-specific proteolytic activity was assayed with 1% casein as substrate (Walter, 1984). The pH values were previously optimized for intestine, and 0.1 M Tris – HCl (pH 9.0) for middle. Reactions were carried out at 25° C for 1 h, stopped with 15% TCA, and the optical density of the supernatant recorded at 280nm against tyrosine as standard. Both a substrate free control and an enzyme free control were run. Specific activity was expressed in micromole of hydrolysed substrate protein (U/mg protein).

Amylase was assayed according to (Bernfeld 1955) slightly modified. Briefly, the reactions were done in 0.2 M citrate/phosphate buffer pH 7.0 with 5% starch solution as substrate and 0.5% NaCl as cofactor. The amount of crude homogenate was previously adjusted. Reactions were performed at 25°C for 35 min and stopped by addition of 5% ZnSO<sub>4</sub> and 0.3 N Ba (OH)<sub>2</sub>. Precipitate was discarded after centrifugation at  $1000 \times g$  for 3 minutes and the reaction product was read at 690 nm (Park & Johnson 1949).

Lipase determination was adapted from (Albro 1985). Reactions were incubated with 0.4 mM r-nitrophenyl myristate in 24 mM ammonium bicarbonate (pH 7.8) with 0.5% Triton X-100. Control reactions were run without enzyme and without substrate. The reaction was stopped by addition of NaOH to a final concentration of 10 mM and the optical density was registered at 405 nm for 30 min. One unit was defined as micromole of substrate hydrolyzed per min and expressed per milligram protein (U/mg protein).

**Protein:** Soluble protein from tissue homogenates was determined according to the method of (Bradford1976), using bovine serum albumin (Sigma) as standard.

The Alkaline Phosphatase, were measured using colorimetric methods (Alkaline phosphatase OSR6004), analysis by spectrophotometry (semi-automatic spectrophotometer Bioplus S-200),

The liver sub-samples were homogenized in buffer as described earlier. Homogenates were centrifuged at 600 g for 3 min, and the supernatants centrifuged at 6000 g for 8 min at 3 °C. Resultant supernatants were used as crude enzyme source. Enzyme protocol assays were: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assays were end-point type, read at 430 nm following the protocol: ALAT – 222 mM alanine or ASAT – 44.4 mM aspartate, 11.6 mM a-ketoglutarate, 0.22 mM arsenate, 0.27 mM pyridoxal phosphate and phosphate buffer pH 7.0 (Reitman & Frankel, 1957).

**Histopathological analysis intestine:** After the trial, fifteen fish per treatment (five per tank) were sampled to determine the histopathology analysis. For intestine collection were selected from the anterior intestine three piece of 1 cm, for further processing. Intestine fragments were immersed in Bouin solution for 24 hours and subsequently washed in 70% alcohol. After fixation, the fragments were dehydrated in graded ethanol solutions (70%, 80%, 90% and 100%), diaphanized and embedding in paraffin with plastic polymer Histosec® (Merck). Microtomy was performed in order to obtain 2 slices with 5 µm thickness, which were stained by hematoxylin-eosin (HE) and by histochemical method of periodic acid Schiff hematoxylin (PAS-H) (McManus, 1948). The microscopic analysis and documentation of the material were performed using a light microscope (Olympus, BX41).

First, qualitative histological analyses were done to observe main treatment effects. Based on those observations, quantitative analyses were

defined. For the morphometry of the material seven slices per treatment were selected and seven sections were photographed. Size of the hepatocytes and height of intestinal villi were measured. Intensity of reaction of the Goblet cells in the intestine, indicated by PAS-H dyeing (McManus, 1948; Ortiz-Delgado *et al.*, 2003), was classified as strong (+ + +), moderate (+ +), weak (+) or absent (-). Intestinal villi height was classified as short (+), moderate (+ +) or high (+ + +) (Hirji & Courtney *et al.*, 1982; Gawlicka *et al.*, 1995; Tengjaroenkul *et al.*, 2000; Moretti *et al.*, 2014).

**Statistical procedures:** Data normality and variance homogeneity of gastroprotective and hepatic parameters was 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  $\pm$  standard deviation (SD). Statistical analyses were performed using R-Studio software (version 1.1.423 – © 2009-2018 R-Studio, Inc.).

## Results

There were no significant differences in the water quality parameters in any treatment. The physical and chemical variables of the water in the experimental units were: dissolved oxygen  $5.4 \pm 0.42$  mg L<sup>-1</sup>, temperature  $27.0 \pm 0.5$  °C, electrical

conductivity  $230.37 \pm 15.47$   $\mu$ S cm<sup>-1</sup>, average pH  $7.0, \pm 0.41$ , and total NH<sub>3</sub> 0.5 mg L<sup>-1</sup>.

The productive parameters and nutrient retention efficiency (Table 2) resulted in no significant differences in weight gain, length, SGR, and the proportion of crude protein in the weight gain. Weight gain varied between 1.97 g in DBH and 2.51 g in DH + DD. The protein production value varied between 2.35 in DBH and 2.71 in DH + DD.

For the parameters of PER, feed conversion, and survival, a significant difference was observed between the treatments. For PER, all treatments were different, DBH (0.33 g·kg<sup>-1</sup>), BH + DD (0.52 g·kg<sup>-1</sup>), and DD (1.21 g·kg<sup>-1</sup>). The DD feed conversion (2.97g) was significantly lower than the other treatments, with DBH (6.63) and BH + DD (6.10). The survival of BH + DD (94.32 %) was significantly higher than the other treatments DBH (85.23 %) and DD (84.09 %).

The enzyme results (Table III) show no significant differences were observed for the metabolic enzymes (ALT and AST) or the relationship between them and the albumin protein dosage. The digestive activity of nonspecific proteases, lipase, and amylase was lower in the DBH fed fish. The DD treatment contained the highest values for digestive enzyme lipase. While the BH + DD diet resulted in increased nonspecific proteases, amylases, and alkaline phosphatases. The height of the intestinal villi was also greater in the BH + DD treatment group.

**Table 2.** Growth performance and efficiency of nutrient utilization of *Symphysodon aequifasciatus*.

| <b>Growth performance</b>                      | <b>DBH</b>  | <b>BH+DD</b>            | <b>DD</b>   |
|------------------------------------------------|-------------|-------------------------|-------------|
| Weight gain (g·fish <sup>-1</sup> )            | 1.97±0.76a  | 2.51±0.32a              | 1.98±0.72b  |
| Length gain (cm·fish <sup>-1</sup> )           | 0.36±0.29b  | 0.56±0.19a              | 0.40±0.30b  |
| Specific growth rate (%·day <sup>-1</sup> )    | 1.23±0.17a  | 1.20±0.39a              | 1.05±0.31b  |
| Protein efficiency ratio (g·kg <sup>-1</sup> ) | 0.33±0.001b | 0.52±0.002b             | 1.21±0.006a |
| Feed conversion                                | 6.63±1.22a  | 6.10±2.81a              | 2.97±1.21b  |
| Survival (%)                                   | 85.23±5.9b  | 94.32±1.97 <sup>a</sup> | 84.09±9.37b |
| Fish homogeneity (%)                           | 61.33       | 68.67                   | 80.55       |
| Relative condition factor (K)                  | 62          | 13                      | 13          |
| <b>Nutrients-retention efficiency</b>          |             |                         |             |
| Protein productive value                       | 2.35±0.33   | 2.54±0.51               | 2.71±0.54   |
| Proportion of crude protein in the weight gain | 1.95±0.31   | 1.95±0.72               | 2.30±0.80   |

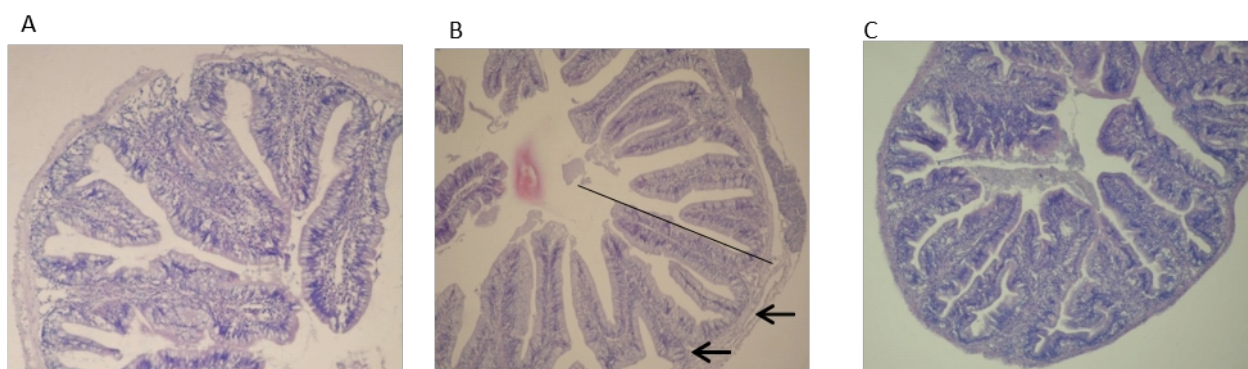
DBH - Diets wet (beef hamburger); BH + DD - 50% Diets wet (beef hamburger) + 50% Commercial dry diet; DD - Commercial dry diet

Mean values with different superscript letters in the same row are significantly different by Tukey test ( $P < 0.05$ ). Values are mean  $\pm$  SEM

Anterior intestine of *S. aequifasciatus* is characterized by four layers: mucosa, submucosa, muscularis and serosa. The mucosa layer is composed of cylindrical epithelium with brush border and enterocytes interspersed by Goblet cells, with lamina containing intraepithelial lymphocytes; the submucosa is formed by cells, collagen fibers and blood vessels; the muscularis layer is composed of circular smooth muscle fibers and externally to

the serosa layer, characterized by loose connective tissue PAS (positive) and pavement cells (Figure 2).

The highest intestinal villus heights were observed in fish fed the BH + DD and DD diets (Table III). The variation in the intensity of glycoprotein secretion by goblet cells was directly correlated with the diet type. There was greater goblet cell production in the intestine of the fish fed DBH (Table IV).



**Figure 2.** The intestinal a) the epithelium region (EP) (DBH, H/E – 10x); b) showing villus height (continuous line) and serous (arrows) (BH+DD, PAS – 10x); c) lumen (\*) and height of the intestinal villus (continuous line) (DD, H/E 10x).

**Table 4.** Histochemistry of the Goblet cells of the anterior intestine of *Symphysodon aequifasciatus*, under experimental diets.

| Methods | Diets      |     |       |    |
|---------|------------|-----|-------|----|
|         |            | DBH | BH+DD | DD |
| HE      | Neutral    | ++  | +     | +  |
|         | Acid       | +   | ++    | -  |
|         | Conjugated | +++ | ++    | +  |
| PAS     | Neutral    | +++ | ++    | +  |
|         | Acid       | -   | -     | -  |
|         | Conjugated | -   | -     | -  |

DBH - Diets wet (beef hamburger); BH + DD - 50% Diets wet (beef hamburger) + 50% Commercial dry diet; DD- Commercial dry diet. Intensity of reaction: weak (+), moderate (++), strong (+++); absent (-).

## Discussion

The higher percentage of soluble protein in the DBH diet was followed by BH + DD and was associated with higher consumption of these diets due to the chemical stimuli promoted. Little is known about the development of chemoreceptor systems in fish; however, for *Piaractus mesopotamicus* larvae, the stimuli could be increased, resulting in a higher intake rate (Tesser & Portela 2006). Kolkovski *et al.* (1997) describes the stimulating consumption effects of using L-amino acids and betaine together with inert diets for *Sparus aurata*. The inclusion of krill hydrolyzed in the

water increased the inert diet intake rate by 200% in larvae of *Perca flavescens* (Kolkovski 2000).

The best growth rates were observed in fish fed the BH + DD diet, this result was also reported by (Wen *et al.* 2018), who combined a wet diet with and hearts (1: 1) and found them to be good growth promoters for *S. aequifasciatus*. The presence of free amino acids in the diet stimulates feeding chemoreceptors (Santos *et al.* 2021, Camilo *et al.* 2021). In a study with larvae of *Piaractus mesopotamicus* it was observed that the use of water from the hatching of brine shrimp stimulated the behavior of capturing food, these results being attributed to the presence of dissolved substances

(Tesser e Portella 2006). Our results agree with previous research that identified food conditioning with moistened rations provides a better nutritional balance that is reflected in higher survival rates. The use of DD showed more homogeneous batches with less tendency to establish hierarchy (Chong *et al.* 2000) reported that *S. aequifasciatus* has territorial characteristics, which results in heterogeneous growth.

The condition factor results of the group fed DBH was superior to the other treatments. The *S. aequifasciatus* fed exclusively with diet had Kn of 0.99 while those fed with 75% heart meat had Kn of 1.02 (Beerli *et al.* 2009). The value reflects the recent nutritional conditions and/or expenditures of the reserves in cyclical activities, possibly relating it to the environmental conditions and the behavioral aspects of the species (Gomiero *et al.* 2010). The weight/length ratio is used to estimate the general state of the fish in a new environment the health of the fish (Tavares-Dias *et al.* 2000), and to estimate the degree of food activity (Weatherley & Gill 1987). The weight/length relationship associated with the other results collaborates with the indication of the food strategy to be adopted. It was observed that for *Pseudoplatystoma* sp fed with increasing levels of protein, the best growth indices and digestive functions reflected in better K indices. (Honorato *et al.* 2015).

Simplistically, the development of fish depends on the body's ability to digest and process the food (Przybyla *et al.* 2014). The best specific growth rate and weight gain were observed in fish fed the DBH and BH + DD diets. This may be a reflection of the intake process being limited by the high energy density of the dry diets (Yufera, 1996 e 1999), the lower palatability of the dry diet (Papadakis *et al.* 2008), and the longer hydration time for dry diets (Kristiansen & Rankin *et al.* 2001; Varsamos *et al.* 2004) and also the altering of the gastric evacuation (Ruohonen *et al.* 1997).

Some studies have shown that efficient nutrient use is more effective in wet diets than dry diets (Mazzola *et al.* 2000; Chatzifotis *et al.* 2005; Papadakis *et al.* 2008). These results are attributed to a greater digestibility of wet diets (Grove *et al.* 2001). Digestibility is the result of the actions of digestive enzymes and the body's ability to absorb nutrients. Therefore, we observed the responsiveness of alkaline phosphatase to BH + DD. Stimulating the production of enzymes by the presence of a specific food is associated with the production, such as modulation in the hepatopancreas (Fuentes-Quesada

*et al.* 2018). Proper protein use is very important for fish nutrition and depends heavily on proteases (González-Félix, 2010). We observed an increase in the activity of digestive protease and alkaline phosphatase in the digestive tract *S. aequifasciatus*. This observation was concomitant with an improvement in the architecture of the intestinal villi. The heights of the villus and the muscular layer were higher for fish fed the BH + DD diet. This result was 53 % higher than the fish fed the DD. The increase in the height of the villus and the muscular layer is an adaptation mechanism to optimize the diet absorption in *S. aequifasciatus*.

The BH + DD treatment showed a larger villus size, which may be due to components of plant origin in the dry diet, which stimulates the growth of intestinal villi. (Omnes *et al.* 2015) evaluated two sources of vegetable proteins in the diet and concluded that the stimulation of the villus development of the epithelium of the proximal intestine of *Sparus aurata* is an adaptation that improves absorption function. In another study (Goulart *et al.* 2018), the effect of dietary fiber concentrates in the diet of *Rhamdia quelen* was evaluated, the authors reported an improvement in the growth performance and intestinal morphology. Thus, a greater height of the intestinal villi contributes to greater digestion and absorption of nutrients; this is reflected positively in animal performance, which may explain the increase in weight gain of fish fed the BH + DD diet.

In the present study, there was no difference in the activity of the enzymes in the hepatic protein metabolism (ALT and AST), which indicates that the largest supply of nutrients was used appropriately for fish growth. The increased activity of AST and ALT are considered the body's response to stressors and the nutritional metabolism is considered indicative of an injured or damaged organ (Meng *et al.* 2018).

Although the use of dry diets (less than 10 % humidity) is recommended in commercial aquaculture because of the advantages of storage and distribution logistics, we must consider that ornamental fish and breeders provide wet diets. In this study, we demonstrated that the routine use of a humid diet for *S. aequifasciatus* in association with a dry diet helps in digestive function, improves the histopathological aspects of the intestine, and stimulates the production of intestinal alkaline phosphatase and nonspecific protease effects. These results can be attributed to the synergistic action of

the higher proportion of soluble protein in the wet diet.

The combination of a wet diet with a dry diet (commercial) resulted in a higher rate of development, reflecting increased activity in the digestive enzymes associated with the absorption of nutrients by the intestinal villi. In conclusion, for discus species it is recommended the utilization BH + DD diet.

### Ethical statement

The experimental protocol was approved by the Ethics Committee for Research with Animals of the Centro Universitário da Grande Dourados/CEUA, protocol: 004/14.

### References

- Albro PW, Hall RD, Corbett JT, Schroeder J, 1985. Activation of nonspecific lipase by bile salts. **Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism**, 835(3): 477-490. [https://doi.org/10.1016/0005-2760\(85\)90117-1](https://doi.org/10.1016/0005-2760(85)90117-1).
- AOAC Associations of Official Analytical Chemists. **Official Methods of Analyses of the Association of Analytical Chemists**. Ed. 17. v.2, 2000.
- Beerli, Eduardo Lopes, 2009. Feeding strategy and stocking density for discus fish (*Symphysodon aequifasciatus*). PhD. Thesis. Universidade Federal de Goiás, Goiânia, Brasil. 78p.
- Bernfeld P, 1955. Amylase  $\alpha$  and  $\beta$  In: Colowick, S. P., Kaplan, N. (Methods in Enzymology. New York: **Academic Press**, 1:149-158.
- Breadford MM, McRorie RA, William WL, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, 72(1-2): 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Camilo, R.Y., Honorato, C.A., Santos, R.F.B., Avilez, I.M., De Almeida, L.C. e Moraes, G., 2021. Macronutrients and essential amino acids on digestive process of the freshwater teleost Matrinxã Medicina Veterinária (UFRPE), 15: 413-421.
- Chatzifotis, S., Papadakis, I. E., & Divanach, P, 2005. Effect of dietary water on growth of dentex *Dentex dentex*. **Fisheries Science**, 71(6), 1243-1248.
- Chong, A. S. C., Hashim, R., Ali, A. B, 2000. Dietary protein requirements for discus (*Symphysodon* spp.). **Aquaculture Nutrition**, 6(4): 275-278. <https://doi.org/10.1046/j.1365-2095.2000.00151.x>.
- Crampton, W.G.R. 2008. Ecology and life history of an Amazon floodplain cichlid: the discus fish *Symphysodon* (Perciformes: Cichlidae). **Neotropical Ichthyology**, 6(4): 599-612.
- Evans JJ, Pasnik DJ, Peres H, Lim C, Klesius PH, 2005. No apparent differences in intestinal histology of channel catfish (*Ictalurus punctatus*) fed heat-treated and non-heat-treated raw soybean meal. **Aquaculture Nutrition**, 11: 123-129. <https://doi.org/10.1111/j.1365-2095.2004.00329.x>.
- Fuentes-Quesada JP, Viana MT, Rombenso AN, Guerrero-Rentería Y, Nomura-Solís M, Gomez-Called, V, Lazoa JP, Mata-Sotres JA, 2018. Enteritis induction by soybean meal in *Totaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. **Aquaculture**, 495: 78-89. <https://doi.org/10.1016/j.aquaculture.2018.05.025>.
- Gawlicka A, Teh S, Hung SSO, Hinton E, de Li, Noüe, J, 1995. Histological and histochemical changes in the digestive tract of white sturgeon larvae during ontogeny. **Fish Physiology Biochemistry**, 14: 357-371. DOI: 10.1007/BF00003374.
- Gomiero, L. M.; Villares-Junior, G. A.; Braga F. M. S, 2010. Relação peso-comprimento e fator de condição de *Oligosarcus hepsetus* (Cuvier, 1829) no Parque Estadual da Serra do Mar - Núcleo Santa Virgínia, Mata Atlântica, estado de São Paulo, Brasil. **Biota Neotropica**, 10(1): 101-105. <http://dx.doi.org/10.1590/S1676-06032010000100009>.
- González-Félix, M.L., Castillo-Yañez, F.J., Ocaño-Higuera, V.M., Perez-Velazquez, M., Cota-Moreno, V. Lozano-Taylor, J, 2010. Effect of dietary protein source and time on alkaline proteolytic activity of Nile tilapia (*Oreochromis niloticus*). **Fish Physiology Biochemistry**, 37: 779-785. DOI 10.1007/s10695-009-9352-4.
- Goulart, Fr.; Lovatto, Nm; Klinger, Ac.; Adorian, Tj.; Mombach, Pi.; Pianesso, D.; Martinelli, Sg.; Veiga, Ml.; Silva, Lp, 2018. Effect of dietary fiber concentrates on growth performance, gut morphology and hepatic

- metabolic intermediates in jundiá (*Rhamdia quelen*). **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, 70(5): 1633-1640. <http://dx.doi.org/10.1590/1678-4162-10218>.
- Grove D, Genna R, Paralika V, Boraston J, Hornyold MG, Siemens R, 2001. Effects of dietary water content on meal size, daily food intake, digestion and growth in turbot, *Scophthalmus maximus* (L.). **Aquaculture Research**, 32:433-442. <https://doi.org/10.1046/j.1365-2109.2001.00585.x>.
- Hirji KN, Courtney Wam, 1982. Leucine aminopeptidase activity in the digestive tract of perch, *Perca fluviatilis* L. **Journal Fish Biology** 21: 615-622. <https://doi.org/10.1111/j.1095-8649.1982.tb02865.x>.
- Honorato, C.A., Ushizima, T.T., Santamaria, F.M., Flores-Quintana, C.I., Marcondes, V.M. e Nascimento, C.A., 2015. Growth performance and economic of *Pseudoplatystoma* sp fed with protein levels reared in net cages **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, 67(5):1408-1414. <https://doi.org/10.1590/1678-4162-7238>
- Inoue LAKA, Neto CS, Moraes G. 2003. Clove oil as anaesthetic for juveniles of matrinxã *Brycon cephalus* (Gunther, 1869). **Ciências Rural**, 33: 943-947. <http://dx.doi.org/10.1590/S0103-84782003000500023>.
- Kolkovski, S.; Arieli, A.; Tandler, A, 1997. Visual and chemical cues stimulate microdiet ingestion in sea bream larvae. **Aquaculture International**, 5: 527-536. <https://doi.org/10.1023/A:1018305416501>.
- Kolkovski, S.; Czesny, S.; Dabrowski, K, 2000. Use of krill hydrolysate as a feed attractant for fish larvae and juvenile. **Journal of the World Aquaculture Society**, 31: 81-88. <https://doi.org/10.1111/j.1749-7345.2000.tb00701.x>.
- Kristiansen H.R., Rankin J.C, 2001. Discrimination between endogenous and exogenous water sources in juvenile rainbow trout fed extruded dry feed. **Aquatic Living Resources**. 14: 359-366. [https://doi.org/10.1016/S0990-7440\(01\)01131-7](https://doi.org/10.1016/S0990-7440(01)01131-7).
- Lima-Junior SE, Cardone IB, Goitein R, 2002. Determination of a method for calculation of Allometric Condition Factor of fish. **Acta Scientiarum: Biological and Health Sciences**, 24(2): 397-400. <http://dx.doi.org/10.4025/actasciobiolsci.v24i0.2311>.
- Liquori, GE, Mastrodonato M, Zizza S, Ferri D, 2007. Glycoconjugate histochemistry of the digestive tract of *Triturus carnifex* (Amphibia, Caudata). **Journal of Molecular Histology**, 38: 191-199. DOI:10.1007/s10735-007-9087-4.
- Mazzola, A., Favaloro, E., & Sarà, G, 2000. Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), in submerged cages in the Western Mediterranean Sea. **Aquaculture**, 181(3-4): 257-268. [https://doi.org/10.1016/S0044-8486\(99\)00243-4](https://doi.org/10.1016/S0044-8486(99)00243-4).
- Mcmanus JFA, 1948. Histological and histochemical uses of periodic acid. **Stain Technology**, 23(3): 99-108. <https://doi.org/10.3109/10520294809106232>.
- Meng, X. L., Li, S., Qin, C. B., Zhu, Z. X., Hu, W. P., Yang, L. P., & Nie, G. X. (2018). Intestinal microbiota and lipid metabolism responses in the common carp (*Cyprinus carpio* L.) following copper exposure. **Ecotoxicology and Environmental Safety**, 160: 257-264. <https://doi.org/10.1016/j.ecoenv.2018.05.050>.
- Moretti DB, Nordi WM, Cruz TM, Cyrino JE, Machado-Neto R, 2014. Histochemical distribution of intestinal enzymes of juvenile pacu (*Piaractus mesopotamicus*) fed lyophilized bovine colostrum. **Fish Physiology and Biochemistry**, 40(5): 1487-93. DOI: 10.1007/s10695-014-9942-7.
- National Research Council - NRC, 2006. Nutrients requirements of fish. Washington: National Academic Press, 114p.
- Omnes, MH.; Silva, FCP.; Moriceau, J.; Aguirre, P.; Kaushik, S.; Gatesoupe, Fj, 2015. Influence of lupin and rapeseed meals on the integrity of digestive tract and organs in gilthead seabream (*Sparus aurata* L.) and goldfish (*Carassius auratus* L.) juveniles. **Aquaculture Nutrition**, 21 (2): 223-233. <https://doi.org/10.1111/anu.12162>.
- Ortiz-Delgado, JB, Darias, MJ, Cañavate JP, Yúfera M, Sarasquete C, 2003. Organogenesis of the digestive tract in the white seabream, *Diplodus sargus*. Histological and histochemical approaches. **Histology and histopathology**, 18 1141-1154. <http://www.hh.um.es>.
- Ota ECO, Honorato CA, Heredia-Vieira SC, Flores-Quintana CI, Silva TSCDA, Inoue LAKA,

- Cardoso CAL (2019) Hepatic and gastroprotective activity of *Serjania marginata* leaf aqueous extract in Nile tilapia (*Oreochromis niloticus*). **Fish Physiology and Biochemistry**, 45: 1-15. <https://doi.org/10.1007/s10695-019-00622-9>
- Park, J.T. & Johnson, M.J., 1949. A sub micro determination of glucose. **Journal of Biological Chemistry**, 181: 149-151.
- Planas, M.; Cunha, I., 1999. Larviculture of marine fish: problems and perspectives. **Aquaculture**, 177: 171-190. [https://doi.org/10.1016/S0044-8486\(99\)00079-4](https://doi.org/10.1016/S0044-8486(99)00079-4).
- Papadakis Ie, Chatzifotis, S, Divanach P, Kentouri M, 2008. Weaning of greater amberjack (*Seriola dumerilii* Risso 1810) juveniles from moist to dry pellet. **Aquaculture International**, 16: 13-25. <https://doi.org/10.1007/s10499-007-9118-x>.
- Przybyla C, Fievet J, Callier M, Blancheton J, 2014. Effect of dietary water content on European sea bass (*Dicentrarchus labrax*) growth and disease resistance. **Aquatic Living Resources**, 27(2): 73-81. DOI: <https://doi.org/10.1051/alr/2014007>.
- Reitman S, Frankel S, 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. **American Journal of Clinical Pathology**, 28:56 <https://doi.org/10.1093/ajcp/28.1.56>.
- Ruohonen K, Grove DJ, MC Ilroy JT, 1997. The amount of food ingested in a single meal by rainbow trout offered chopped herring, dry and wet diets. **Journal of Fish Biology**, 51: 93-105. <https://doi.org/10.1111/j.1095-8649.1997.tb02516.x>.
- Salaro, A.L.; Luz, R.K.; Nogueira, G.C.C., 2003. Diferentes densidades de estocagem na produção de alevinos de trairão (*Hoplias cf. lacerdae*). **Revista Brasileira de Zootecnia**, 32(5): 1033-1036. <https://doi.org/10.1590/S1516-35982003000500001>.
- Camilo, R. Y., C. A. Honorato, R. F. B. Santos, I. M. Avilez, L. C. De Almeida, e G. Moraes. 2021. Macronutrients and essential amino acids on digestive process of the freshwater teleost Matrinxã. *Medicina Veterinária (UFRPE)* 15(4):413-421.
- Honorato, C. A., T. T. Ushizima, F. M. Santamaria, C. I. Flores-Quintana, V. M. Marcondes, e C. A. Nascimento. 2015. Growth performance and economic of *Pseudoplatystoma* sp fed with protein levels reared in net cages. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 67(5).
- Santos, R. F. B., M. S. Siqueira, R. G. A. Reis, W. V. da SILVA, H. M. Ziemniczak, e C. A. Honorato. 2021. Influence of a blend of dietary essential amino acids on growth, enzymatic activity, and intestinal histology of blue discus. *Boletim do Instituto de Pesca* 47:1-9.
- Tesser, M. B., e M. C. Portella. 2006. Ingestão de ração e comportamento de larvas de pacu em resposta a estímulos químicos e visuais. *Revista Brasileira de Zootecnia* 35(5):1887-1892.
- Satoh, S.; Tanoue H.; Ruitton, S.; Mohri M.; Komatsu, T, 2017. Morphological and behavioral ontogeny in larval and early juvenile discus fish *Symphysodon aequifasciatus*. **Ichthyological Research**, 64: 37-44. DOI 10.1007/s10228-016-0530-y.
- Sørensen, M.; Berge, Gm.; Reitan, Ki.; Ruyter, B, 2016. Microalga *Phaeodactylum tricornutum* in feed for Atlantic salmon (*Salmo salar*)—Effect on nutrient digestibility, growth and utilization of feed. **Aquaculture**, 460: 116-123. <https://doi.org/10.1016/j.aquaculture.2016.04.010>.
- Tavares-Dias, M.; Martins, M. L.; Moraes, F. R, 2000. Relação hepatossomática e esplenossomática em peixes teleósteos de cultivo intensivo. **Revista Brasileira de Zoologia**, 17(1): 273-281. <https://doi.org/10.1590/S0101-81752000000100024>.
- Tengjaroenkul, B., B. J. Smith, T. Caceci, And S. A, 2000. Smith Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. **Aquaculture Nutrition**, 182 (3-4): 317-327. [https://doi.org/10.1016/S0044-8486\(99\)00270-7](https://doi.org/10.1016/S0044-8486(99)00270-7).
- Tesser, Mb.; Portella, Mc, 2006. Ingestão de ração e comportamento de larvas de pacu em resposta a estímulos químicos e visuais. **Revista Brasileira de Zootecnia**, 35(5): 1887-1892. <http://dx.doi.org/10.1590/S1516-35982006000700002>.
- Varsamos S., Bonga S.E.W., Charmantier G., Flik C, 2004. Drinking and Na<sup>+</sup>/K<sup>+</sup> ATPase activity

- during early development of European sea bass, *Dicentrarchus labrax* – Ontogeny and shortterm regulation following acute salinity changes. **Journal of Experimental Marine Biology and Ecology**, 311: 189–200. <https://doi.org/10.1016/j.jembe.2004.04.019>.
- Walter, H. E., 1984. Proteinases: methods with hemoglobin, casein and azocoll as substrates. In: Bergmeyer, H.U. (Ed.), *Methods of Enzymatic Analysis*, Verlag Chemie, Weinheim, 5: 270-277. NAID: 10005517514.
- Wattley, J., 1991. *Discus para o perfeccionista*. 1<sup>a</sup> ed. Publicações TFH, Neptune City, 128p.
- Weatherley, Ah.; Gill, Hs, 1987. *The biology of fish growth*. London: Academic Press, 443 p.
- Wen, B., Chen, Z., Qu, H., & Gao, J, 2018. Growth and fatty acid composition of discus fish *Symphysodon haraldi* given varying feed ratios of beef heart, duck heart, and shrimp meat. **Aquaculture and fisheries**, 3(2): 84-89. <https://doi.org/10.1016/j.aaf.2018.01.002>.
- Yúfera, M., Pascual, E., Fernández-Díaz, C., 1999. A highly efficient microencapsulated food for early rearing of marine fish larvae. **Aquaculture Nutrition**, 177: 249-256. [https://doi.org/10.1016/S0044-8486\(99\)00088-5](https://doi.org/10.1016/S0044-8486(99)00088-5)
- Yúfera, M., Polo, A., Pascual, E., 1993. Changes in chemical composition and biomass during the transition from endogenous to exogenous feeding of *Sparus aurata* L. (Pisces, Sparidae) larvae reared in the laboratory. **Journal of Experimental Marine Biology and Ecology**, 167:2, 149-161. [https://doi.org/10.1016/0022-0981\(93\)90028-M](https://doi.org/10.1016/0022-0981(93)90028-M).

Received: December 2020

Accepted: July 2021

Published: December 2022