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Dietary glutamine-glutamate supplementation enhances growth performance and intestinal villi development in cage-farmed Nile tilapia fingerlings

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ABSTRACT - The objective of this research was to evaluate the effect of increasing levels of associated glutamine and glutamic acid on growth performance and intestinal development of Nile tilapia, *Oreochromis niloticus*, fingerlings. Five isoproteic (~344.70 g kg^{-1} crude protein) and isocaloric diets (~3,925 kcal kg^{-1} gross energy) were developed containing 0, 5, 10, 15, or 20 g kg^{-1} of associated glutamine and glutamic acid in extruded diets. Fish (n = 2,000, mean body weight of 2.12±0.53 g) were distributed into twenty 1-m^3 floating net cages in an entirely randomized design with five treatments and four replicates, and each replicate comprised one floating net cage with 100 fish. Fish were hand-fed seven days per week, three times a day until apparent satiety for 45 days. There was a quadratic effect on final body weight, body weight gain, daily weight gain, feed conversion ratio, protein retention efficiency, net protein utilization, and intestinal villi height with optimized values for supplementation of associated glutamine and glutamic acid at 10.77, 10.67, 10.00, 8.85, 9.85, 10.15, and 10.98 g kg^{-1}, respectively. There was no effect of associated glutamine and glutamic acid supplementation on feed intake, survival, and body composition. We conclude that 10.67 g kg^{-1} of associated glutamine and glutamic acid is adequate for growth performance optimization, and supplementation at 10.98 g kg^{-1} exerts trophic action and improves intestinal morphometry in cage-farmed Nile tilapia fingerlings.

Keywords: aminoacid, aquaculture, fish nutrition, *Oreochromis* sp.

1. Introduction

Non-essential amino acids play an important role in maintaining normal physiological function and nutritional status of the body. Many non-essential amino acids, including glutamine and glutamic acid, regulate key metabolic functions of cells crucial for survival, growth, and reproduction of animals (Wu, 2014).

Glutamine and glutamic acid are related amino acids naturally found in ingredients of feed commonly used in animal nutrition, which can be converted to each other in the intestine, liver, and kidneys (Wu, 2010). Glutamine is considered a prevalent amino acid in the blood, corresponding 30 to 50% of the plasmatic amino acids and whole-body free amino acid (Wu et al., 2013). This amino acid is

an important substrate for energy in rapidly dividing cells like enterocytes, lymphocytes, and kidney cells, producing ATP for the "turnover" of intracellular proteins for nutrient transport, growth, and cell migration (Li et al., 2007).

Dietary glutamine supplementation has been described to increase growth (Yan and Qiu-Zhou, 2006; Cheng et al., 2012; Pereira et al., 2017), improve feed efficiency (Han et al., 2014) and protein retention (Caballero-Solares et al., 2015), and enhance intestinal development of fish (Cheng et al., 2011). Recently, glutamine and glutamate supplementation have been considered beyond growth performance and their effects on disease resistance (Zhang et al., 2017) and use for fish reared under conditions of temperature stress and hypoxia have also been considered (Liu et al., 2015).

Tilapia is a well-known freshwater fish species popular worldwide as a food source and is a farmed fish in rural communities and on a commercial scale in many countries. High-density cage-farmed tilapias are constantly subjected to environmental challenge, particularly high temperature and suboptimal dissolved oxygen conditions. To sustain fast growth performance and health status, the use of non-essential amino acid such as glutamine and glutamate has been suggested in fish nutrition (Li et al., 2009).

Adding products with trophic effects on the intestinal mucosa of livestock, such as glutamine and glutamate, to the tilapia diet might be beneficial in challenging situations. Such supplementation could improve the physiological processes of digestion and the absorption of nutrients from the diet, thus enhancing growth performance. The intestine is one of the most important sites for nutrient digestion and absorption, and improving nutrient utilization by using functional amino acids may be effective in improving fish profitability.

Although the requirements of non-essential amino acids for tilapia are properly described in NRC (2011), the effects of dietary associated glutamine and glutamic acid on growth performance and intestinal villus development of Nile tilapia, *Oreochromis niloticus*, raised in floating net cages are poorly documented. Thus, the objective of the present research was to evaluate the effects of associated glutamine and glutamate on growth performance and their role in intestinal villus development of Nile tilapia fingerlings.

2. Material and Methods

This study was conducted on an experimental farm in Guaíra, Paraná, Brazil (Latitude: 24°4'46" S and 54°15'27" W). Research on animals was conducted according to the institutional committee on animal use (case no. 013/07).

2.1. Diets and fish management

Five isoproteic (~344.70 g kg^{-1} crude protein) and isoenergetic (~3,925 kcal kg^{-1} gross energy) diets were formulated to contain 0, 5, 10, 15, and 20 g kg^{-1} of combined L-glutamine and L-glutamate. Crystalline essential amino acids were supplemented to meet the quantitative essential amino acid requirements for tilapias described in NRC (2011). Crystalline L-glutamine and L-glutamate were supplied as industrial AminoGut® (Ajinomoto, São Paulo Indústria e Comércio de Alimentos Ltda., São Paulo, SP, Brazil) containing a mixture (1:1) of L-glutamine and L-glutamate. Dietary glutamine and glutamate were included at the expense of L-alanine amino acid, and protein and energy equivalences were considered. In addition, dietary corn levels were adjusted to maintain isoenergetic values of diets (Table 1). All ingredients were finely ground into a 600-µm mesh, prior to mixing in an automatic "V" mixer (MA200; Marconi, Piracicaba, SP, Brazil), extruded in a single-screen experimental feed mill (Extec, Ribeirão Preto SP, Brazil) to produce 1.5-mm pellets and dried for 24 h in a ventilated oven at 52 °C.

Two thousand Nile tilapia fingerlings (2.12±0.53 g) were distributed into twenty 1000-L floating net cages (1×1×1 m) allocated in an earthen pond of 3,000 m^2, with an average depth of 1.8 m, automated

aeration, and daily renewal rate of 0.5%. Fish were cultured under water temperature ranging from 26 to 31 °C, and dissolved oxygen ranging from 5.5 to 6.1 mg L^{-1} (YSI® 550A, Florianópolis, SC, Brasil). Data of water temperature and dissolved oxygen were measured twice a day at 08:30 and 17:00 h. Fish were hand-fed three times a day at 08:30, 14:00, and 17:00 h until apparent satiety for 45 days.

At the end of the experimental phase, fish were fasted for 24 h prior to counting and weighing to calculate growth performance. Then, ten fish per cage were sampled for proximate whole-body composition analysis, and three fish per cage were used for histological analysis. Fish were euthanized with benzocaine (100 mg L^{-1} water). Whole-body samples were ground in a meat grinder and kept at −20 °C until subsequent analysis.

2.2. Calculations

Growth performance data were calculated according to the following equations:

Weight gain (g) = final whole-body weight (g) – initial whole-body weight (g);

Daily weight gain = final whole-body weight (g) – initial whole-body weight (g)/time of experiment (days);

Feed intake = total amount of feed offered (g);

Table 1 - Formulation and analyzed composition of the experimental diets

Item	Associated glutamine and glutamic acid (g kg^{-1})				
	0	5	10	15	20
Corn	306.03	303.53	301.03	298.53	296.03
Broken rice	65.00	65.00	65.00	65.00	65.00
Soybean meal	478.00	478.00	478.00	478.00	478.00
Poultry byproduct meal	80.00	80.00	80.00	80.00	80.00
Soy oil	30.00	30.00	30.00	30.00	30.00
Dicalcium phosphate	10.00	10.00	10.00	10.00	10.00
DL-methionine (995 g kg^{-1})	1.75	1.75	1.75	1.75	1.75
L-lysine HCl (785 g kg^{-1})	0.69	0.69	0.69	0.69	0.69
L-threonine (785 g kg^{-1})	2.53	2.53	2.53	2.53	2.53
L-alanine (990 g kg^{-1})	20.00	17.50	15.00	12.50	10.00
Vitamin and mineral mix[1]	5.00	5.00	5.00	5.00	5.00
L-glutamine and L-glutamic acid[2]	0.00	5.00	10.00	15.00	20.00
Antifungic[3]	1.00	1.00	1.00	1.00	1.00
Analyzed composition (g kg^{-1}, dry-matter basis)					
Crude protein	344.55	344.62	344.70	344.77	344.85
Digestible energy (kcal kg^{-1})[4]	3,927	3,924	3,923	3,923	3,929
Crude fiber	38.30	38.25	38.19	38.14	38.09
Crude lipids	58.18	58.09	58.00	57.90	57.81
Calcium	10.39	10.38	10.35	10.34	10.32
Total phosphorus	8.83	8.92	8.82	8.73	8.89

[1] Composition (IU or mg kg^{-1} of diet): Vitamin A (retinal palmitate), 6,000 IU; vitamin D_3, (cholecalciferol), 1,000 IU; vitamin E (DL -α-tocopherol), 60 mg; vitamin K_3 (menadione), 12 mg; vitamin B_1 (thiamine HCl), 24 mg; vitamin B_2 (riboflavnin), 24 mg; vitamin B_6 (pyridoxine HCl), 20 mg; vitamin B_{12} (cyanocobalamin), 24 mg; folic acid, 6 mg; D-calcium pantothenate, 60 mg; vitamin C (ascorbic acid), 240 mg; D-biotin, 0.24 mg; choline chloride, 325 mg; niacin, 120 mg; ferrous sulfate ($FeSO_4.H_2O0.7H_2O$), 50 mg; copper sulphate ($CuSO_4.7H_2O$), 3 mg; manganous sulfate ($MnSO_4.H_2O$), 20 mg; zinc sulfate ($ZnSO_4.7H_2O$), 30 mg; cobalt sulfate ($CoSO_4.4H_2O$), 0.01 mg; sodium selenite (Na_2SeO_3), 0.1 mg; butylated hydroxytoluene (BHT), 0.075 mg; butylated hydroxyanisole (BHA), 0.075 mg.
[2] Associated L-glutamine-L-glutamate (1:1) (Ajinomoto do Brasil Indústria e Comércio de Alimentos Ltda, São Paulo, SP, Brazil).
[3] Mold Zap® - Composition: ammonium dipropionate, acetic acid, sorbic acid, and benzoic acid (Alltech, Maringá, PR, Brazil).
[4] Considering values of digestible energy of feed ingredients previously established for Nile tilapia (Pezzato et al., 2002; Guimarães et al., 2008a; Guimarães et al., 2008b; Gonçalves et al., 2009). Equivalent protein values of crystalline amino acids were considered, and gross energy was considered as totally converted to digestible energy (Rostagno et al., 2017).

Feed efficiency ratio = weight gain (g)/feed intake (g);

Protein retention efficiency (%) = [(final weight × final nutrient content) − (initial weight × initial nutrient content)] × 100/nutrient intake;

Survival rate (%) = 100 × (final number of fish/initial number of fish).

2.3. Laboratorial analysis

Analysis of dry matter (method 927.05), crude protein (method 984.13), crude lipid (method 920.39), crude fiber (method 985.29), and mineral matter (method 942.05) of experimental diets and whole-body of fish were performed (AOAC, 2007). Moisture was determined by oven-drying at 105 °C until constant weight. Crude protein (N×6.25) was performed by using Kjeldahl method after acid digestion. Mineral matter content was determined by muffle furnace at 550 °C for 24 h. Crude lipid was determined by the ether-extraction method using a Soxtec System. Calcium and phosphorus were determined by inductively coupled plasma optical emissions spectrometry using an internally validated method (PerkinElmer 8000, Waltham, MA, USA) analysis (method 985.01(A, B, D)) (AOAC, 1990). All analyses were performed in duplicate.

2.4. Intestinal morphometry analysis

After the end of the trial, the middle part of the intestines of three fish from each cage (12 fish per treatment) were collected and fixed in 10% buffered formalin for 24 h. Fragments of the medium part of the intestine were fixed in *Bouin* solution for 8 h, embedded in blocks of paraffin, semi-serial 5-µm cross-sectioned, and stained with hematoxylin-eosin (HE). For the villus height measurement, a total of 100 apparently intact villi were measured per fish.

The villus height was measured from the villus top to the bottom. An average of 100 villi per fish were measured and expressed as the mean villus height for each fish, and an average of 300 villi from each cage was considered as the mean villi height for each experimental unit, totaling 1,200 villi measured per treatment. The sections were examined under an optical microscope, attached to an Olympus camera (Brazeiss Representações Ltda, São Paulo, SP, Brazil) for image capture. The analyses of height of the villi were performed from the apex of the villi to the beginning of the muscularis mucosae layer and determined using the software Image-Pro Plus.

2.5. Statistical analysis and design

All treatments were replicated four times, and the experimental unit was a floating net cage with 100 fish. Survival values were transformed using the arcsine. The experiment was conducted as a completely randomized design according to the following general model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

in which Y_{ij} = dependent variable, μ = general mean, T_i = effect of the i-th level of associated glutamine and glutamic acid (i = 0, 5, 10, 15, or 20 g kg^{-1}), and e_{ij} = residual error. All the data were subjected to ANOVA; an α- value of 0.05 was used to assess significance, and orthogonal polynomial contrast were performed to find a linear or quadratic response. All the data were analyzed using SAS software (Statistical Analysis System, version 9.3).

3. Results

The results of the dietary proximate analysis of the experimental diets match the values expected when the experiment was designed. At the end of the trial, no visually external morphological pathology was observed.

No difference (P>0.05) on feed intake and survival of fish fed the experimental diets were observed. The increase in inclusion levels of associated glutamine and glutamic acid resulted in quadratic effect on final whole-body weight ($y = 42.170 + 2.799x - 0.130x^2$, $r^2 = 0.765$), daily weight gain ($y = 0.886 + 0.060x - 0.003x^2$, $r^2 = 0.753$), feed intake ($y = 47.906 + 0.797x - 0.032x^2$, $r^2 = 0.340$), and feed efficiency ratio ($y = 1.218 - 0.049x + 0.002x^2$, $r^2 = 0.753$), with optimized values for supplementation of 10.77, 13.28, 10.00, and 11.21 g kg^{-1}, respectively (Table 2).

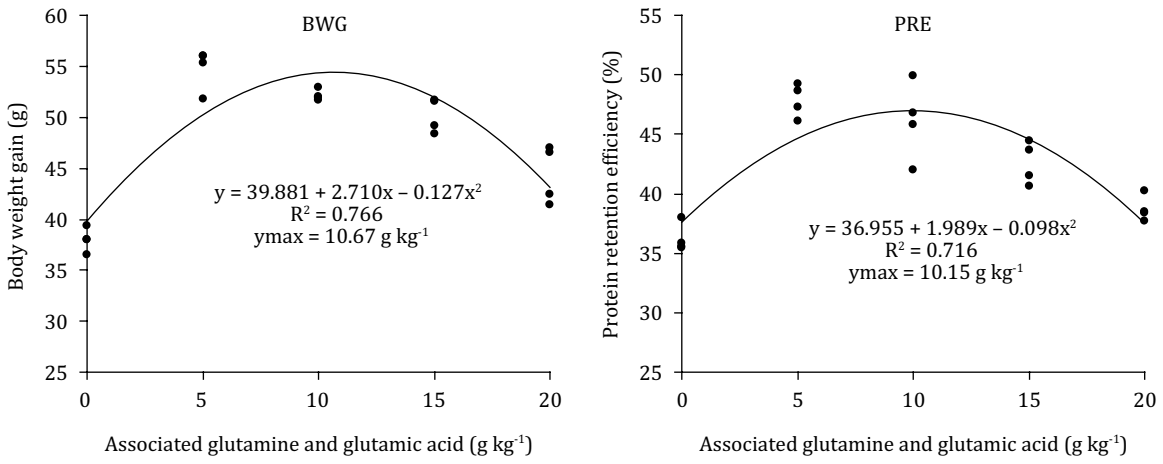
A quadratic effect on whole-body weight gain and protein retention efficiency in fish fed graded levels of associated glutamine and glutamic acid was observed (Figure 1). Associated dietary glutamine and glutamic acid affected villus height, and the maximum effect was found at 10.98 g kg^{-1} of glutamine and glutamic acid (Figure 2). There was no difference (P<0.05) in whole-body composition of fish fed graded levels of associated glutamine and glutamic acid (Table 3).

The intestinal wall showed a typical structural arrangement. The tunica mucosa, submucosa, muscularis, and serosa were all in good condition, even in the fish fed diet without glutamine and glutamic acid supplementation (Figure 3). The intestinal mucosa showed foliaceus villi of irregular heights, covered by a simple cylindrical epithelium with goblet cells. Intestinal crypts were not

Table 2 - Growth performance of Nile tilapia fingerlings fed the experimental diets[1]

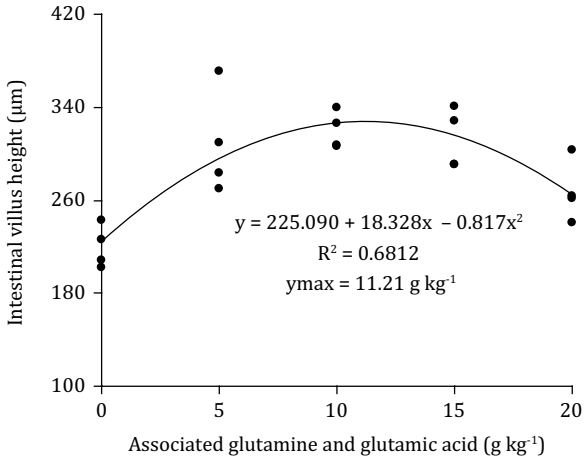
Treatment[2]	IBW (g)	FBW (g)	DWG (g)	FI (g)	FER	PRE (%)	SUR (%)
0	2.26±0.22	40.24±0.36	0.84±0.06	47.54±1.92	0.80±0.02	36.43±0.86	99.67±0.22
5	2.67±0.14	57.44±0.96	1.22±0.08	52.02±5.46	1.05±0.04	47.81±2.82	99.75±0.22
10	2.55±0.06	54.69±0.22	1.16±0.02	52.03±5.44	1.00±0.04	46.81±6.52	99.33±0.44
15	2.73±0.04	52.9±0.72	1.11±0.08	52.47±7.60	0.96±0.04	42.56±3.64	100±0.00
20	2.47±0.16	46.82±1.42	0.99±0.12	51.07±4.98	0.87±0.02	38.70±2.16	99.5±0.44
SEM	0.068	1.388	0.030	0.642	0.021	1.056	0.138
P-value							
Linear	0.369	0.419	0.434	0.119	0.585	0.793	0.938
Quadratic	0.083	<0.001	<0.001	0.033	<0.001	<0.001	0.949

IBW - initial body weight; FBW - final body weight; DWG - daily weigh gain; FI - feed intake; FER - feed efficiency ratio; PRE - protein retention efficiency; SUR - survival; SEM - pooled standard error of the mean.
[1] Values are mean ± standard deviation of four replicates of floating net cages of 100 masculinized Nile tilapia, and the glutamine and glutamate were provided as L-glutamine and L-glutamic acid.
[2] Associated glutamine and glutamic acid (g kg^{-1}).



Each point is the means of each floating net cage of 100 fish.

Figure 1 - Body weight gain (BWG) and protein retention efficiency (PRE) of Nile tilapia fingerlings fed diets containing graded levels of associated glutamine and glutamic acid.



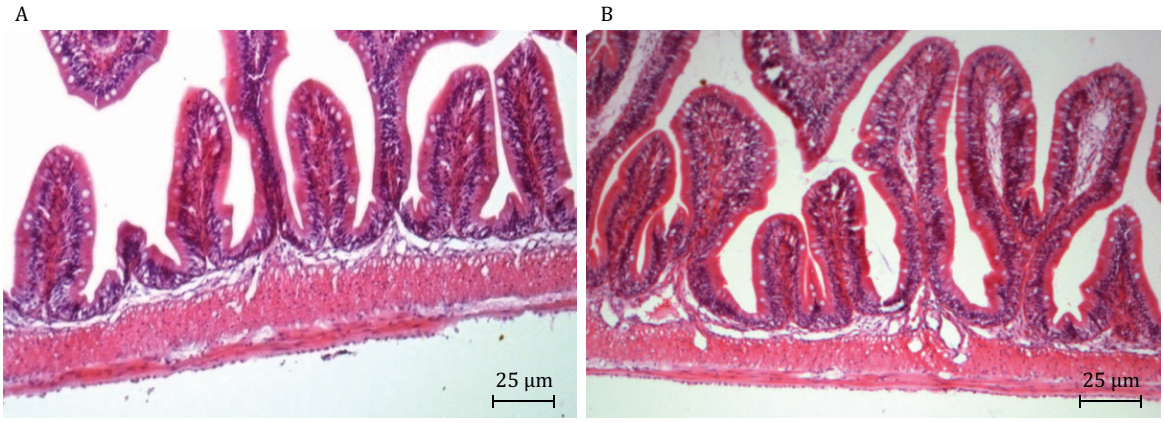
Each point represents the mean of each floating net cage of 100 fish.

Figure 2 - Intestinal villus height of Nile tilapia fingerlings fed diets containing graded levels of associated glutamine and glutamic acid.

Table 3 - Whole-body composition of Nile tilapia fingerlings fed the experimental diets[1]

Treatment[2]	Whole-body composition (g kg^{-1})			
	Moisture	Crude protein	Crude lipids	Ash
0	722.04±0.18	150.82±0.22	91.72±0.06	36.56±0.06
5	730.01±0.26	154.47±0.08	83.92±0.04	36.66±0.18
10	724.64±0.12	156.24±0.26	82.43±0.14	36.72±0.02
15	727.83±0.28	151.19±0.16	85.28±0.20	37.11±0.04
20	722.22±0.18	151.63±0.02	88.77±0.04	36.17±0.08
SEM	0.119	0.09	0.095	0.041
P-value				
Linear	0.844	0.798	0.505	0.947
Quadratic	0.068	0.091	0.103	0.637

SEM - standard error of the mean.
[1] Values are mean ± standard deviation of four replicates of floating net cages of 100 masculinized Nile tilapia, and glutamine and glutamic acid were provided as L-glutamine and L-glutamic acid.
[2] Associated glutamine and glutamic acid (g kg^{-1}).
Initial body composition (g kg^{-1}): moisture, 787.00; crude protein, 120.10; crude lipids, 52.40; ash, 35.80.



HE coloring.
200X.

Figure 3 - Representative photomicrograph of the midgut of Nile tilapia fingerlings fed diet without supplementation of associated glutamine and glutamic acid (A) and fish fed associated glutamine and glutamic acid at 15 g kg^{-1} (B).

observed in this work. Fish fed diet without glutamine and glutamic acid supplementation had lower villus heights than those fed a diet containing 10 g kg^{-1} of associated glutamine and glutamic acid.

4. Discussion

Glutamine and glutamic acid are considered conditionally essential amino acids (Li et al., 2009) for farm animals fed low protein, plant-based diets (Wu, 2014) or subjected to environmental challenges (Liu et al., 2015). In the present work, the increased weight gain of fish fed glutamine and glutamic acid diets can be attributed to the more efficient protein utilization compared with that of fish fed the control diet. This result is in agreement with studies on red drum (*Sciaenops ocellatus*; Cheng et al., 2011), hybrid striped bass (*Morone chrysops* × *Morone saxatilis*; Cheng et al., 2012), Atlantic cod (*Gadus morhua*; Ingebrigtsen et al., 2014), hybrid sturgeon (*Acipenser schrenckii* ♀ × *Huso dauricus* ♂; Qiyou et al., 2011), and Nile tilapia (Pereira et al., 2017) fed diets supplemented with glutamine. Glutamate has been considered to improve growth of Atlantic salmon (*Salmo salar*; Oehme et al., 2010). The positive effects on protein were previously attributed to the specific functional properties of glutamine and glutamate and the induced metabolic changes resulting from regulation of glycolysis and lipogenesis through the mediation of different cell signaling pathways, as demonstrated in gilthead seabream (*Sparus aurata*; Caballero-Solares et al., 2015).

Nitrogen retention appears to be the best parameter for the evaluation of dietary amino acid response in fish diets, considering that weight gain is not only a result of protein accretion, but also involves the deposition of other whole-body components. However, in this study, dietary glutamine and glutamic acid did not affect the whole-body composition of the fish and, as previously described in Jian carp (*Cyprinus carpio* var. Jian; Yan and Qiu-Zhou, 2006), weight gain was used as the main variable to response criteria for optimizing amino acid supplementation in the tilapia diets.

In the present work, fish fed 10.67 g kg^{-1} of associated glutamine and glutamic acid showed improved growth compared with fish fed a diet with no supplementation. However, fish fed 20 g kg^{-1} glutamine and glutamic acid diet showed reduced weight gain, a poor feed efficiency ratio, and lower protein retention efficiency when compared with fish fed 10.67 g kg^{-1} of associated glutamine and glutamic acid. This result may be because excessive glutamine and glutamic acid cause disproportionate amino acid utilization and negative effects on growth performance. Lower weight gain and poor feed conversion may occur in fish fed excessive quantities of amino acids (Li et al., 2009). In this study, the basal diet before glutamine and glutamic acid supplementation already contained all crystalline essential amino acids required to meet the essential dietary amino acid requirement described for tilapias in NRC (2011). This made it possible to evaluate the effects of glutamine and glutamic acid supplementation in diets already containing well-balanced levels of essential amino acids.

Positive effects of glutamine or glutamate on protein utilization have also been attributed to modulation of gene expression of key enzymes of hepatic metabolism in gilthead seabream (Caballero-Solares et al., 2015), positive changes in intestinal functionality (Yan and Qiu-Zhou, 2006; Cheng et al., 2012), and immune response of fish (Pohlenz et al., 2012; Li et al., 2017; Pereira et al., 2017). To date, glutamine acts as a regulator of metabolic demands by increasing protein synthesis, reducing protein degradation in skeletal muscle, and consequently promoting positive effects on weight gain and feed efficiency ratio of fish (Caballero-Solares et al., 2015).

In this study, no effect of dietary associated glutamine and glutamic acid on the whole-body composition of fish was observed, and this concurs previous results described in tongue sole (*Cynoglossus semilaevis*; Liu et al., 2015) and Nile tilapia (Pereira et al., 2017). However, a significant difference in lipid content was noted in the analysis of whole-body composition of channel catfish (*Ictalurus punctatus*; Pohlenz et al., 2012). Although glutamine regulates protein formation for muscle synthesis (Wu, 2010), the absence of differences in whole-body composition of fish fed glutamine and glutamic acid may be attributed to differences in fish species and size, diet composition, and feed allowance. In general, the effect of supplementing essential amino acids on whole-body composition is more evident in adult than in juvenile fish due to higher whole-body lipid content (Michelato et al.,

2016; Silva et al., 2015). Glutamine has predominantly cellular effects, mainly acting as an energy substrate for rapid cell proliferation and synthesis of other amino acids. From a fish production perspective, the maintenance, development, and health of the gastrointestinal tract are important, since it possesses the functions of food storage, digestion, and absorption of nutrients. In the present study, intestinal crypts were not observed. Similar results were described in catfish (Pohlenz et al., 2012) and Nile tilapia (Gargiulo et al., 1998).

In the present work, the trophic action of the dietary glutamine and glutamic acid on villus heights was observed in fish fed 10.98 g kg^{-1} of associated glutamine and glutamic acid. Glutamine is a trophic agent that enhances cell renewal and stimulates the development of intestinal villi, and positive effects on weight gain may be associated to the improvement in the intestinal structure, as observed in Jian carp (Yan and Qiu-Zhou, 2006), hybrid striped bass (Cheng et al., 2012), and catfish (Pohlenz et al., 2012).

The increase in the length of the villi implies an increase in surface area for the greater absorption of nutrients. This trophic effect of the dietary glutamine and glutamic acid in the intestinal mucosa epithelium may be explained by their action in cell renewal by increasing mitosis in the villus base, which results in the increased proliferation of intestinal mucosa epithelial cells (Wu, 2010). The digestive tract mucosa cells, as well as other rapidly proliferating cells, have high requirement of glutamine because of its role as a supplier of half the nitrogen requirements for purine and pyrimidine synthesis through carbamoyl phosphate synthetase action, thus allowing constant cell renewal (Lobley et al., 2001).

Glutamine and glutamate act as conditionally essential amino acids in fish subjected to nutritional, environmental, or disease challenge (Liu et al., 2015). Fish farmed in net floating cages are constantly subjected to acute nictemeral variations in temperature and dissolved oxygen, especially when stocked at high densities. Thus, "functional amino acids" such as glutamine and glutamic acid have become alternatives to improve growth performance and modulate intestinal development. In the present study, dietary associated glutamine and glutamic acid demonstrated positive effects on growth performance and intestinal development of cage-farmed Nile tilapia fingerlings raised under high-temperature condition.

5. Conclusions

Supplementation of associated glutamine and glutamic acid at 10.67 g kg^{-1} optimizes growth performance. Dietary glutamine and glutamic acid at 10.98 g kg^{-1} exerts trophic action and improves intestinal morphometry in cage-farmed Nile tilapia fingerlings.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Investigation: E.S. Macêdo and T.S.G. Franco. Methodology: M.R.M. Natali. Project administration: P.A.P. Panaczevicz. Supervision: A.R. Rudnik and J.A.G. Miranda. Validation: W.M. Furuya. Writing-original draft: E.S. Macêdo and T.S.G. Franco. Writing-review & editing: W.M. Furuya.

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