Dietary histidine requirement of grow-out Nile tilapia (*Oreochromis niloticus*), based on growth performance, muscle development, expression of muscle-growth-related genes, and blood parameters

Micheli Zaminhan-Hassemer\(^1\), Mariana Michelato\(^2\), Wilson Rogério Boscolo\(^3\), Allan Vinnícius Urbich\(^4\), Thais Pereira da Cruz\(^5\), Fernanda Losi Alves de Almeida\(^6\), Valéria Rossetto Barriviera Furuya\(^2\), Wilson Massamitu Furuya\(^1,2^*\)

\(^1\) Universidade Estadual de Maringá, Programa de Pós-Graduação em Zootecnia, Maringá, PR, Brasil.
\(^2\) Universidade Estadual de Ponta Grossa, Programa de Pós-Graduação em Zootecnia, Ponta Grossa, PR, Brasil.
\(^3\) Universidade Estadual do Oeste do Paraná, Toledo, PR, Brasil.
\(^4\) Universidade Estadual de Maringá, Departamento de Ciências Morfológicas, Maringá, PR, Brasil.

**ABSTRACT** - This research was conducted to determine optimal dietary histidine requirement of grow-out Nile tilapia, *Oreochromis niloticus*, based on muscle development, expression of muscle-growth-related genes, and blood parameters. Fish (n = 288, initial body weight of 64.17±0.53 g) were fed extruded diets with graded levels of histidine (4.23, 5.44, 7.17, 8.91, and 11.57 g kg\(^{-1}\)), containing approximately 289 g kg\(^{-1}\) crude protein and 3565 kcal kg\(^{-1}\) digestible energy. The study followed a completely randomized design with five treatments and four replicates each, for 65 days. There was a quadratic effect of dietary histidine on final body weight, feed conversion, and net protein utilization, and the best values were optimized at 8.09, 7.88, and 7.33 g kg\(^{-1}\), respectively. Feed intake, hepatosomatic index, survival, body composition, and blood parameters of total protein, glucose, triglycerides, cholesterol, hematocrit, and hemoglobin were not affected by dietary treatments. Predominance of hypertrophic growth and higher mRNA levels of myogenin and MyoD were observed in fish fed histidine from 5.44 to 11.57 g kg\(^{-1}\) compared with fish fed histidine at 4.23 g kg\(^{-1}\). The mRNA expression of myostatin was not affected by dietary treatments. The dietary requirement of histidine for grow-out Nile tilapia was determined at 8.09 g kg\(^{-1}\), considering growth performance, muscle development, and gene expression responses.

**Keywords**: amino acids, aquaculture, fish nutrition, genomics

**Introduction**

Many advances in amino acid nutrition have been reached by understanding their availability in feed ingredients and determining their dietary requirements for Nile tilapia, *Oreochromis niloticus*. These approaches have been valuable in developing well-balanced diets that improve not only growth and feed efficiency, but also fish health and fillet yield.
Fish recruits new muscle fibers throughout their lifetime (Rowlerson and Veggetti, 2001), and hypertrophic and hyperplasic muscle growth may be influenced by nutrition (Dal Pai et al., 2000). Genomics assays have been used to better understand how nutrients can affect muscle-growth-related genes and metabolic responses in fish (Alami-Durante et al., 2010; De Paula et al., 2017). Muscle growth processes are regulated by the activation and proliferation of satellite cells and myogenic regulatory factors (MRF), such as MyoD and myogenin, and MyoD regulates myogenic activity in cell differentiation (Watabe, 1999). While MRF are positively associated with fish growth, myostatin negatively regulates myogenesis, inhibiting myoblast cell proliferation and differentiation, and are used as an indicator of negative energy and nutrient balance in fish (Rescan et al., 2001).

Dietary protein utilization efficiency depends on the content and balance of amino acids in the diet, and a well-balanced diet is important for optimum growth performance. Well-balanced diets also reduce nitrogen excretion and environmental impacts of fish production. Knowledge on the optimum amino acid contents is required for formulating cost-effective diets that improve growth performance and health, and that are environmentally friendly.

Histidine, which is found in high concentration in hemoglobin (Khan and Abidi, 2014), acts as an antioxidant in the lens, and thereby prevents cataracts in some salmonid species (Waagboø et al., 2010). It plays an important role in protein synthesis (Li et al., 2009), stimulating skeletal muscle growth and muscle-growth-related genes in Nile tilapia juveniles (Michelato et al., 2017).

The dietary histidine requirements has been determined for Nile tilapia fingerlings (Santiago and Lovell, 1988; Diógenes et al., 2016; Michelato et al., 2017). However, the dietary histidine requirement for grow-out Nile tilapia remains poorly documented. Thus, reliable data about histidine requirements are required to elaborate well-balanced and low-cost diet for this fish species. Therefore, the objective of this research was to determine the dietary histidine requirement of grow-out Nile tilapia, based on growth performance, muscle development, expression of muscle growth-related genes, and blood parameters as criteria responses.

Material and Methods

This research was conducted according to the local ethical and animal welfare guidelines in Toledo, Paraná, Brazil (24°43′12″ S, 53°44′6″ W), approved under case no. 05/14.

Five diets containing graded levels of L-histidine were formulated. Increased L-histidine supplementation levels was compensated by decreasing equal amounts of L-alanine (Table 1). Diets were elaborated to contain approximately 289 g kg\(^{-1}\) crude protein and 3565 kcal kg\(^{-1}\) digestible energy, while meeting the dietary amino acid requirements of Nile tilapia (Furuya, 2010; NRC, 2011), except for histidine. The histidine levels based on laboratorial analysis were 4.23, 5.44, 7.17, 8.91, and 11.57 g kg\(^{-1}\). The digestible energy contents of the experimental diets were estimated from data previously established for Nile tilapia (Gonçalves et al., 2009; Guimarães et al., 2012) (Table 2). The proximate composition and amino acids content of the experimental diets were confirmed by laboratorial analysis (Table 2).

Diets were ground into 800-µm mesh, prior to mixing all ingredients in an “V” mixer (MA200; Marconi, Piracicaba, SP, Brazil), extruded in a single screen experimental feed mill (Extec, Ribeirão Preto, SP, Brazil) to produce 3-mm diameter pellets, and dried for 24 h in ventilated oven at 55 °C.

Two hundred and eighty-eight masculinized Nile tilapia (initial body weight 64.17 g) were randomly distributed into 24 250-L tanks at 12 fish per tank. Each tank was equipped with a continuous water flow-through system (1.5 L min\(^{-1}\)). Five treatment diets were randomly assigned to 24 tanks at four replicates per diet. A constant and natural photoperiod of 12 h light:12 h dark was kept during the experimental trial. Fish were hand-fed four days per week at 8.00, 11.00, 14.00, and 17.00 h until apparent satiety, for 65 days. Water quality parameters were monitored daily during the feeding trial and the parameter values (mean±standard error of the mean) were as follow: water temperature, 28.00±0.20 °C; pH, 7.00±0.10; and dissolved oxygen, 6.00±0.20 mg L\(^{-1}\).
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Table 1 - Ingredient composition of the experimental diets (g kg⁻¹, as fed-basis)

| Ingredient              | Dietary histidine (g kg⁻¹) |
|-------------------------|---------------------------|
|                         | 4.23                      | 5.44                      | 7.17          | 8.91          | 11.57         |
| Rice flour              | 658.7                     | 658.7                     | 658.7         | 658.7         | 658.7         |
| Poultry byproduct meal  | 145.7                     | 145.7                     | 145.7         | 145.7         | 145.7         |
| Feather meal            | 100.0                     | 100.0                     | 100.0         | 100.0         | 100.0         |
| Meat and bone meal      | 50.0                      | 50.0                      | 50.0          | 50.0          | 50.0          |
| Soybean oil             | 5.8                       | 5.8                       | 5.8           | 5.8           | 5.8           |
| L-alanine               | 13.0                      | 11.0                      | 9.0           | 7.0           | 5.0           |
| L-histidine             | -                         | 2.0                       | 4.0           | 6.0           | 8.0           |
| L-lysine                | 6.4                       | 6.4                       | 6.4           | 6.4           | 6.4           |
| DL-methionine           | 4.5                       | 4.5                       | 4.5           | 4.5           | 4.5           |
| L-threonine             | 2.0                       | 2.0                       | 2.0           | 2.0           | 2.0           |
| L-tryptophan            | 0.9                       | 0.9                       | 0.9           | 0.9           | 0.9           |
| Min. and vit. mix¹      | 10.0                      | 10.0                      | 10.0          | 10.0          | 10.0          |
| Salt                    | 3.0                       | 3.0                       | 3.0           | 3.0           | 3.0           |

¹ Mineral and vitamin mix - composition per kilogram of feed (IU or mg kg⁻¹ of diet): vitamin A (retinal palmitate), 24,000 IU; vitamin D₃ (cholecalciferol), 6,000 IU; vitamin E (DL-α-tocopherol), 30 mg; vitamin K₃ (menadione), 30 mg; vitamin B₁ (thiamine HCl), 40 mg; vitamin B₂ (riboflavin), 40 mg; vitamin B₆ (pyridoxine HCl), 35 mg; vitamin B₁₂ (cyanocobalamin), 80 mg; folic acid, 12 mg; D-calcium pantothenate, 100 mg; vitamin C (ascorbic acid), 600 mg; D-biotin, 2 mg; choline chloride, 1000 mg; niacin, 120 mg; ferrous sulfate (FeSO₄.7H₂O), 200 mg; copper sulphate (CuSO₄.7H₂O), 35 mg; manganous sulfate (MnSO₄.H₂O), 100 mg; zinc sulfate (ZnSO₄.7H₂O), 240 mg; cobalt sulfate (CoSO₄.4H₂O), 0.8 mg; sodium selenite (Na₂SeO₃), 0.1 mg; butylated hydroxytoluene (BHT), 0.075 mg; butylated hydroxyanisole (BHA), 0.075 mg.

Table 2 - Analyzed composition of the experimental diets (g kg⁻¹, as dry matter basis)

| Item                      | Dietary histidine (g kg⁻¹) |
|---------------------------|---------------------------|
|                          | 4.23                      | 5.44                      | 7.17          | 8.91          | 11.57         |
| Dry matter                | 924.42                    | 923.93                    | 923.71        | 924.97        | 923.05        |
| Digestible energy (kcal kg⁻¹)¹ | 3606.04                  | 3597.23                   | 3588.08       | 3580.04       | 3572.13       |
| Crude protein             | 288.82                    | 288.74                    | 288.64        | 288.86        | 288.64        |
| Crude lipids              | 44.34                     | 44.93                     | 44.33         | 44.71         | 44.33         |
| Crude fiber               | 2.21                      | 2.13                      | 2.14          | 2.27          | 2.14          |
| Calcium                   | 12.93                     | 12.07                     | 13.12         | 13.21         | 13.11         |
| Phosphorus                | 9.41                      | 9.32                      | 9.21          | 9.46          | 9.41          |
| Arginine                  | 17.47                     | 18.42                     | 17.47         | 18.49         | 18.26         |
| Histidine                 | 4.23                      | 5.44                      | 7.17          | 8.91          | 11.57         |
| Isoleucine                | 11.03                     | 10.84                     | 10.93         | 10.71         | 10.68         |
| Leucine                   | 20.93                     | 20.71                     | 20.84         | 20.94         | 20.17         |
| Lysine                    | 17.77                     | 17.53                     | 17.66         | 18.19         | 18.01         |
| Methionine                | 9.38                      | 9.64                      | 9.91          | 9.28          | 9.16          |
| Phenylalanine             | 12.83                     | 13.27                     | 13.21         | 13.46         | 13.26         |
| Threonine                 | 12.06                     | 12.14                     | 12.72         | 12.73         | 12.71         |
| Tryptophan                | 3.21                      | 3.23                      | 3.22          | 3.34          | 3.18          |
| Valine                    | 14.44                     | 15.25                     | 15.28         | 14.92         | 14.90         |
| Alanine                   | 31.71                     | 29.13                     | 25.65         | 22.54         | 20.02         |
| Aspartic acid             | 21.64                     | 21.36                     | 21.3          | 21.75         | 21.06         |
| Cystine                   | 6.07                      | 5.90                      | 6.13          | 5.94          | 6.17          |
| Glycine                   | 24.23                     | 24.46                     | 24.52         | 25.08         | 24.10         |
| Glutamic acid             | 37.26                     | 36.46                     | 36.66         | 38.13         | 36.38         |
| Serine                    | 16.85                     | 16.9                      | 16.75         | 16.96         | 16.21         |
| Tyrosine                  | 8.88                      | 8.93                      | 9.35          | 9.39          | 9.11          |

¹ Rice flour (Gonçalves et al., 2009); meat and bone meal, poultry byproduct meal, and feather meal (Guimarães et al., 2012); and gross energy of crystalline amino acids (Rostagno et al., 2017) were considered to be totally converted to digestible energy.
At the beginning of the experiment, sixteen fish were fasted for 24 h and randomly sampled for whole-body composition. Fish were euthanized with an overdose of 100 mg L$^{-1}$ of benzocaine of prior to sampling. At the end of the feeding trial, fish were fasted for 24 h, counted, and weighed. Six fish from each tank were collected for whole-body nutrient composition and retention assays.

Body weight gain, feed intake, feed conversion ratio, net protein utilization, hepatosomatic index, and survival rate were assessed. For body weight gain calculation, fish were weighed at the beginning and end of the experimental trial. Feed intake was recorded daily, on a dry matter basis. Feed conversion ratio was determined by dividing feed intake by body weight gain. Net protein utilization was assessed by dividing the mean body protein retained by protein intake. Hepatosomatic index was calculated by dividing the mean liver weight by body weight. Survival rate was assessed considering the number of fish that died during the experimental trial.

Three fish per aquarium were collected to determine hepatosomatic index parameter. Proximate composition of diets and whole-body fish were determined according to standard method (AOAC, 1990). Moisture was analyzed by oven-drying at 105 °C until constant weight; crude lipid analyses were performed by the ether-extraction method using a Soxhlet extractor (Tecnal, TE-044, Piracicaba, SP, Brazil). Crude protein (N x 6.25) followed the Kjeldahl method (Tecnal, MA-036, Piracicaba, SP, Brazil) after acid hydrolysis. Ash analyses was obtained by combustion in a muffle furnace at 550 °C overnight (Tecnal, 2000B, Belo Horizonte, MG, Brazil). Amino acid profiles of the diets were determined by High-Performance Liquid Chromatograph (HPLC) (Hitachi, Tokyo, Japan) after acid hydrolysis (Rayner, 1985). Tryptophan was analyzed after alkaline hydrolysis of the sample with lithium hydroxide.

At the end of the feeding trial, three fish from each aquarium were randomly collected to process the morphometric analysis of white skeletal muscle (n = 12 fish per treatment). Samples of muscle tissue were collected from the epaxial region, frozen in liquid nitrogen, and stored in freezer at −80 °C. Histological sections of white skeletal muscle (5-7 µm) were performed using −20 °C cryostat (CM1950, Leica Microsystems, Berlin, Germany) and stained with hematoxylin and eosin (HE) following standard methodology (Bancroft and Steven, 1990).

The smallest diameter (n = 200 muscle fibers) from each fish was measured (Dubowitz and Brooke, 1973) and distributed into three diameter classes (<20, 20-50, or >50 µm) according to previously established methodology (Almeida et al., 2008). All images were captured by high-resolution camera (Q Color 3, Olympus, Melville, NY, USA) coupled to a light microscope (Olympus BX40, Melville, NY, USA). Morphometric measurements were realized using Image Pro Plus® 4.5 image analysis software (Media Cybernetics, Silver Spring, MD, USA). Mean values of muscle fiber diameter were determined, and the frequency of occurrence was expressed as number of fiber per diameter class relative to the total number of measured fibers. After anesthesia with benzocaine (100 mg L$^{-1}$), blood samples were collected from the caudal vein of fish (n = 3 fish per aquarium), using a sterile syringe (1.0 mL) rinsed with an anticoagulant solution (3% ethylene dimethyl tetra amine – EDTA). All fish were fasted for 24 h prior to sampling.

Hemoglobin was determined by the cyanmethemoglobin colorimetric method (Collier, 1944), by using a commercial kit (Gold Analisa Diagnóstica®, Belo Horizonte, MG, Brazil). The hematocrit percentage was determined by the microhematocrit method (Goldenfarb et al., 1971). The total plasma proteins were measured by using the standard method (Jain, 1986). Blood biochemical analysis were performed using a partial blood aliquot (1 mL) placed in non-heparinized tubes and centrifuged at 3000 g for 5 min (Quimis, Diadema, SP, Brazil). After centrifugation, the plasma was used to determine glucose, triglycerides, and total cholesterol using a commercial kit (Gold Analisa Diagnóstica®, Belo Horizonte, MG, Brazil).

At the end of feeding, total RNA was extracted from white skeletal muscle tissue from three fish per aquarium) using TRIzol® (Invitrogen, Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s protocol. Samples of mRNA from the same fish used for white skeletal muscle morphometric analysis were collected. The cDNA synthesis was performed from total RNA (2 µg) using the First Strand Synthesis Kit (GE Healthcare Bio-Sciences, Piscataway, NJ, USA), as recommended by...
the manufacturer's protocol. Primer pairs for MyoD, myogenin, myostatin, and 18S ribosomal RNA were designed with reference to cDNA nucleotide sequence from *Oreochromis niloticus*, available in GenBank (http://www.ncbi.nlm.nih.gov/pubmed/nucleotide) (Table 3).

The quantitative real-time-polymerase chain reaction (qRT-PCR) was carried out in a thermocycler (Stratagene MxPro3005P, La Jolla, CA, USA), using SYBR Green PCR Master Mix Kit (Stratagene, La Jolla, CA, USA). A total of 20 ng of cDNA template and 10 µM of each primer in a 20 µL of total volume qRT-PCR program consisted of denaturation step at 95°C for 10 min, followed by 40 cycles of 15 s denaturation at 95°C, 30 s annealing at 60°C, 30 s extension at 72°C, ended with a dissociation curve. Analysis of qRT-PCR was performed in duplicate for each sample and the threshold cycle (Ct) values obtained by amplification were measured and a relative change in the expression level of one specific gene was presented as $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). The gene expression was normalized relative to a housekeeping gene 18S ribosomal RNA – 18SrRNA.

Data were tested for normality analysis with the Shapiro-Wilk test (P>0.05) and transformed when necessary. The study was conducted as a completely randomized design according to the following general model:

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \epsilon_{ij},$$

in which $Y_i$ represents the observation, $\beta_1$ represents the equation constant, $\beta_2 X_i$ represents the linear regression coefficient, $\beta_2 X_i^2$ represents the quadratic regression coefficient, and $\epsilon_{ij}$ represents the residual error.

Morphometric data of muscle fibers and gene expression showed non-normal distribution and were analyzed by a nonparametric Kruskal-Wallis test (P<0.05) complemented with Dunn’s multiple comparison test (P<0.05). All data were analyzed using SAS software (Statistical Analysis System, version 9.3).

### Table 3 - Primers used for qRT-PCR analysis in this study

| Gene                     | GenBank          | Primer sequence (5’-3’)                                      |
|--------------------------|------------------|---------------------------------------------------------------|
| Myogenic Factor (MyoD)   | GU246722         | Forward: CCACCTGGTCAGACACACCAGA                                |
|                          |                  | Reverse: ACTGCGTTCGCTCTTCCAGAC                               |
| Myogenin                 | GU246725         | Forward: CTCAACCAGCAGGACACTGAG                                |
|                          |                  | Reverse: ATCTCGCTGCTGCTAGCTCT                                 |
| Myostatin 1              | FJ972683         | Forward: TCCACATGACCTCGAGAC                                  |
|                          |                  | Reverse: TGCAACACAAGACTCTCTCAGC                               |
| 18S ribosomal DNA (18SrDNA) | JF698683     | Forward: GACGGGAGAAGGATTCGAG                                 |
|                          |                  | Reverse: GTTCGTTATGAGAATTAACCAGAC                            |

### Results

The feeding trial produced no unexpected results. The lower mortality was unrelated to experimental treatments. No external pathological signs were observed in fish from any groups. The increase in inclusion levels of histidine resulted in quadratic effect on body weight gain ($y = 0.555 + 43.451X - 2.684X^2$, $R^2 = 0.798$) (Table 4), and the maximum body weight gains were estimated in fish fed histidine at 8.09 g kg$^{-1}$ (Figure 1). Similarly, a quadratic effect of histidine on feed conversion ratio ($y = 1.868 - 0.205X + 0.013X^2$, $R^2 = 0.663$) and net protein utilization ($y = 23.545 + 6.782X - 0.462X^2$, $R^2 = 0.513$) were observed, and the best results were obtained in fish fed histidine at 7.88 and 7.33 g kg$^{-1}$, respectively. Feed intake, hepatosomatic index, and survival rate were not significantly (P>0.05) affected by the graded histidine levels tested (Table 4).

Whole body moisture, crude protein, crude lipids, and ash were not affected by dietary treatments (Table 5). Similarly, blood glucose, triglycerides, cholesterol, total plasma protein, hematocrit, and hemoglobin parameters were not influenced by dietary histidine levels (Table 6).
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**Table 4** - Growth performance of Nile tilapia fed the experimental diets

| Histidine (g kg⁻¹) | IBW (g) | BWG (g) | FI (g) | FCR (g/g) | NPU (%) | HIS (%) | SUR (%) |
|--------------------|--------|--------|-------|-----------|---------|--------|--------|
| 4.23               | 63.78  | 126.13 | 240.31| 1.27      | 41.54   | 3.35   | 97.22  |
| 5.44               | 64.11  | 158.12 | 250.23| 1.13      | 48.12   | 3.19   | 97.22  |
| 7.17               | 64.11  | 168.20 | 244.03| 1.05      | 50.39   | 3.63   | 100.00 |
| 8.91               | 64.44  | 177.32 | 259.06| 1.07      | 46.77   | 3.18   | 97.22  |
| 11.57              | 64.08  | 168.44 | 253.36| 1.09      | 45.66   | 3.15   | 100.00 |
| SEM                | 0.125  | 3.977  | 2.666 | 0.021     | 0.949   | 0.101  | 0.683  |
| P-value            |        |        |       |           |         |        |        |
| Linear             | 0.962  | 0.527  | 0.527 | 0.652     | 0.152   | 0.659  | 0.199  |
| Quadratic          | 0.165  | <0.001 | 0.521 | <0.001    | 0.002   | 0.519  | 0.955  |

IBW - initial body weight; BWG - body weight gain; FI - feed intake; FCR - feed conversion ratio; NPU - net protein utilization; HIS - hepatosomatic index; SUR - survival; SEM - pooled standard error of the mean.

1 Data are means of four aquaria of 12 Nile tilapia each at the age of 100-165 days.

**Figure 1** - Dietary histidine level to optimize body weight gain, estimated by the quadratic equation for grow-out Nile tilapia.

\[ y = 0.555 + 43.451x - 2.684x^2 \]

\[ R^2 = 0.798 \]

\[ \text{His} = 8.09 \text{ g kg}^{-1} \]

**Table 5** - Body composition of grow-out Nile tilapia fed the experimental diets

| Histidine (g kg⁻¹) | Moisture (g kg⁻¹) | Crude protein (g kg⁻¹) | Crude lipid (g kg⁻¹) | Ash (g kg⁻¹) |
|--------------------|------------------|------------------------|----------------------|--------------|
| 4.23               | 728.96           | 145.03                 | 99.96                | 46.86        |
| 5.44               | 719.22           | 151.76                 | 93.91                | 46.15        |
| 7.17               | 722.29           | 151.93                 | 99.36                | 48.07        |
| 8.91               | 727.29           | 151.82                 | 97.84                | 51.05        |
| 11.57              | 722.15           | 150.63                 | 99.23                | 44.07        |
| SEM                | 111.51           | 22.94                  | 14.37                | 6.68         |
| P-value            |                  |                        |                      |              |
| Linear             | 0.995            | 0.381                  | 0.774                | 0.915        |
| Quadratic          | 0.450            | 0.771                  | 0.731                | 0.531        |

SEM - standard error of the mean.

1 Data are means of four aquaria of 12 Nile tilapia at the age of 100-165 days.
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Muscles were characterized by fibers with different diameters giving a typic mosaic appearance. Fish fed histidine at 4.23 g kg\(^{-1}\) had more (P<0.05) muscle fibers of <20 µm diameter than those fed histidine at 5.44 to 11.57 g kg\(^{-1}\). However, dietary histidine supplementation had no effect (P>0.05) on the abundance of fibers in the 20-50 µm and >50 µm diameter size classes (Table 7).

Fish fed histidine at 5.44 to 11.57 g kg\(^{-1}\) showed higher mRNA expression of MyoD and myogenin than those fed at 4.23 g kg\(^{-1}\). However, mRNA expression of MyoD and myogenin did no differ between fish fed histidine from 5.44 to 11.57 g kg\(^{-1}\). The mRNA expression of myostatin was not influenced by treatments (Figure 2).

**Discussion**

In this study, the dietary histidine requirement was established at 8.09 g kg\(^{-1}\) based on body weight gain and was found to be higher than previously established for Nile tilapia fingerlings fed pelleted purified diets (Santiago and Lovell, 1988), and Nile tilapia fingerlings fed extruded practical diets (Michelato et al., 2017). Differences on growth response to histidine supplementation may be related to diet type and composition, particularly related to amino acids content, balance, and availability. Amino acids content and availability are markedly variable between feed ingredients (Guimarães et al.,

| Histidine (g kg\(^{-1}\)) | GLU (mg dL\(^{-1}\)) | TG (mg dL\(^{-1}\)) | CHO (mg dL\(^{-1}\)) | TP (g dL\(^{-1}\)) | Ht (%) | Hb (g dL\(^{-1}\)) |
|---------------------------|---------------------|---------------------|---------------------|------------------|--------|------------------|
| 4.23                      | 92.17               | 253.05              | 200.44              | 4.18             | 38.5   | 10.27            |
| 5.44                      | 93.03               | 215.7               | 167.54              | 4.08             | 39.12  | 11.01            |
| 7.17                      | 91.41               | 257.53              | 197.94              | 4.38             | 39.23  | 11.74            |
| 8.91                      | 96.13               | 237.33              | 175.84              | 4.02             | 38.25  | 11.67            |
| 11.57                     | 92.33               | 226.47              | 188.27              | 4.16             | 38.38  | 11.21            |
| SEM                       | 0.698               | 6.426               | 5.468               | 0.051            | 0.176  | 0.221            |

P-value

| Linear | 0.438 | 0.677 | 0.873 | 0.978 | 0.912 | 0.215 |
|--------|-------|-------|-------|-------|-------|-------|
| Quadratic | 0.793 | 0.854 | 0.389 | 0.894 | 0.911 | 0.066 |

GLU - glucose; TG - triglycerides; CHO - cholesterol; TP - total plasma protein; Ht - hematocrit; Hb - hemoglobin; SEM - pooled standard error of the mean.

1 Data are means of four aquaria of 12 Nile tilapia at the age of 100-165 days.

| Fiber class | <20 µm | 20-50 µm | >50 µm |
|-------------|--------|----------|--------|
| Histidine (g kg\(^{-1}\)) |        |          |        |
| 4.23        | 20.92b | 52.67    | 26.41  |
| 5.44        | 17.50ab| 64.75    | 17.75  |
| 7.17        | 15.14a | 47.44    | 37.42  |
| 8.91        | 14.75a | 57.25    | 28.00  |
| 11.57       | 14.00a | 54.75    | 31.25  |
| SEM         | 1.122  | 2.563    | 2.694  |

**Table 7**: Occurrence (%) of white muscle fibers of grow-out Nile tilapia fed the experimental diets

| Fiber class | <20 µm | 20-50 µm | >50 µm |
|-------------|--------|----------|--------|
| Histidine (g kg\(^{-1}\)) |        |          |        |
| 4.23        | 20.92b | 52.67    | 26.41  |
| 5.44        | 17.50ab| 64.75    | 17.75  |
| 7.17        | 15.14a | 47.44    | 37.42  |
| 8.91        | 14.75a | 57.25    | 28.00  |
| 11.57       | 14.00a | 54.75    | 31.25  |
| SEM         | 1.122  | 2.563    | 2.694  |

SEM - pooled standard error of the mean.

1 Data are means of four aquaria of 12 Nile tilapia at the age of 100-165 days.

Different letters indicate differences within each fiber class size by Dunn’s multiple comparison test (P<0.05).
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Dietary histidine requirement by analyzing the availability of amino acids should be considered to improve accuracy to determine the dietary requirements of amino acids. Many recent studies have evaluated the dietary requirements of amino acids such as leucine (Gan et al., 2016), lysine (Michelato et al., 2016), tryptophan (Zaminhan et al., 2017), and valine (Xiao et al., 2018); the revised dietary requirement figures provided by these studies are higher than those previously established for Nile tilapia. This trend is expected because genetic improvements have been successfully applied to improve the growth rate of Nile tilapia (Bentsen et al., 2017). In addition, appropriate fish management, combined with advances in feed evaluation, formulation, and processing, has markedly contributed to global Nile tilapia production.

Dietary histidine at 11.63 g kg\(^{-1}\) was found to optimize body weight gain in this research. This value is higher than previously established by Santiago and Lovell (1988) and should be updated for Nile tilapia nutrition. To date, the optimum histidine requirement found in this study is close to the level used in commercial feeds currently used for Nile tilapia in Brazil (Montanhini Neto and Ostrensky, 2015).

Histidine has been described as a limiting amino acid for salmonids; furthermore, histidine is known to prevent cataracts, acting as a potent antioxidant in the lens (Remø et al., 2017). However, by the end of this study, using visual assessment, cataract was not detected. Cataract occurrence is fish is more frequent in cold-warm species than in warm-water species (Khan and Abidi, 2014). Consistent with the findings of this study, cataract was not observed in African catfish (*Clarias gariepinus*; Khan and Abidi, 2009) and Catla (*Catla catla*; Zehra and Khan, 2016) fed histidine-deficient diets.

As observed in this study, excess histidine induces amino acid catabolism reducing dietary protein utilization in fish (Aragão et al., 2004). Reduced dietary protein in response to excess supplemental histidine has been described in Indian major carp (*Cirrhinus cirrhosis*; Ahmed and Khan, 2005) and in Singhi (*Heteropneustes fossilis*; Khan and Abidi, 2014).

In this work, hepatosomatic index of fish was not affected by dietary histidine levels. In contrast, Farhat and Khan (2013) and Zehra and Khan (2016) found that histidine dosage affected hepatosomatic index in stinging catfish (*Heteropneustes fossilis*) and Catla. Hepatosomatic index, as an indicator of condition and nutritional status of fish, reflects the accumulation of visceral fat caused by imbalanced diets (Michelato et al., 2017). Liver weight is affected by many factors and is highly variable. However, this is a not reliable indicator of fish health and condition (Chellappa et al., 1995).

Reduced feed intake has been documented in fish fed amino acid-deficient diets; this has been demonstrated in grass carp (*Ctenopharyngodon idella*; Gao et al., 2016). However, in this study, fish feed intake was not affected by dietary treatments. Similar results were described for Singhi (Khan and Abidi, 2014) and Japanese flounder (*Paralichthys olivaceus*) juveniles (Han et al., 2013).
Whole body protein of fish used in this research was not affected by dietary histidine supplementation, consistent with findings for Japanese flounder (Han et al., 2013) and grass carp (Gao et al., 2016), but in contrast to findings observed in Catla fed supplemental histidine (Zehra and Khan, 2016), because histidine positively stimulates protein synthesis and, consequently, leads to increased body weight in fish. This positive effect has been observed in blunt snout bream (Megalobrama amblycephala; Wilson-Arop et al., 2018).

The presence of the narrowest muscle fibers (<20 µm diameter) indicated that hyperplasic growth was still occurring in skeletal muscle of fish used in this research. However, predominance of the widest fibers (>50 µm of diameter) confirmed the occurrence of active hypertrophic growth in many fish species (Rowlerson and Veggetti, 2001), including Nile tilapia juveniles (Michelato et al., 2007). Similarly, pacu (Piaractus mesopotamicus) juveniles have been shown to have more of the narrowest (<20 µm of diameter) than the widest (>50 µm diameter) muscle fibers; the opposite pattern was observed in adult pacu (Almeida et al., 2008).

High mRNA myostatin levels are associated with the inhibition of myoblasts and satellite cell proliferation in a quiescent and undifferentiated state, and are negatively associated with muscle growth (Watabe, 1999). In the present work, dietary histidine did not affect (P>0.05) mRNA levels of myostatin.

Blood biochemical and hematological parameters of fish were not affected by histidine levels in this study. All biochemical and hematological parameters evaluated fell within the reference intervals for cultured tilapia (Hrubec et al., 2000). In accordance, no effects on triglycerides, glucose, hematocrit, and hemoglobin were described in Japanese flounder juveniles fed diets containing graded levels of histidine (Han et al., 2013). Histidine is highly concentrated in hemoglobin protein (Khan and Abidi, 2014). No anemia was observed in fish analyzed in this study, in contrast with the lower hematocrit and hemoglobin described in Catla fed a histidine-deficient diet (Zehra and Khan, 2016).

In summary, the dietary requirements of grow-out Nile tilapia were estimated at 8.09, 7.88, and 7.33 g kg\(^{-1}\) to optimize body weight gains, feed conversion ratio, and net protein utilization, respectively. In addition, fish fed histidine from 5.44 to 11.57 g kg\(^{-1}\) demonstrated predominance of hypertrophic muscle-growth, in line with higher mRNA expression of myogenin and MyoD. Histidine may be a limiting amino acid in plant-based diets and the dietary requirements determined in the current study may be considered to formulate diets for grow-out Nile tilapia.

Conclusions

The dietary requirement of histidine for grow-out Nile tilapia was found to be 8.09 g kg\(^{-1}\) based on growth performance, muscle development, and gene expression responses.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Data curation: M. Zaminhan-Hassemer, M. Michelato and W.M. Furuya. Conceptualization: M. Zaminhan-Hassemer, M. Michelato, V.R.B. Furuya and W.M. Furuya. Formal analysis: M. Zaminhan-Hassemer, M. Michelato and W.M. Furuya. Funding acquisition: W.M. Furuya. Investigation: M. Zaminhan-Hassemer, W.R. Boscolo, A.V. Urbich and T.P. Cruz. Methodology: M. Michelato and F.L.A. Almeida. Project administration: W.M. Furuya. Resources: V.R.B Furuya and W.M. Furuya. Software: M. Zaminhan-Hassemer. Supervision: W.M. Furuya. Validation: V.R.B. Furuya and W.M. Furuya. Visualization: M. Zaminhan-Hassemer and M. Michelato. Writing-original draft: M. Zaminhan-Hassemer, M. Michelato and W.M. Furuya. Writing-review & editing: M. Michelato and W.M. Furuya.
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