Estimating Optimum Levels of Dietary Histidine Needed to Attain Improved Performance of Juvenile Nile tilapia

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ABSTRACT

Background: Optimum levels of essential amino acids in the diet have a critical role in fish growth and health. This study investigated the effects of dietary histidine levels on the growth performance, and whole-body composition of Nile tilapia (Oreochromis niloticus) juveniles. Methodology: The experimental design included six diets with varying histidine levels (0.56%, 0.68%, 0.81%, 0.96%, 1.09%, and 1.25%), with five replicates consisting of 17 Nile tilapia juveniles per tank. The fish were fed twice a day until satiety for 55 days. Data were analysed by one-way ANOVA using the SAS GLM procedure, and differences among dietary treatments were compared using Tukey's multiple comparison test. Results: Growth performance data showed that fish fed with 0.56% dietary histidine exhibited the highest body weight gain compared to other treatments, with the 1.09% histidine diet showing the lowest body weight gain (p < 0.05). A similar pattern was observed for the thermal growth coefficient and feed intake, where values decreased as dietary histidine level increased (p < 0.05). Feed conversion ratio and protein retention did not differ significantly across the varying histidine levels (p > 0.05). Results indicated that only the body protein content was significantly affected by dietary histidine levels, increasing proportionally with higher histidine concentrations (p < 0.05). Conclusion: The study recommended the dietary histidine supplementation of 0.56% for Nile tilapia, as this level yielded the best growth performance under the tested conditions. This finding can guide the formulation of more cost-effective and nutritionally balanced diets, optimising tilapia nutrition and enhancing the sustainability of aquaculture production.

aquaculture production.

**Keywords:* Essential amino acids; balanced diet; dose-response; histidine; Nile

INTRODUCTION

tilapia

The aquaculture industry plays an essential role in the global food supply chain, with Nile tilapia (Oreochromis niloticus) being a prominent species. Ranking second, it accounts for 9% of the world's total inland finfish production (FAO, 2024). The production of Nile tilapia occurs in over 120 countries, utilizing a diverse range of production methods, from small-scale traditional practices to large-scale industrial operations (FAO, 2024). Aquafeed is essential for successful fish farming, with its cost sometimes accounting for up to 70% of total expenses in intensive systems (Thompson et al., 2005, El-Sayed, 2020). Among the essential nutrients are amino acids, which are the building blocks of protein. These nutrients are essential for a fish's ability to deposit protein, directly affecting its weight gain and how efficiently it converts feed into growth (Kaushik and Seiliez, 2010). Formulating balanced diets requires a deep understanding of a species'

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specific nutritional needs, as the right balance of amino acids is key to optimal performance.

Histidine serves as a precursor for bioactive compounds like histamine, which is involved in allergic and inflammatory reactions (Glover and Wood, 2008; Khan and Abidi, 2014). It also participates in vital biochemical processes such as osmoregulation, muscle pH buffering, and the detoxification of reactive carbonyl species (Waagbø et al., 2010). Research has demonstrated that supplementing the diet with adequate levels of histidine can promote growth in juvenile Nile tilapia by suppressing the expression of myostatin mRNA (Michelato et al., Furthermore. studies have shown haematological and biochemical parameters were not adversely affected in either the juvenile or grow-out phase tilapia fed diets with increasing levels of histidine (Michelato et al., 2016; Zaminhan-Hassemer et al., 2020). Dietary histidine requirements in fish vary with developmental stage and farming conditions, making it essential to determine precise levels to avoid deficiencies and maximise productivity. This variability is evident in studies on Nile tilapia, where recommended values differ significantly across growth phases. For example, Santiago and Lovell (1988) established a requirement of 0.48% for fry, while more recent studies have reported higher values, such as 0.54% (Diógenes et al., 2016), 0.82% (Michelato et al., 2016), and 0.81% (Zaminhan-Hassemer et al., 2020). The wide range of reported values underscores the need to refine optimal inclusion levels. Accurately determining these requirements is crucial for formulating cost-effective diets that enhance performance without causing nutritional imbalances. Therefore, this study aimed to evaluate the effects of varying dietary histidine levels on the zootechnical performance of Nile tilapia (Oreochromis niloticus) and to determine the optimal concentration for maximizing growth.

MATERIALS AND METHODS Ethical approval

The present study was conducted according to the ethical guidelines of the Brazilian College of Animal Experimentation (COBEA). They were approved by the Ethics Committee on Animal Care and Use of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University (UNESP) via Protocol No. 009999/14.

Fish and experimental design

Nile tilapia juveniles of the GIFT (Genetically Improved Farmed Tilapia) strain that had been sexually inverted to male were purchased from a local commercial fish farm (Fish Project, Sales de Oliveira, State of São Paulo, Brazil). Before the feeding trial, fish were acclimatised to laboratory conditions for two weeks and fed with a commercial feed twice a day. After the adaptation period, a total of 510 fish (Nile tilapia) juveniles were divided into 17 groups, each having 30 experimental units. During the feeding trial, fish were kept in a closed recirculating aquaculture system (RAS), equipped with a biological filter, a heat exchange system, and independent aeration. Fish were fed twice a day until apparent satiety for 55 days. During the feeding trial, the average water temperature was 27.05 ± 1.04 °C, dissolved oxygen was 5.43 ± 0.99 mg L⁻¹, pH was 7.60 ± 0.56 , and total ammonia was 0.06 \pm 0.07 mg L⁻¹. Photoperiod was set at 12 hours dark:12 hours light. The water quality variables were maintained within the optimum for this species (El-Sayed, 2020).

Experimental diets and feeding management

Before manufacturing diets, all ingredients were individually ground in a hammer mill (1.0 mm mesh) and manually sieved (0.6 mm). Samples of all raw materials were sent to Evonik (Guarulhos, SP, Brazil) for AA analysis. A basal diet master batch was formulated to be isoenergetic and to meet the nutritional requirements of Nile tilapia (Furuya et al., 2010; NRC, 2011). The aliquots of this batch were supplemented with increasing levels of histidine and decreasing levels of glutamic acid to keep the dietary nitrogen level, and to create the six experimental

diets. The diets were formulated to contain histidine levels of 0.56, 0.68, 0.81, 0.96, 1.09, and 1.25% (Table 1).

Diets were extruded using a single-thread extruder (Exteec, Ribeirao Preto, São Paulo, Brazil). During the extrusion process, the temperature of extruder barrel was maintained at 100°C and the moisture of the mixtures at 21%. The resulting floating pellets (2-3 mm) were dried in an oven with forced air circulation at 55°C and packaged and stored in a cold chamber at -12°C.

Sample collection and chemical analyses

At the beginning of each feeding trial, 30 fish were fasted for 24 h and then euthanised with 175 mg L⁻¹ of benzocaine (4-Aminobenzoic acid ethyl ester; Sigma-Aldrich, Brazil) to analyse the proximate composition of the whole body. Ten fish per tank were randomly collected at the end of the trial and stored at -20 °C for further analysis. The frozen samples were ground using a meat grinder, homogenised, weighed, and subsequently frozen at -80°C (in a lyophilizer) for pre-drying. After that, freeze-drying was carried out for 72 hours at -80°C and -10 ATM using an Edwards SuperModulo (Thermo Fisher Scientific, Waltham, MA), followed by drying, weighing, and milling using a micro grinder. The diets and fish were analysed using standard methods outlined by AOAC International (2005). The total body lipid content of the fish was determined by extracting with petroleum ether using a fat extractor (Ankon technology®, Macedon, NY) (method 920.39), while the lipid content of the diets was determined via acid hydrolysis. The crude protein content was analysed using the Dumas method with a Leco 528 LC apparatus (Etheridge et al. 1998). Moisture content was determined by oven-drying the samples at 105°C until a constant weight was achieved (method 920.39). Ash content was determined by combustion in a muffle furnace at 550°C (method 942.05). Amino acid content analysis of the diets and fish body were conducted using High Performance Liquid Chromatography (HPLC) by Evonik Operations GmbH – Nutrition & Care (Essen, Germany) after acid hydrolysis. Tryptophan content was determined after alkaline hydroxylation of the sample with lithium hydroxide.

Growth performance assessment

At the beginning of the feeding trial, all fish were individually weighed and sorted, maintaining a coefficient of variation of 5% within each experimental unit and among all treatments. At the end of the trial, the fish were individually weighed per tank to assess growth performance. Body weight gain (BWG), thermal growth coefficient (TGC), protein retention (PR), feed intake (FI), feed conversion ratio (FCR), and survival (SR) were calculated according to the following formulae

Body weight gain (g) = final body weight – initial body weight

Thermal growth coefficient (%) = $\frac{\text{final body weight}^{0.3333} - \text{initial body weight}^{0.3333}}{a \text{verage daily temperature} \times \text{days}} \times 100$

$$\begin{aligned} & \text{Protein retained} \\ & \text{Protein intake} \\ & \text{Feed intake (g/fish)} = \frac{\text{dry feed consumed}}{\text{number of fish}} \\ & \text{Feed conversio ratio (g/g)} = \frac{\text{feed intake}}{\text{body weight gain}} \end{aligned}$$

Survival (%) =
$$\frac{\text{number of final fish}}{\text{number of initial fish}} \times 100$$

Statistical analysis

Data were analysed by one-way ANOVA using the SAS GLM procedure (SAS Inst. Inc., Cary, NC, USA) (SAS, 2014). Differences among dietary treatments were compared using Tukey's multiple comparison test. The significance level adopted was 5%.

Table 1: Ingredient composition of experimental diets

| Ingredients (%) | Treatments | | | | | | |
|-----------------------------|------------|-------|-------|-------|-------|-------|--|
| | 0.56 | 0.68 | 0.81 | 0.96 | 1.09 | 1.25 | |
| Wheat flour | 27.90 | 27.90 | 27.90 | 27.90 | 27.90 | 27.90 | |
| CGM, 60% CP | 13.00 | 13.00 | 13.00 | 13.00 | 13.00 | 13.00 | |
| Feather meal | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | |
| Gelatin | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | |
| Rice bran | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | |
| L-Glutamic acid | 0.75 | 0.63 | 0.48 | 0.33 | 0.18 | 0.02 | |
| Biolys® (L-Lys Sulphate) | 2.39 | 2.39 | 2.39 | 2.39 | 2.39 | 2.39 | |
| Celulose | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | |
| Fish oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | |
| MetAMINO® (DL-Met) | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | |
| ThreAMINO® (L-Thr) | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | |
| L-Valine | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | |
| L-Isoleucine | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | |
| L-Histidine | 0.03 | 0.15 | 0.30 | 0.45 | 0.60 | 0.76 | |
| TrypAMINO® (L-Trp) | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | |
| MCP | 3.20 | 3.20 | 3.20 | 3.20 | 3.20 | 3.20 | |
| Vit-Min premix ¹ | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | |

¹moisture (%) 2.0; Ash (%) 71.6442; Choline (mg/kg) 30000; Magnesium (%) 0.0085; sulfur (%) 1.1589; Iron (mg/kg) 25 714; Copper (mg/kg) 1.960; Manganese (mg/kg) 13,345; Zinc (mg/kg) 30,000; Iodine (mg/kg) 939; Selenium (mg/kg) 30; Vitamin A (IU/kg) 600 000; Vitamin D3 (IU/kg) 600 000; Vitamin E (mg/kg) 12.000; Vitamin K3 (mg/kg) 631; Thiamine B1 (mg/kg) 1176; Riboflavin B2 (mg/kg) 1536; Pyridoxine B6 (mg/kg) 1,274; Vitamin B12 (mg/kg) 4000; Niacin (mg/kg) 19800; Pantothenic acid B3 (mg/kg) 3920; Folic acid (mg/kg) 192 Biotin (mg/kg) 20; Vitamin C (mg/kg) 40,250.

Table 2: Nutrient composition of experimental diets (% dry diet)

| Nutrients | Treatment | | | | | |
|-------------------------|-----------|---------|---------|---------|---------|---------|
| Nutrients | 0.56 | 0.68 | 0.81 | 0.96 | 1.09 | 1.25 |
| Dry matter (%) | 90.62 | 90.45 | 90.76 | 90.57 | 90.03 | 90.07 |
| Crude protein (%) | 37.81 | 37.98 | 38.23 | 38.30 | 37.97 | 38.38 |
| Crude ether extract (%) | 4.95 | 4.99 | 4.98 | 4.99 | 4.96 | 4.99 |
| Crude energy (kcal/kg) | 4344.51 | 4443.85 | 4548.10 | 4521.70 | 4427.25 | 4470.16 |
| Ash | 3.75 | 3.55 | 3.69 | 3.64 | 3.68 | 3.63 |
| Crude Methionine | 0.95 | 0.96 | 0.95 | 0.95 | 0.96 | 0.96 |
| Crude Lysine | 2.33 | 2.33 | 2.31 | 2.32 | 2.30 | 2.27 |
| Crude Threonine | 1.51 | 1.53 | 1.52 | 1.52 | 1.50 | 1.51 |
| Crude Arginine | 1.99 | 2.01 | 1.99 | 1.97 | 1.99 | 2.00 |
| Crude Isoleucine | 1.65 | 1.65 | 1.64 | 1.66 | 1.64 | 1.64 |
| Crude Leucine | 3.04 | 3.04 | 3.03 | 3.03 | 2.98 | 3.02 |
| Crude Valine | 1.92 | 1.92 | 1.91 | 1.93 | 1.93 | 1.91 |
| Crude Histidine | 0.56 | 0.68 | 0.81 | 0.96 | 1.09 | 1.25 |
| Crude Phenylalanine | 1.58 | 1.58 | 1.57 | 1.57 | 1.55 | 1.57 |
| Crude Tryptophan | 0.46 | 0.48 | 0.48 | 0.47 | 0.49 | 0.48 |

RESULTS Growth performance

Data on the growth performance of Nile tilapia juveniles are presented in Table 3. The mortality rate was less than 1% and showed no correlation with the experimental diets. Statistical differences were observed for BWG, TGC, and FI (p < 0.05) in tilapia fed with increasing levels of histidine. According to Tukey's test, fish fed with 0.56% dietary histidine had a superior BWG compared to other treatments, with the 1.09% treatment showing the lowest BWG (Fig. 1). A similar pattern was observed for TGC

and FI (Fig. 2), with values decreasing as dietary histidine concentration increased. The remaining variables, such as FCR and PR, showed no statistical difference in fish fed increasing levels of histidine (p > 0.05).

Whole-body composition

In the whole-body composition analysis, only protein was statistically affected (p < 0.05) by dietary histidine levels, as shown in Table 4. The body protein content increased proportionally with the graduated levels of dietary histidine.

Table 3: Growth performance of Nile tilapia fed with graded levels of dietary histidine

| | 0.56 | 0.68 | 0.81 | 0.96 | 1.09 | 1.25 | p -value |
|-------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------|
| IW (g) | 8.22 ± 0.08 | 8.27 ± 0.13 | 8.24 ± 0.08 | 8.24 ± 0.07 | 8.16 ± 0.08 | 8.24 ± 0.10 | 0.5353 |
| BWG (g) | 121.76 ± 2.05^{a} | 113.55 ± 2.03^{b} | 109.34 ± 1.73^{b} | 108.74 ± 4.52^{b} | 102.71 ± 1.09^{c} | 106.58 ± 0.97^{b} | < 0.0001 |
| TGC (%) | 0.20 ± 0.00^a | 0.20 ± 0.00^{ab} | 0.19 ± 0.00^{b} | 0.19 ± 0.00^{b} | 0.19 ± 0.00^{b} | 0.19 ± 0.00^b | 0.0018 |
| FI (g/fish) | 99.12 ± 2.10^{a} | 97.47 ± 1.97^{a} | 91.88 ± 1.00^{ab} | 90.68 ± 4.98^{ab} | 85.03 ± 2.63^{b} | 89.87 ± 2.18^{ab} | 0.0027 |
| FCR (g/g) | 0.81 ± 0.02 | 0.86 ± 0.01 | 0.84 ± 0.01 | 0.83 ± 0.02 | 0.83 ± 0.02 | 0.84 ± 0.01 | 0.1262 |
| PR (%) | 43.62 ± 1.5 | 44.60 ± 0.67 | 43.99 ± 0.42 | 45.26 ± 0.94 | 48.18 ± 1.72 | 45.26 ± 0.77 | 0.5720 |
| S (%) | 100 ± 0.00 | 98.82 ± 2.63 | 97.65 ± 5.26 | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 | 0.5656 |

Abbreviations: IW, initial weight; BWG, body weight gain; TGC, thermal growth coefficient; FI, feed intake; FCR, feed conversion ratio; PR, protein retention; S, survival.

Table 4: Whole-body proximate composition (g 100 g⁻¹) of Nile tilapia fed with graded levels of histidine

| | 2 1 | (8 : 8) | | |
|------------|------------------|-----------------|-----------------|-----------------------------|
| Treatments | Dry matter | Ash | Fat | Crude protein |
| Initial | 23.05 | 3.97 | 4.63 | 13.72 |
| 0.56 | 27.54 ± 0.71 | 3.34 ± 0.05 | 9.62 ± 0.39 | 13.61 ± 0.54^{b} |
| 0.68 | 27.57 ± 0.63 | 3.43 ± 0.32 | 9.28 ± 0.69 | 14.30 ± 0.38^a |
| 0.81 | 27.44 ± 0.25 | 3.24 ± 0.18 | 9.53 ± 0.34 | 14.07 ± 0.24^{ab} |
| 0.96 | 27.53 ± 0.84 | 3.53 ± 0.49 | 9.33 ± 0.82 | $14.35\pm0.20^{\mathrm{a}}$ |
| 1.09 | 27.67 ± 0.21 | 3.32 ± 0.07 | 9.44 ± 0.30 | 14.11 ± 0.42^{ab} |
| 1.25 | 27.49 ± 0.61 | 3.39 ± 0.18 | 9.49 ± 0.50 | $14.44\pm0.39^{\mathrm{a}}$ |
| P Value | 0.9901 | 0.6703 | 0.9491 | 0.0018 |

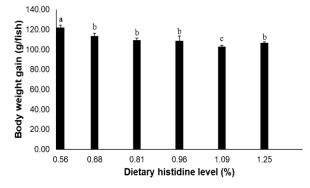


Figure 1: Effect of different dietary histidine levels on the body weight gain of juvenile Nile tilapia (*Oreochromis niloticus*) after a 55-day trial. Values are presented as mean ± standard deviation of the mean.

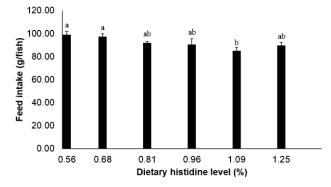


Figure 2: Effect of different dietary histidine levels on the feed intake of juvenile Nile tilapia (*Oreochromis niloticus*) after a 55-day trial. Values are presented as mean ± standard deviation of the mean.

DISCUSSION

In recent years, determining the dietary amino acid requirements has become a primary focus of fish nutrition research. This emphasis is driven by the aim to develop cost-effective diets with high-performance aquafeeds. In study, increasing dietary histidine present supplementation did not improve the growth performance of juvenile Nile tilapia, as the lowest level tested (0.56%) was sufficient to achieve the best results compared to the other treatments. In a previous study, Santiago and Lovell (1988) estimated the histidine requirement at 0.48% (1.70%) dietary protein) for fingerling stage Nile tilapia. In addition, Diógenes et al. (2016) established the histidine requirement of 20 g tilapia at 0.54% (1.80% dietary protein) based on the deletion technique. In agreement with this work, both studies indicated that the histidine requirement is not higher than 0.56%. However, in the study by Michelato et al. (2016), it was determined that the requirement for juvenile tilapia with an initial weight of 4.80 g was 0.82% (3.10% dietary protein) based on weight gain. More recently, a requirement of 0.81% (2.80%) dietary protein) histidine was estimated for growing tilapia with an initial weight of 64.17 g (Zaminhan-Hassemer et al., 2020). These last two studies diverged from the value found in the present study of 0.56% histidine (1.48% dietary protein). Variations in feed intake, which dictate the total histidine intake, may explain the observed differences. In the present study, 4.56 mg of histidine was ingested for each gram of body weight gain. This value indicates a higher efficiency of histidine utilization for growth compared to the findings of Michelato et al. (2016) and Zaminhan-Hassemer et al. (2020), that histidine intake was 10.15 and 9.53 mg/g, respectively.

In the presente study, as the dietary histidine level increased, body weight gain and thermal growth coefficient decreased. The same trend was observed for feed intake, which also decreased. Excess dietary histidine can lead to an amino acid (AA) imbalance, causing a reduction in growth and feed intake (Moro, 2020). A study with juvenile red drum (Sciaenops ocellatus) observed that excess histidine impaired erythrocyte fragility. This was potentially attributed to elevated histamine production resulting from the excess histidine, as histamine can induce pro-inflammatory cytokines that may disturb erythrocyte integrity (Peachey et al., 2018). Similarly, excessive histidine has been shown to compromise the gill structure in juvenile grass carp (Ctenopharyngodon idella) (Jiang et al., 2016). Although the present study did not assess specific parameters that could indicate the deleterious effects of excess histidine on Nile tilapia the observed decrease in growth performance at higher dietary levels suggests a potential negative impact from excess histidine. These responses to increasing dietary histidine levels may indicate that the histidine requirement under the experimental conditions of this study is 0.56% or lower. However, future studies are needed to evaluate histidine levels lower than those tested, as the concentration range

used in the present study did not allow for the determination of a conclusive optimal requirement.

In the present study, although there was no improvement in growth, the body protein content of tilapia increased as dietary histidine was raised from 0.56% to 0.68%, remaining constant at higher levels. However, the reason for this specific effect on protein is unclear, particularly since other body composition variables, such as fat, moisture, and ash, were unaffected. These findings underscore the need for further research to elucidate the physiological and biochemical responses of Nile tilapia to varying levels of dietary histidine, from deficiency to excess. Given the discrepancies in recommended requirements found in recent literature, new feeding trials are essential to precisely define the optimal inclusion level of histidine for this species.

CONCLUSION

The inclusion of dietary histidine at 0.56% resulted in significant improvements in growth performance in Nile tilapia. Since increased levels of histidine supplementation offered no additional benefits to growth, indicating that the 0.56% level was sufficient to meet the tilapia's dietary requirement. Based on these results, future research should focus on levels below 0.56% to more precisely determine the minimum requirement. This finding is crucial for nutritionists formulating cost-effective, balanced diets aimed at enhancing the productivity and sustainability of tilapia farming.

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Conflict of interest

The authors declare that there is no conflict of interest.

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