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EFFECT OF GUANAMIN® SUPPLEMENTATION ON THE NUTRITION OF JUVENILE NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

EFEITO DA SUPLEMENTAÇÃO DE GUANAMINO® NA NUTRIÇÃO DE JUVENIS DE TILÁPIA DO NILO (OREOCHROMIS NILOTICUS)

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Abstract
GAA is a product composed of amino acids that acts primarily in energy metabolism. Its use reduces production costs compared to the use of creatinine. The aim of this study was to evaluate the effects of GAA supplementation on the proximate composition, productive performance, body indices, and oxidative and enzymatic responses of juvenile Nile tilapia. One hundred and twenty fish were used, distributed in a completely randomized design, with three treatments and three replicates, totaling 20

Resumo
O GAA é produto composto por aminoácidos que atua principalmente no metabolismo energético. Sua utilização reduz os custos de produção em comparação ao uso de creatinina. O objetivo deste estudo foi avaliar os efeitos da suplementação de GAA sobre a composição centesimal, no desempenho produtivo, nos índices corporais e respostas oxidativas e enzimáticas de juvenis de tilápias do Nilo. Foram utilizados 120 peixes, distribuídos em um delineamento inteiramente casualizado, com

animals per experimental unit. The experimental diets contained 36% CP and were supplemented with 0, 0.050, and 0.150% GAA. The data obtained were subjected to analysis of variance and linear and quadratic polynomial regression. Fish supplemented with 0.15% GAA showed higher protein content and lower lipid reserves, better results for AFW, WG, DWG and SGR, as well as increased catalase, sucrase and trypsin activity. However, there was a reduction in PER and FY, as well as a worsening in FC. Fish supplemented with 0.05% GAA showed improved FC and total protein intake, as well as reduced VFI and HI in the fish. Supplementation with 0.05% GAA is recommended for Nile tilapia juvenile diets.

Keywords: Aquaculture. Guanidineacetic Acid. Energy Metabolism. Muscle Tissue.

três tratamentos e três repetições, totalizando 20 animais por unidade experimental. As dietas experimentais continham 36% de PB e suplementadas com 0, 0,050 e 0,150% de GAA. Os dados obtidos foram submetidos à análise de variância e regressão polinomial linear e quadrática. Os peixes suplementados com 0,15% de GAA apresentaram maior teor de proteína e menor reserva de lipídios, melhores resultados para PFM, GP, GPD e TCE, além de aumento na atividade da catalase, sacarase e tripsina. Entretanto, houve redução na TEP e RF, bem como piora na CAA. Já os peixes suplementados com 0,05% de GAA apresentaram melhora na CAA e TEP, além de reduzir os IGV e IHS nos peixes. Recomenda-se a suplementação de 0,05% de GAA para dietas de juvenis de tilápia do Nilo.

Palavras-chave: *Aquicultura. Ácido Guanidinoacético. Metabolismo Energético. Tecido Muscular.*

1 INTRODUCTION

Guanamino® or guanidinoacetic acid (GAA) is a natural additive derived from the amino acids arginine and glycine, being the main pathway of creatine (Cr), acting in energy metabolism and energy production (khajali *et al.*, 2020). In addition, GAA also promotes an amino acid-sparing effect, improving the performance (Ahmadipour *et al.*, 2018) and digestibility of fish (Portocarero & Braun, 2021), As well as strengthening their antioxidant defenses (Zhao *et al*., 2021; Amiri *et al.*, 2019). Its supplementation in animal feed can also reduce production costs when compared to the use of creatinine (Crn) (Baker, 2009). Furthermore, guanidine compounds are also able to act in the regulation of homeostasis, strengthening the immune system and increasing muscle glucose uptake, in addition to promoting increased Cr availability and improved energy efficiency (Diniz *et al.*, 2024).

However, studies evaluating the effects of GAA supplementation in fish, especially Nile tilapia, are limited, with most available research primarily focused on terrestrial species such as poultry, swine, and cattle.

Thus, the objective of this study was to evaluate the effect of GuanAMINO® on the proximate composition, productive performance, body indices, and oxidative and enzymatic responses of juvenile Nile tilapia.

2 THEORETICAL FRAMEWORK

Nile tilapia is one of the most widely farmed fish in the world, with an estimated production of 5.3 million tons (FAO, 2024). In Brazil, it represents 65% of total fish production, with 579,080 tons in 2023 (FAO, 2024). This omnivorous species stands out for its good productive performance in extensive, semi-intensive and intensive production systems (Santos *et al.,* 2019) and rapid growth, being able to reach commercial size in approximately six months, depending on production conditions (Aguiar *et al.,* 2023; Vijayaram *et al.,* 2023). Furthermore, they exhibit resistance to various types of diseases and adapt well to environments with low levels of dissolved oxygen and high concentrations of ammonia (Li *et al.,* 2024).

In this context, nutritional additives have contributed significantly to the growth of fish production, promoting improvements in productive performance, animal health, the sustainability of the activity, and the reduction of production costs.

GAA is a natural additive derived from the amino acids arginine and glycine, being the main pathway of Cr, acting in energy metabolism and energy production (Khajali *et al.,* 2020). Furthermore, GAA also promotes an amino acid-sparing effect, improving the performance (Ahmadipour *et al.,* 2018) and digestibility of fish (Portocarero & Braun, 2021), as well as strengthening their antioxidant defenses (Zhao *et al.,* 2021; Amiri *et al.,* 2019).

Its supplementation in animal feed can also reduce production costs when compared to the use of Crn (Baker, 2009). Furthermore, guanidine compounds are also able to act in the regulation of homeostasis, strengthening the immune system and increasing muscle glucose uptake, in addition to promoting increased Cr availability and improved energy efficiency (Diniz *et al.,* 2024).

3 METHODOLOGY

The study was conducted at the facilities of the Aquaculture Studies and Management Group (GEMAq), at the State University of Western Paraná (UNIOESTE), Toledo campus, PR, Brazil. All procedures adopted in conducting this study were approved by the Ethics Committee on the Use of Production Animals of UNIOESTE (protocol no. 16/2022 CEUAP).

Three experimentais diets were formulated with 36% crude protein (CP) and supplemented with: 0, 0.05 and 0.15% GAA supplied by the company EVONIK – Leading Beyond Chemistry (Table 1).

Table 1. Percentage composition of ingredients in experimental diets, based on dry matter, supplemented with GuanAMINO® and fed to juvenile Nile tilapia for 60 days.

Ingredients	**Treatments**		
	C	**0.05%**	**0.15%**
Corn	32.61	32.61	32.61
Soybean Meal	15.00	15.00	15.00
Wheat flour	0.00	0.00	0.00
Poultry By-product Meal	19.42	19.42	19.42
Meat/Bone Meal	6.81	6.81	6.81
Rice grains	5.00	5.00	5.00
Distilled Alcohol Yeast	1.00	1.00	1.00
Feather meal	8.00	8.00	8.00
Soy Protein Concentrate	6.35	6.35	6.35
Soybean oil	3.60	3.60	3.60
Antifungal	0.10	0.10	0.10
Antioxidant	0.02	0.02	0.02
DL-Methionine	0.29	0.29	0.29
L-Lysine	0.27	0.27	0.27
L-Threonine	0.30	0.30	0.30
Premix	0.50	0.50	0.50
Common Salt	0.50	0.50	0.50
Hill	0.10	0.10	0.10
Vitamin C	0.13	0.13	0.13
TOTAL	100.00	100.00	100.00
Nutritional Levels			
Histidine	0.90	0.87	0.89
Isoleucine	1.36	1.30	1.36
Leucine	2.94	2.89	2.90
Lysine	2.10	2.03	2.04
Methionine	0.85	0.89	0.85
Phenylalanine	1.66	1.62	1.67
Threonine	1.79	1.78	1.78
Valina	1.94	1.90	1.91
Alanine	2.25	2.26	2.20
Arginine	2.36	2.29	2.34
Aspartic acid	3.18	3.01	3.09

Cysteine	0.59	0.60	0.58
Glutamic acid	5.02	4.78	4.98
Glycine	2.79	2.84	2.76
Proline	2.44	2.44	2.22
Serina	1.90	1.86	1.88
GuanAMINO®	0.00	0.05	0.15
Methionine/Cysteine	1.45	1.49	1.44

The experimental diets were processed at the feed mill using a 0.3 mm sieve, a hammer mill for grinding and particle size reduction, and a Y-type mechanical mixer for homogenizing the diets for 15 minutes.

Extrusion was performed using an Ex-Micro® model extruder with a capacity of 10 kg h-1 in a 1.0 mm diameter die and dried in a forced-air oven at 120 °C for 2 hours. Subsequently, the diets were stored in a cold chamber at 4 °C for better preservation until use.

The experimental trial was conducted under laboratory conditions using 120 juvenile Nile tilapia, with an average initial weight of 35.80 ± 1.58 g. After the acclimation period, the fish were randomly distributed into six 500 liter aquariums in a completely randomized design, consisting of three treatments and three replicates, totaling 20 animals per experimental unit, for 60 days.

The experimental structures consisted of a continuous flow water recirculation system, a mechanical filter, aeration by means of an air blower, and heating by an electric thermostat.

The fish were fed four times a day (7:40 AM, 11:40 AM, 2:40 PM, and 5:00 PM) until they appeared satiated. At the end of each day, the aquariums were siphoned and the removed water was replaced.

During the experimental period, the physical and chemical variables of the water were monitored using a portable multiparameter probe (model YSI Pro Plus) and water quality kits for measuring temperature (27.22 ± 0.59 °C), dissolved oxygen (3.6 ± 0.6 mg/L), pH (7.17 ± 0.13), total ammonia (0.35 ± 0.16 mg/L) and nitrite (0.6 ± 0.22 mg/L). All physical and chemical variables of the water remained within the values recommended for freshwater fish production (Arana, 2004).

Following the experimental trial, all fish were fasted for 24 hours and anesthetized in a 75 mg L^{-1} eugenol solution (Deriggi *et al.*, 2006). Productive performance was assessed according to NRC (2011), considering the following equations:

$$\text{AFW (g)} = \frac{\sum Final\ weight}{Number\ of\ fish} \qquad (1)$$

WG (g) = Average final weight – Average starting weight

$$\text{DWG (g)} = \frac{Final\ weight\ gain}{Number\ of\ experimental\ days} \qquad (2)$$

$$\text{FC (g)} = \frac{Feed\ consumption}{Weight\ gain} \qquad (3)$$

$$\text{PER (\%)} = \frac{Weight\ gain}{Crude\ protein\ consumed} \text{ x } 100 \qquad (4)$$

$$\text{SGR (\% dia}^{-1}\text{)} = \frac{[(\ln Average\ final\ weight) - (\ln Average\ starting\ weight)]}{Trial\ period} \qquad (5)$$

$$\text{SU (\%)} = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \text{ x } 100 \qquad (6)$$

$$\text{FY (\%)} = \frac{Fillet\ weight}{Total\ body\ weight} \text{ x } 100 \qquad (7)$$

onde:

AFW = Average final weight;
WG = Weight gain;
DWG = Daily weight gain;
FC = Feed conversion;
PER = Protein efficiency ratio;
SGR = Specific growth rate;
SU = Survival;
FY = Fillet yield.

Three animals from each experimental unit were euthanized in a 200 mg/L eugenol solution (Okamura *et al.*, 2010) for collection of coelomic cavity material, where body indices were determined:

$$\text{HI (\%)} = \frac{Liver\ weight}{Final\ weight} \text{ x } 100; \qquad (8)$$

$$\text{VFI (\%)} = \frac{\text{Visceral fat weight}}{Final\ weight} \text{ x } 100. \qquad (9)$$

onde:

HI = Hepatosomatic indices;
VFI = Visceral fat indices.

Three samples of the diets from each experimental unit (Table 3) were sent for proximate analysis to the Commercial Laboratory (CBO) Laboratoriais Ltda., Valinhos-SP, Brazil.

Three whole, gutted fish from each experimental unit (Figure 1) were euthanized for the performance of centesimal analyses of moisture, crude protein, dry matter, lipids and mineral matter according to the methodology described by the Adolfo Lutz Institute (2008) and Silva (1990).

Table 3. Proximate composition of experimental diets, supplemented with GuanAMINO® and fed to juvenile Nile tilapia during 60 experimental days.

Parameters	**Treatment**		
	C	**0.05%**	**0.15%**
[1]EE (%)	6.70	8.50	8.60
[2]CF (%)	2.30	2.40	1.80
[3]ASH (%)	10.20	9.60	10.00
[4]STA (%)	28.70	30.80	27.40
[5]ADF (%)	5.10	5.10	3.40
[6]NDF (%)	21.00	19.90	17.40
[7]SUG (%)	2.30	2.00	2.10
[8]P (%)	12.437	13.344	11.421
[9]GE (Kcal)	0.00	0.00	4.493

[1]Ether extract; [2]Crude fiber; [3]Ashes; [4]Starch; [5]Acid detergent fiber; [6]Neutral detergent fiber; [7]Sugars; [8]Phosphorus; [9]Energy.

Three fish from each experimental unit were fed one hour before being subjected to anesthesia and euthanasia, for the immediate removal of a portion of the liver and the anterior part of the intestine. Subsequently, the samples were stored in microtubes, which were immediately frozen individually in an ultra-freezer at -80 °C and maintained under these conditions until the time of analysis.

Liver and intestine samples were homogenized in 50 mM TRIS-HCl buffer, pH 7.4, using a homogenizer and centrifuged at 12,000 x g for 10 min (4 °C). The supernatant was then used to determine the antioxidant status of the liver and the activity of digestive enzymes in the intestine. Protein quantification was performed using the Bradford method (1976), using bovine serum albumin as a standard. All analyses were performed in triplicate.

Trypsin activity was determined using the Hummel method (1959) adapted for microplates. The substrate α-ρ-toluenesulfonyl-L-arginine methyl ester hydrochloride (TAME) was used to determine the activity of this enzyme. The extracts were incubated for two minutes in flat-bottomed microplates with readings every 10 seconds, using Tris/CaCl2 buffer, pH 8.1. The readings were performed using a microplate reader set to 256 nm and 30 °C. One trypsin unit was used to determine the amount of enzyme needed to form 1 μmol of Na-p-Tosyl-L-Arginine/minute. The molar extinction coefficient used to calculate the enzyme was 540 M, and the result is expressed in μmol/min/mg of protein.

The determination of disaccharidase/glycosidase activity was performed in a 96-well flat-bottom microplate with readings at 505 nm. Samples were incubated in a water bath with temperature control at 25 °C. For the initial assay, 50 μL of the supernatant from the intestinal homogenates was incubated with 20 μL of 86.7 mM maleate buffer (pH 6.0) for 5 minutes. Subsequently, 30 μL of substrate (sucrose) at a concentration of 0.112 μM was added, and the reaction medium was incubated for 5 minutes at 25 °C. To identify enzyme activity, glucose levels were measured at the end of the incubation period using a commercial colorimetric kit. The results were expressed as U/mg of protein, where 1 U = amount of enzyme that forms 1 μmol of glucose per minute per mg of protein (Dahlqvist, 1984).

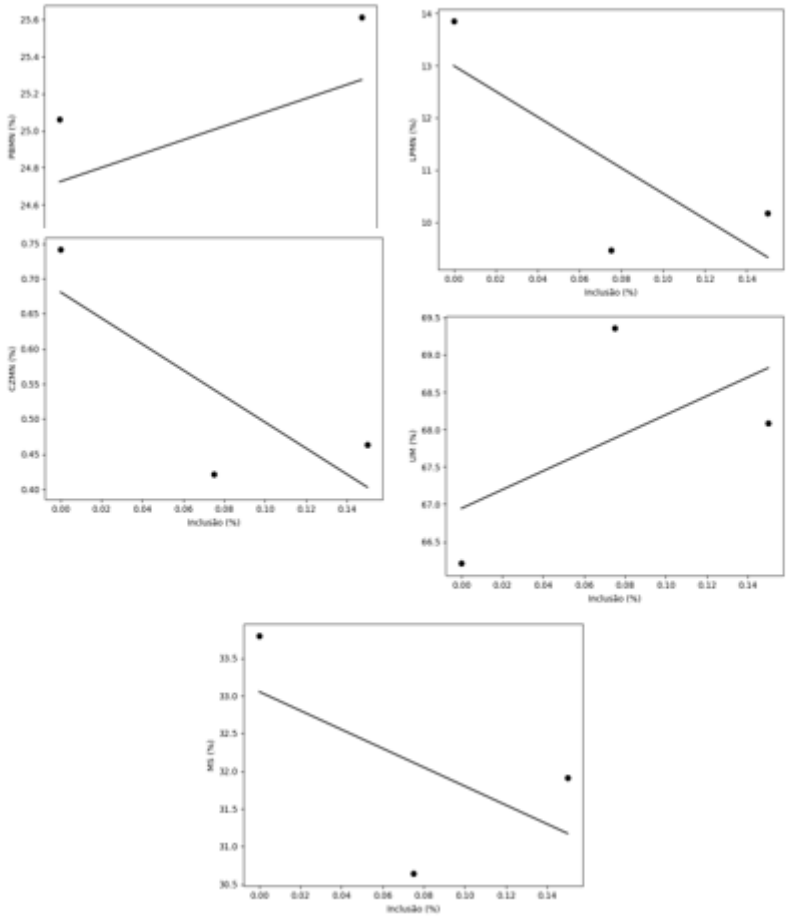
The antioxidant status in fish liver was assessed by catalase activity (Aebi, 1984) and by GSH levels (Aebi, 1984; Federici *et al.*, 2007) and lipid peroxidation (TBARS) (Federici *et al.*, 2007).

At the end of the study, the data were subjected to analysis of variance and, when a significant effect of the treatments was observed ($p < 0.05$), polynomial regression models were fitted as a function of the treatment levels. Linear and quadratic models were tested, selecting the one with the highest coefficient of determination (R^2) and statistical significance. The statistical software R was used.

4 RESULTS AND DISCUSSION

The percentages of crude protein and moisture increased as a function of the increasing inclusion levels of GAA evaluated (Figure 1). The quadratic regression with R^2 of 0.86 and 0.10 respectively, indicated a positive slope of the line, demonstrating that fish fed diets containing 0.15% GAA had higher levels of crude protein and moisture compared to the other treatments evaluated.

Figure 1. Proximate composition of Nile tilapia juveniles, based on natural matter, subjected to experimentais diets supplemented with GuanAMINO® for 60 days.



The percentages of lipids, ash, and dry matter decreased as a function of increasing GAA supplementation. Quadratic regression with R^2 values of 0.53, 0.40, and 0.10, respectively, indicated a negative slope of the line, demonstrating that fish fed diets containing 0.15% GAA exhibited the lowest levels of lipids, ash, and dry matter compared to the other treatments evaluated.

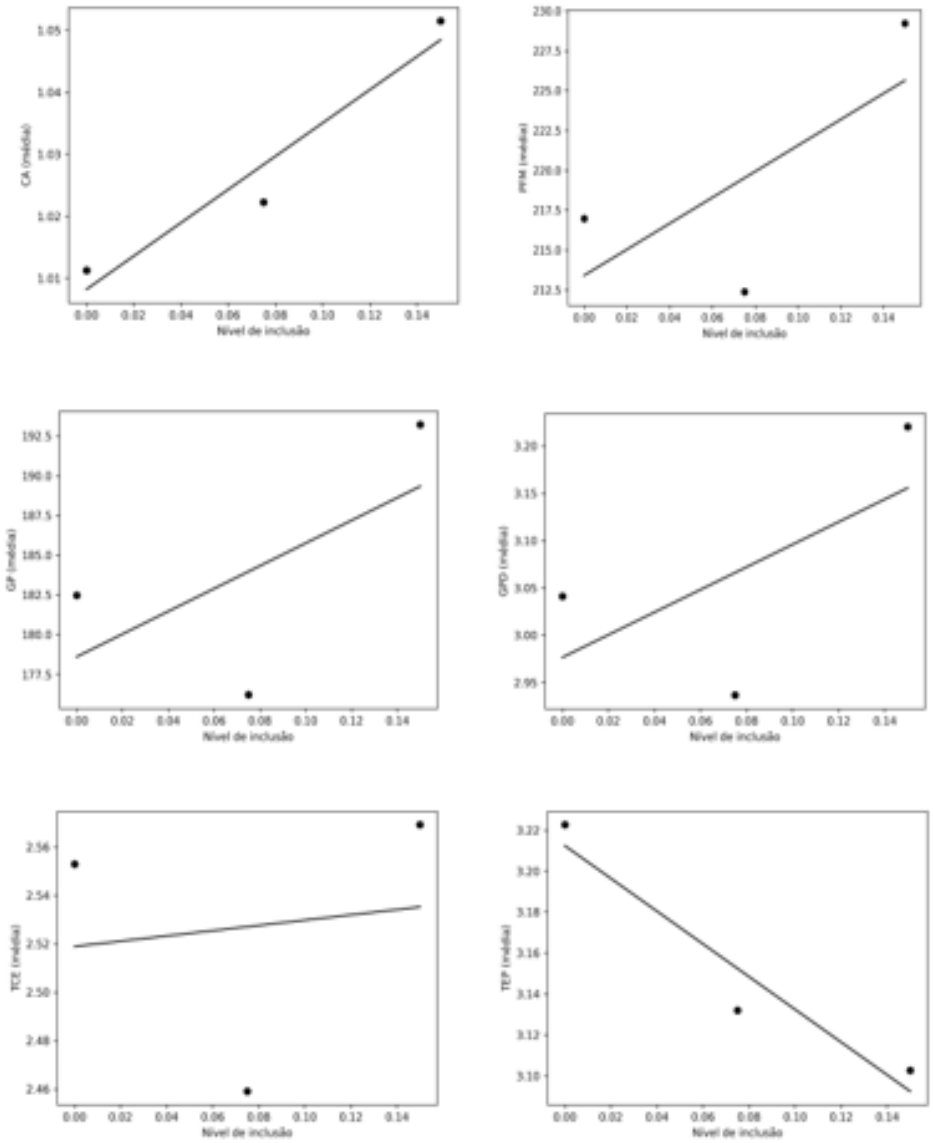
In this study, fish fed with 0.15% GAA showed a higher percentage of protein and low lipid reserves, indicating better utilization of proteins and lipids in energy and protein metabolism.

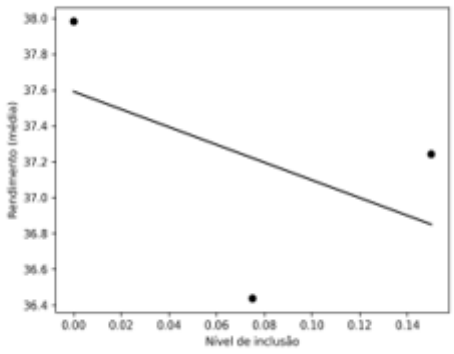
They also demonstrate an increased capacity for water retention in the tissue, resulting from greater protein deposition and greater integrity between muscle fibers, factors that favor energy metabolism in fish. However, they also indicate alterations in

mineral deposition, reducing the mineral fraction in the fillet and increasing the percentages of humidity in the fish.

In this study, the AFW, WG, DWG, and SGR variables increased as a function of increasing levels of GAA inclusion evaluated (Figure 2). Linear and quadratic regression with R^2 of 0.91, 0.90, 0.88, and 0.85, respectively, indicated a positive slope of the line, demonstrating that fish fed diets containing 0.15% GAA showed the highest AFW, WG, and DWG results compared to the other treatments evaluated.

Figure 2. Productive performance of Nile tilapia juveniles subjected to experimental diets supplemented with GuanAMINO® for 60 days.





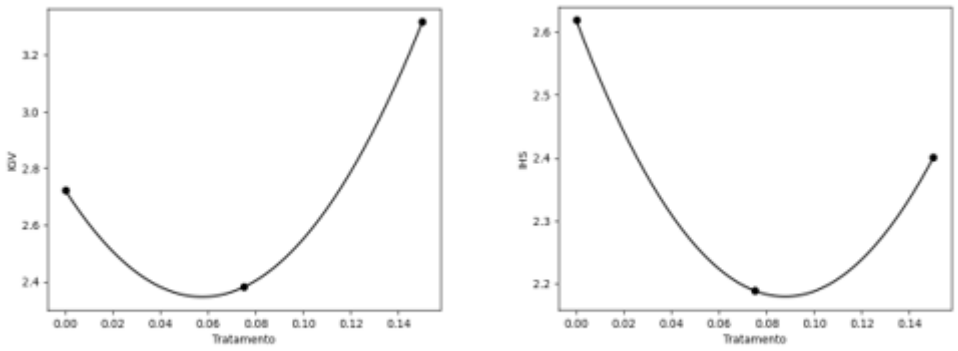
FC increased as a function of increasing levels of GAA inclusion evaluated. Linear regression indicated a positive slope of the line with an R^2 of 0.72, demonstrating that fish fed diets containing 0.15% GAA showed a decrease in FC compared to the other treatments evaluated.

The PER decreased as a function of increasing levels of GAA inclusion evaluated. Quadratic regression indicated a negative slope of the line with an R^2 of 0.84, demonstrating that fish fed diets containing 0.15% GAA showed the lowest PER results compared to the other treatments evaluated.

Thus, fish fed diets containing 0.05% GAA showed the best FC and PER results compared to the other treatments. Under these conditions, there was also an increase in AFW, WG, DWG, and SGR values, and lower results for PER and FY, indicating a reduction in the efficiency of utilization of available nutrients, in addition to an increase in FC. This demonstrates that the fish required a greater quantity of nutrients to increase WG, resulting in an increase in VFI, justifying the higher results found in the AFW, WG, DWG, and SGR variables.

In this study, the HI and VFI of the fish increased as a function of increasing levels of GAA inclusion evaluated (Figure 3). Quadratic regression indicated an increasing curve, with R^2 of 0.63 and 0.90, demonstrating that fish fed diets containing 0.15% GAA showed the highest VFI results compared to the other treatments evaluated. While fish fed control diets, without GAA supplementation, showed the highest HI results. Conversely, fish supplemented with diets containing 0.05% GAA showed the best HI and VFI results compared to the other treatments.

Figure 3. Body indices of juvenile Nile tilapia subjected to experimental diets supplemented with GuanAMINO® for 60 days.



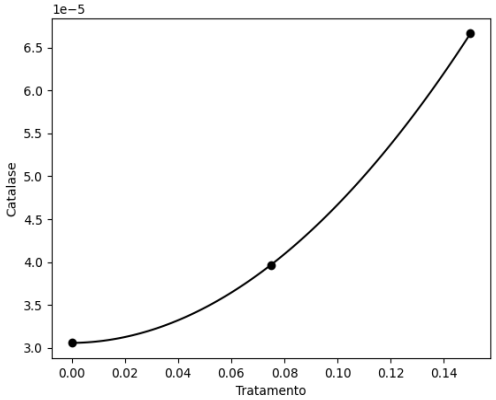
Furthermore, fish fed a supplement of 0.15% GAA showed greater metabolic activity in the intestine and liver, due to the greater supply of nutrients and the greater energy reserve in the liver, as well as greater cellular defense in the liver, justifying the values observed for VFI and catalase.

On the other hand, fish fed with 0.05% GAA showed the best results for FC and PER, in addition to lower HI and VFI values, and a reduction in intestinal enzymatic activity and liver oxidative status. These results suggest lower feed intake with better nutrient utilization, reducing energy reserves and favoring growth and fillet yield in fish.

In this study, the hepatic oxidative status of catalase showed an increase as a function of the increasing levels of GAA inclusion evaluated (Figure 4). Quadratic regression indicated an increasing curve with an R^2 of 0.45, demonstrating that fish fed diets containing 0.15% GAA showed greater oxidative activity compared to the other treatments evaluated.

Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) present in the liver did not show a pattern of behavior with GAA supplementation in the fish diet.

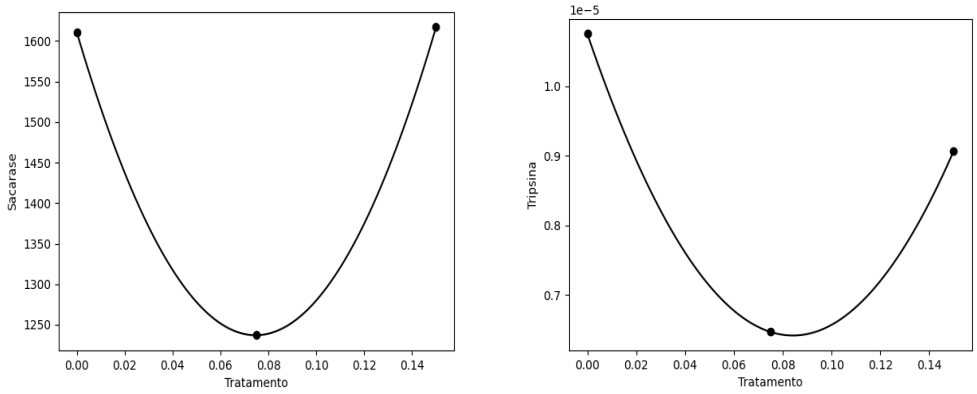
Figure 4. Antioxidant status of the liver of juvenile Nile tilapia subjected to experimental diets supplemented with GuanAMINO® during 60 experimental days.



In this study, trypsin and sucrase showed quadratic regressions with R^2 of 0.65 and 0.55, respectively, indicating a parabola with a minimum point in the treatment with 0.05% GAA, demonstrating an increase in enzymatic activity from this supplementation level compared to the other treatments evaluated.

However, it was observed that fish fed without GAA supplementation and those fed with 0.15% supplementation showed higher sucrase activity. The highest trypsin activity was observed in fish without GAA supplementation.

Figure 5. Activity of digestive enzymes in the intestines of juvenile Nile tilapia subjected to experimental diets supplemented with GuanAMINO® during 60 experimental days.



Therefore, supplementation with 0.05% GAA is recommended for juvenile Nile tilapia, as it provides a balance between crude protein and lipid levels, favoring the energy and protein metabolism of the fish. In addition to promoting good results in productive

performance, fish growth, and fillet yield, it also provides lower body mass indexes in fish, favoring better metabolic use of nutrients and less fat accumulation in the liver and energy reserves, as a result of energy metabolism.

Similar results were found in studies conducted by El-Sayed and colleagues (2019) who evaluated the effect of GAA supplementation in juvenile Nile tilapia and observed that supplementation with 0.06% GAA improved the productive performance and growth of the fish.

Lin *et al*. (2025) found superior results to those observed in this study when evaluating the effect of GAA supplementation in juvenile *Litopenaeus vannamei*. The authors observed that supplementation with 0.10 and 0.13% GAA improved weight gain, specific growth rate, and feed conversion.

Other studies with grass carp (*Ctenopharyngodon idella*) (Yang, *et al.*, 2020) and Nile tilapia have also shown that supplementation with GAA improves the productive performance and growth of the fish.

5 CONCLUSION

Supplementation with 0.15% GuanAMINO® impaired the performance and growth of juvenile Nile tilapia.

Based on the results listed in this study, supplementation with 0.05% GuanAMINO® is recommended for diets of juvenile Nile tilapia.

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