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ANIMAL SCIENCE

# Influence of taurine on the zootechnical performance and health parameters of juvenile Nile tilapia in a recirculating aquaculture system

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**Abstract:** Taurine is considered a conditionally essential amino acid for fish, so its supplementation may improve feed conversion. This study evaluated the supplementation of taurine on growth performance, hematological and immunological parameters, production costs, and survival of Nile tilapia (*Oreochromis niloticus*) juveniles raised in a recirculating aquaculture system (RAS). A control diet was formulated with 360 g $kg^{-1}$ of crude protein without fish meal and without taurine supplementation (Control). From the control diet, another diet supplemented with 9.7 g of taurine per kg of feed (Taurine) was produced. Fish fed diet supplemented with taurine had lower daily average weight gain and final average weight compared to the control diet ($p < 0.05$). It was observed that taurine had no influence on condition factor, survival, or hemato-immunological parameters of Nile tilapia juveniles, but there was a higher mean corpuscular volume and greater nitrogen retention in fish from the control group ($p < 0.05$). It is concluded that Nile tilapia juveniles do not benefit from taurine supplementation in RAS, even when fed diet containing plant-based protein sources.

**Key words:** amino acid, hematology, nutrition, *Oreochromis niloticus*, sustainability.

## INTRODUCTION

The rise of aquaculture production and instability in the supply of fishmeal, market has been seeking alternative sources to reduce dependence on this ingredient, which is the main animal protein source used in commercial tilapia diets (FAO 2022). Plant-based protein sources, especially oilseed plants such as soybean meal are one of the most used sources to replace marine protein, due to their acceptable protein level, adequate amino acid content, economic opportunities, consistent quality, and because they are considered a source of renewable ingredient (De Souza & De Oliveira 2018, Martinelli 2016, Naylor et al. 1998, Tacon et al. 2011).

Despite plant-derived protein sources showing some satisfactory results, especially with omnivorous species, they are limited in several amino acids, including taurine and its precursors, which may be necessary for optimal performance and metabolism in aquatic animals (De Souza & De Oliveira 2018, El-Sayed 2006, Kuzmina et al. 2010, Martinelli 2016, Tacon et al. 2011).

Among the numerous physiological functions in which it is involved, taurine is a very important compound in lipid metabolism, as it acts as the only amino acid conjugated to bile salts in teleost fish, forming acids (taurocholic and taurochenodeoxycholic) that act in the solubilization or emulsification of fats, making

its more accessible for digestion (Chatzifotis et al. 2008, Huxtable 1992).

Taurine is characterized as a non-essential amino acid for some species of fish, such as Nile tilapia (Gonçalves et al. 2011), its effects on fish physiology, metabolism, and nutrition have been increasingly investigated in different species of freshwater and marine fish. Recent studies have shown that the presence of taurine in the diet is essential for the proper development of aquatic species, however, its synthesis differs widely depending on the species, size, feeding habits, and activity of the enzyme cysteine sulfonate decarboxylase (Al-Feky et al. 2016, El-Sayed 2014).

In studies with Nile tilapia larvae, supplementation with taurine resulted in better growth levels and feed efficiency, suggesting 9.7 g $kg^{-1}$ of dietary taurine (Al-Feky et al. 2016). Other studies have shown taurine as a feeding stimulant for species such as European seabass (*Dicentrarchus labrax*) and giant tiger prawn (*Penaeus monodon*), making its supplementation in diets recommendable whenever maximum performance in aquaculture is sought (Carr 1982, Coman et al. 1996, Martinez et al. 2004).

Therefore, the aim of this study was to evaluate the growth performance, hematological and immunological parameters, and survival after experimental infection against *Aeromonas hydrophila* in Nile tilapia juveniles (*Oreochromis niloticus*) fed with a taurine-supplemented diet.

## MATERIALS AND METHODS

The experiment was carried out at Laboratório de Aquicultura of Instituto Federal Catarinense – Campus Araquari, and all procedures carried out in this study were approved by the Ethics Committee on the Use of Animals under protocol number 263/2018.

### Experimental diets

The diets used in this study were produced by NUTRICOL® in São Ludgero, Santa Catarina, Brazil. The dry ingredients, including corn, soybeans, and wheat bran, were ground to a particle size of less than 0.42 mm before being mixed with the other macro and micro ingredients. The mixture was then homogenized in a horizontal paddle mixer for 4 minutes and ground again to a size of 800 µm. It was then extruded at 105 °C using an extruder (FERRAZ®, Ribeirão Preto, SP, Brazil) with a capacity of 3000 kg $h^{-1}$, producing 3 mm diameter extrudates, according to the methodology used by Stockhausen et al. (2022).

The present experiment involved two treatments (Table I): a control diet, formulated with practical ingredients and devoid of fish meal (Stockhausen et al. 2022), and a taurine-supplemented diet, which contained the same practical ingredients and was supplemented with taurine at a level of 9.7 g $kg^{-1}$ of feed (Al-Feky et al. 2016). Both diets were isoproteic and isoenergetic and formulated to meet the nutritional requirements of tilapia, as outlined by the NRC (2011). Samples of the diets were analyzed for their aminogram using high-performance liquid chromatography (HPLC) and proximate composition using the AOAC (2005) methodology (Table II) at CBO ANÁLISES LABORATORIAIS.

### Experimental design

The study used of 200 Nile tilapia juvenile (*O. Niloticus*) with an average weight of 13.3 g. The fishes were placed in eight polyethylene tanks (800 L) with 25 fishes per tank. The tanks were equipped with a water recirculation system, which renewed 150% of the water per day with a biological filter. The experimental units were randomly divided into two groups, with four replicates each. The first group served as a control and was fed a commercial diet made

**Table I. Composition of experimental diets for Oreochromis niloticus with and without addition of Taurine.**

| Ingredients (%) | Diets | |
|---|---|---|
| | Control | Taurine |
| Soybean meal | 46.50 | 46.60 |
| Grain corn | 12.00 | 12.00 |
| Ground beans | 12.00 | 12.00 |
| Wheat bran | 4.12 | 4.00 |
| Bovine meal and bone meal | 13.50 | 13.00 |
| Blood meal | 6.46 | 6.40 |
| Fish oil | 0.86 | 0.86 |
| Soy oil | 1.13 | 1.03 |
| Common salt (NaCl) | 0.30 | 0.30 |
| Calcitic limestone | 2.21 | 2.21 |
| Mineral vitamin supplement[1] | 0.40 | 0.40 |
| DL-Methionine | 0.09 | 0.09 |
| Essential (functional oil)[2] | 0.10 | 0.10 |
| Antifungal[3] | 0.10 | 0.10 |
| Adsorbent[4] | 0.10 | 0.10 |
| Antioxidant[5] | 0.03 | 0.03 |
| Taurine | 0.00 | 0.97 |
| Kaolin | 0.97 | 0.03 |
| Cost (BRL/kg)[6] | 1.17 | 1.35 |

**[1]Mineral vitamin supplement (per Kg of commercial product) = Vitamin A (min.) 800,000 IU; Vitamin D3 (min.) 410,000 IU; Vitamin E (min.) 15,000 IU; Vitamin K3 (min.) 505 mg; Vitamin B1 (min.) 1395.9 mg; Vitamin B2 (min.) 2,000 mg; Vitamin B6 (min.) 1,862 mg; Vitamin B12 (min.) 2,500 mg; Vitamin C (min.) 125g; Niacin (min.) 3,781 mg; Pantothenic Acid (min.) 4,018 mg; Folic Acid (min.) 198 mg; Biotin (min.) 100 mg; Choline (min.) 86.68 g; Copper (min.) 750 mg; Iron (min.) 8,310 mg; Manganese (min.) 1,320 mg; Cobalt (min.) 24 mg; Iodine (min) 264.8 mg; Zinc (min.) 15.05 g; Selenium (min.) 47.55 mg; Inositol (min.) 25 g. [2]Castor Oil and Canola Oil. [3]Propionic Acid. [4]Bentonite, Sepiolite, Calcium Propionate, Sodium Chloride and other ingredients. [5]B.H.T. (Butylhydroxytoluene), Propyl Gallate. [6]Cost considering ingredient prices in the year 2022. (Adapted by Stockhausen et al. 2022).**

with practical ingredients. The second group was fed the same practical diet supplemented with taurine. The experiment lasted for eight weeks.

## Physicochemical parameters of water quality and food management

The animals were fed three times per a day (9:00 am; 11:00 am and 3:30 pm), with 3 to 6% of their total biomass. Whenever necessary, the experimental units were cleaned to remove excess organic matter. Throughout the experiment, the water's dissolved oxygen and temperature were checked twice a day, and toxic levels of ammonia, pH, nitrite, nitrate, and alkalinity were measured weekly using an Alfakit photocolorimeter.

The water quality parameters were monitored continuously during the experiment, and the following parameters were recorded: dissolved oxygen levels 3.88 ± 0.81 mg $L^{-1}$ and a temperature of 26.54 ± 1.56 °C (measured using YSI PRO20 Oximeter); ammonia levels 0.08 ± 0.08 $NH_3$ mg $L^{-1}$; nitrite levels 1.76 ± 2.68 mg $L^{-1}$; nitrate levels 1.71 ± 2.35 mg $L^{-1}$; alkalinity levels 97.84 ± 14.31 mg $CaC0_2$ $L^{-1}$; and a pH of 6.88 ± 0.08.

## Hematological and immunological analyzes

For the hematological analyses, five fish per tank (20 per treatment) were anesthetized with Eugenol (50 mg $L^{-1}$) and aliquots of blood were taken from the caudal vessel, with EDTA anticoagulant, for the performance of blood extensions in duplicate, and determination of hematocrit (Goldenfarb et al. 1971), plasma glucose (Free® G-TECH), total erythrocyte count, hemoglobin concentration, and calculation of hematimetric indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Extensions were stained with Giemsa/MayGrünwald stain (Rosenfeld 1947) for total and differential WBC count.

For immunological analysis, 0.5 mL of blood from five animals per experimental unit was collected by puncturing the caudal vessel,

without anticoagulant to obtain blood serum. After coagulation, the blood was centrifuged at 1400 g for 10 min to separate the serum, which was collected and stored at -20 °C for subsequent immunological analysis. The concentration of total serum protein was measured with the Total Protein Kit (Biochemical Reagent, Total Proteins, LabTest, Brazil), using bovine albumin to prepare the standard curve. Total immunoglobulin concentration was measured according to the method described by Amar et al. (2000), where 100 µL of blood serum was mixed with 100 µL of 12% polyethylene glycol (PEG) solution (Sigma-Aldrich), for subsequent incubation at room temperature for two hours, in order to precipitate the immunoglobulin molecules. The precipitate was removed by centrifugation at 5000g at 6°C for 10 min. After removing the supernatant, the amount of total protein was measured using the Total Protein kit (Lab Test®). The total immunoglobulin concentration is expressed in mg $mL^{-1}$, calculated by the formula:

Total immunoglobulin = (total protein treated with PEG - total plasma protein) / volume (mL)

**Table II. Aminogram and centesimal composition of experimental diets for *Oreochromis niloticus* with and without addition of Taurine.**

| Nutrients (%) | Diets | |
|---|---|---|
| | Control | Taurine |
| Moisture | 9.72 | 8.56 |
| Crude protein | 36.36 | 36.51 |
| Ethereal extract | 5.66 | 6.69 |
| Fibrous matter | 3.25 | 3.91 |
| Mineral matter | 11.48 | 11.19 |
| Calcium | 2.69 | 2.72 |
| Phosphor | 1.27 | 1.25 |
| Arginine | 2.36 | 2.42 |
| Lysine | 2.24 | 1.82 |
| Methionine | 0.50 | 0.47 |
| Cystine | 0.71 | 0.50 |
| Threonine | 1.41 | 1.49 |
| Tryptophan | 0.35 | 0.30 |
| Leucine | 2.93 | 3.02 |
| Isoleucine | 1.32 | 1.32 |
| Valine | 1.89 | 2.16 |
| Histidine | 1.04 | 1.16 |
| Phenylamine | 1.79 | 1.82 |
| Serine | 1.76 | 1.84 |
| Glycine | 2.47 | 2.24 |
| Taurine | 0.04 | 1.04 |
| Alanine | 2.21 | 2.51 |
| Proline | 2.05 | 2.07 |
| Tyrosine | 1.14 | 1.13 |
| Aspartic acid | 3.11 | 3.35 |
| Glutamic acid | 5.47 | 5.50 |

## Body indices and condition factor (k)

To determine the hepatosomatic (IHS) and viscerosomatic (IVS) indices, three fish were taken from the hematological collection sample and their liver + bile, viscera, and eviscerated parts were removed and weighed. The weight/length ratio and the allometric condition factor were calculated using Santos (1978) formulas, as follows:

$$HSI = 100 * \frac{LW}{CW}$$

HSI: hepatosomatic index (%);
LW: liver weight;
CW: carcass weight.

$$VSI = 100 * \frac{GW}{CW}$$

VSI: vicerosomatic index (%);
GW: guts weight;
CW: carcass weight.

## Nitrogen and potassium retention

Twelve fish samples (four before the rearing period, four after the rearing period from the

control treatment and four from fish fed with taurine) were euthanized, frozen and lyophilized to be sent to CBO ANÁLISES LABORATORIAIS to evaluate the concentration of nitrogen and potassium according to AOAC (2005) methodology.

## Experimental challenge

A bacterial inoculum isolated from a strain was plated on PCA plates (plate count agar). After growth at 30 °C for 24 h, a bacterial colony was inoculated into BHI broth and the suspension was incubated at 30 °C for 24 h. After incubation, the culture was serially diluted (1:10) to $1x10^8$ colony forming units (CFU) $mL^{-1}$ and seeded in PCA to determine the bacterial concentration of the initial inoculum. To construct a growth curve, bacterial inoculum was serially diluted (1:2) in triplicate in 96-well microtiter plates for 12 times, and the absorbance of each well was measured at 630 nm using a microplate reader. For the infection experiment, a pure bacterial culture grown in BHI broth at 30 °C for 24 h, in static incubation, was centrifuged for 30 min at 1800 g. The supernatant was discarded, and the pellet was resuspended in a sterile 0.65% saline solution to maintain the bacteria concentration at $2.1x10^8$ CFU $mL^{-1}$. The bacterial suspension was diluted to the desired concentration for the experiment.

For the experimental challenge, five fish per experimental unit (20 per treatment) were inoculated intraperitoneally with 100 µL of Aeromonas *hydrophila* (ATCC 7966) at a concentration of $2.5 \times 10^6$ CFU $mL^{-1}$ (Stockhausen et al. 2022). After 96 hours, fish survival was evaluated.

## Growth performance

At the end of the experiment, all fish were weighed to measure final average weight (g), survival (S), apparent feed conversion (AFC), specific growth rate (SGR), average productivity, (AP) weekly weight gain (WWG), protein efficacy rate (PER) and feed cost to produce 1,000 juveniles ($C_{1000}$). The following equations were used to calculate the performance parameters:

$$S = 100 * \frac{FN}{IN}$$

S: survival (%);
FN: final number of animals;
IN: initial number of animals.

$$AFC = \frac{FC}{(FB - IB)}$$

AFC: apparent feed conversion;
AF: total amount of feed provide (g);
FB: final biomass (g);
IB: initial biomass (g).

$$WWG = \frac{Fw - Iw}{TW}$$

WWG: weekly weight gain (g $day^{-1}$):
FW: final weight (g);
IW: initial weight (g);
TW: experiment time in weeks.

$$SGR = 100 * \frac{IAW - FAW}{TD}$$

SGR: specific growth rate (% $day^{-1}$);
IAW: initial average weight (g);
FAW: final average weight (g);
TD: experiment time in days.

$$AP = \frac{FB}{V}$$

AP: average productivity (kg $m^{-3}$);
FB: final biomass (kg);
V: volume ($m^3$).

$$PER = 100 * \frac{WG}{FC * DCP}$$

PER: protein efficiency rate;
WG: weight gain (g);
FC: feed consumption;
DCP: dietary crude protein (%).

$$C_{1000} = FC * AFC * 1000$$

$C_{1000}$: feed cost to produce 1,000 juveniles;

FC: feed cost;
AFC: average food consumption.

### Statical analysis

Data were submitted to the Kolmogorov-Smirnov test to verify whether the data distribution was within the normality curve and to the Levene test to verify homoscedasticity. The, the data were submitted to the t test (software STATISTICA 10.0). All analyzes with a significance level of 5% (Zar 2010).

## RESULTS

### Growth performance, body indices and N and P retention

Fish fed with the control diet had higher final average weight, average daily gain, apparent feed efficiency, protein efficiency rate, specific growth rate, and cost per kilogram of fish compared to fish fed with taurine-supplemented diet ($p < 0.05$). Also, the productivity was higher in the tanks of the control treatment ($p < 0.05$). Fish survival was not influenced by diet ($p > 0.05$) (Table III).

Regarding body indices, fish fed with taurine-supplemented diet did not show significant changes ($p > 0.05$) in the evaluated parameters compared to the control treatment (Table III).

Nitrogen (N) retention was higher in fish fed the control diet ($p < 0.05$), while phosphorus (P) retention did not differ between treatments ($p > 0.05$) (Figure 1).

### Animal health (hematology, immunology and experimental infection against *Aeromonas hydrophila*)

Among the hematological and immunological parameters evaluated, mean corpuscular volume (MCV) was lower in the treatment with taurine-supplemented diet ($p < 0.05$). The others parameters did not show significant differences between treatments ($p < 0.05$) (Table IV).

Survival after bacterial challenge also did not differ significantly between fish fed with taurine or not ($p > 0.05$) (Figure 2).

**Table III. Growth performance and body indices (mean ± standard deviation) in Nile tilapia (*O. niloticus*) reared in a Recirculation Aquaculture System (RAS) fed a diet supplemented or not with taurine.**

| Variables | Diets | |
|---|---|---|
| | Control | Taurine |
| Final average weight (g) | 77.75 ± 3.95* | 68.35± 1.54 |
| Average daily gain (g) | 0.90 ± 0.05* | 0.67 ± 0.13 |
| Apparent feed conversion | 0.88 ±0.04* | 0.96 ± 0.05 |
| Protein efficiency rate | 0.41 ±0.02* | 0.38 ± 0.02 |
| Specific growth rate (% $day^{-1}$) | 1.36 ± 0.04* | 1.26 ± 0.02 |
| Survival (%) | 100.00 ± 0.00 | 99.0 ± 2.00 |
| Productivity (kg $m^3$) | 2.01 ± 0.12* | 1.69 ± 0.05 |
| Cost per unit | 67.20 ± 1.71* | 71.36 ± 2.06 |
| Condition factor | 1.03 ± 0.06 | 1.01 ± 0.03 |
| Hepatosomatic index | 3.64 ± 0.22 | 4.01 ± 0.28 |
| Viscerosomatic index | 11.65 ± 1.32 | 11.39 ± 1.41 |

***Indicates a statistical difference in the t-test ($p < 0.05$).**

## DISCUSSION

### Experimental diets and physicochemical parameters of water quality

In this study, the experimental diets had similar centesimal composition and amino acid profiles. Of the evaluated amino acids, only methionine was below the recommended levels of 5.0 (Control) and 4.7 (Taurine) - 5.2 g $kg^{-1}$ (Furuya et al. 2010). However, about methionine + cysteine amount, only the taurine-supplemented diet was below the recommended level (9.2 g $kg^{-1}$), according to Furuya et al. (2010).

Methionine is the first limiting amino acid in diets formulated based on soy or soy derivatives

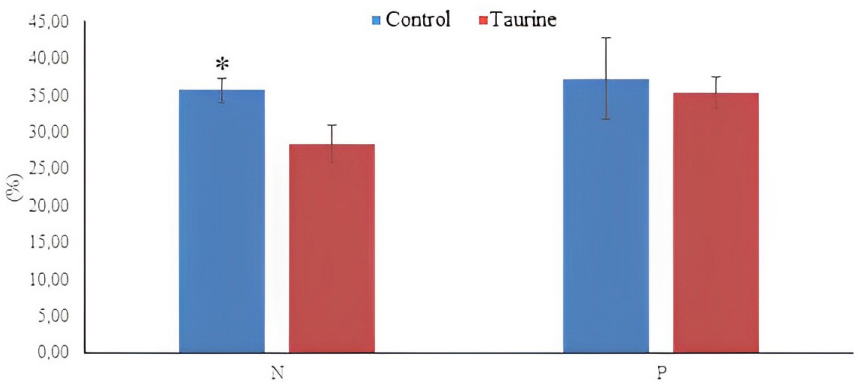


**Figure 1**. **Nitrogen (N) and Phosphorus (P) retention in Nile tilapia juveniles (*O. niloticus*) reared in Recirculation Aquaculture System (RAS) fed a diet supplemented or not with taurine. *Indicates a statistical difference in the t-test ($p < 0.05$).**

(NRC 2011). In diets for tilapia juveniles using levels of methionine supplementation, lower weight gain was observed in fish fed with the lowest level of methionine inclusion (He et al. 2017, Urbich 2020). Other studies have revealed similar data, with a low growth rate and high feed conversion in rainbow trout (*Oncorhynchus mykiss*) (Belghit et al. 2014) and yellowtail (*Seriola dorsalis*) (Garcia-Organista et al. 2019), requiring the addition of this amino acid in crystalline form (Browdy et al. 2012, Michelato et al. 2018, Nunes et al. 2014). Therefore, growth performance is also correlated with methionine level in the diet, which, despite being below the recommended level for the species, good results can be justified by the sum of "methionine + cysteine", because the data obtained in this research are similar to those of fish produced with a commercial diet (Stockhausen et al. 2022)

## Growth performance, body indices and N and P retention

The life stage of the fish is a factor to be considered, as it has been observed in other studies, sometimes the need for taurine occurs in the early stages (post-larvae) (Al-Feky et al. 2014, Kim et al. 2005, Salze et al. 2012). According to Gonçalves et al. (2011), it is difficult to draw distinctions based on feeding habits of the tilapia species already studied, for Nile tilapia taurine proves to be limiting, while for hybrid red tilapia, its requirement can be met by endogenous production.

Taurine supplementation (40.0 g $kg^{-1}$) did not improve the performance of tilapia over eight weeks in an experiment with soy-based diets when dietary methionine levels had not been achieved (Michelato et al. 2018). While in this study, the results showed that taurine supplementation compromised the performance of Nile tilapia juveniles. Considering the increased cost of the diet, the use of taurine was found to be unfeasible as a growth promoter. This result corroborates with Han et al. (2014), who observed impaired growth performance in Red Drum (*Sciaenops ocellatus*) fed a diet supplemented with 1% and 2% taurine. However, even with a smaller effect on final weight gain and average daily gain, taurine did not influence fish survival rate.

Some studies have shown that taurine supplementation, when provided at levels above the optimal for the species, leads to excessive excretion to maintain its optimized metabolic concentration, which requires greater energy expenditure, thereby harming growth and gonadal development (Al-Feky et al. 2014, Yue et al. 2013). Perhaps the dose offered to Nile tilapia juveniles in this study was above the recommended dose for juveniles, as this dose was based on the taurine requirement for the larval phase of tilapia obtained by Al-Feky et

**Table IV. Blood parameters (mean ± standard deviation) of Nile tilapia (*O. niloticus*) reared in Recirculation Aquaculture System (RAS) fed a diet supplemented or not with taurine.**

| Blood parameter | Diets | |
|---|---|---|
| | Control | Taurine |
| Total and differential count | | |
| Thrombocytes (x $10^4$ $\mu L^{-1}$) | 5.74 ± 1.19 | 7.15 ± 1.97 |
| Total leukocytes (x $10^3$ $\mu L^{-1}$) | 75.69 ± 10.40 | 92.56 ± 25.38 |
| Lymphocytes (x $10^3$ $\mu L^{-1}$) | 71.15 ± 9.90 | 86.96 ± 23.90 |
| Basophils (x $10^3$ $\mu L^{-1}$) | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Eosinophils (x $10^3$ $\mu L^{-1}$) | 0.16 ± 0.08 | 0.05 ± 0.07 |
| Monocytes (x $10^3$ $\mu L^{-1}$) | 0.16 ± 0.08 | 3.43 ± 1.60 |
| Neutrophils (x $10^3$ $\mu L^{-1}$) | 1.64 ± 0.57 | 2.11 ± 0.69 |
| Hematimetric parameters | | |
| Red blood cells (x $10^6$ $\mu L^{-1}$) | 2.67 ± 0.38 | 2.98 ± 0.33 |
| Hematocrit (%) | 27.73 ± 1.16 | 26.60 ± 1.50 |
| Hemoglobin concentration (g $dL^{-1}$) | 7.67 ± 0.37 | 7.47 ± 0.26 |
| Mean corpuscular volume ($10^{-5}$ pg) | 10.75 ± 1.41* | 8.54 ± 0.47 |
| Mean corpuscular hemoglobin ($10^{-5}$ pg) | 2.98 ± 0.46 | 2.42 ± 0.15 |
| Mean corpuscular hemoglobin concentration (g $dL^{-1}$) | 2.78 ± 0.07 | 2.83 ± 0.08 |
| Immunological parameters | | |
| Glucose (mg $dL^{-1}$) | 40.90 ± 5.56 | 44.37 ± 8.22 |
| Total protein (mg $L^{-1}$) | 1047.85 ± 3.65 | 1049.91 ± 2.16 |
| Total immunoglobulin (mg $L^{-1}$) | 27.92 ± 4.37 | 29.91 ± 3.18 |

***Indicates a statistical difference in the t-test ($p < 0.05$).**

al. (2016). This fact would justify the low growth performance of fish fed a taurine-supplemented diet.

The same behavior has already been observed in silver catfish (*Rhamdia quelen*) by Martinelli (2016) and Rossato (2015). In the study of Martinelli (2016), silver catfish juveniles supplemented with 2% taurine had a worse feed conversion, negatively affecting productive parameters. Qi et al. (2012) suggest that excessive taurine supplementation may retard growth through reduced fed intake. However, in silver catfish post-larvae fed diets based on soy protein concentrate and supplemented with 0.5% and 1.5% taurine, Rossato (2015) observed a significant improvement in growth. Therefore, in the post-larval phase, the key enzyme in the conversion of cystine to taurine (L-cysteine sulfinate decarboxylase) is insufficiently produced to meet the metabolic demand for taurine, justifying the positive results obtained in the initial phase (Al-Feky et al. 2014, Salze et al. 2012), different from the observed in this study.

The liver is a crucial organ for nutrient metabolism and is considered an excellent indicator of nutritional pathologies in fish. Its main functions include the formation of plasma proteins, deamination of proteins, formation of urea for ammonia removal, amino acid synthesis, and energy storage (Honorato et al. 2013). The lipid storage and utilization cycle are

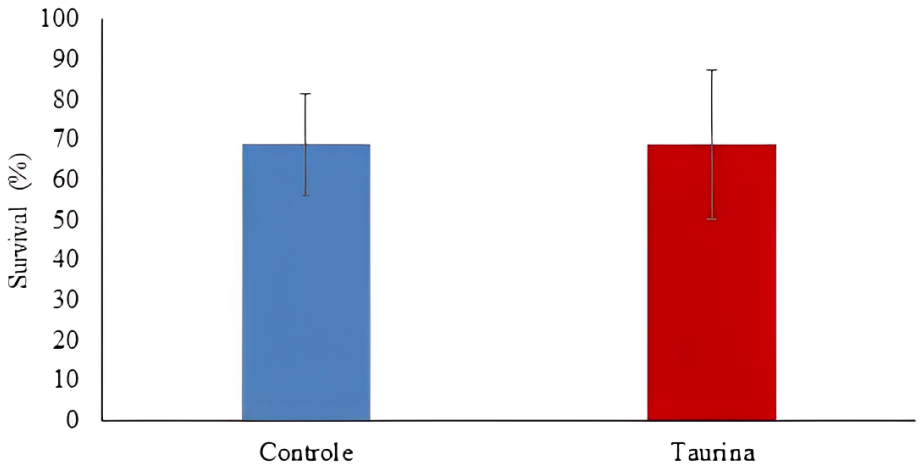


**Figure 2. Survival of Nile tilapia juveniles (*O. niloticus*) fed a diet supplemented or not with taurine 96 h after experimental infection with *Aeromonas hydrophila*.**

usually associated with seasonal changes in food availability or metabolic demand (Soares et al. 2001). Thus, the hepatosomatic index may be related to the mobilization of hepatic energy reserves necessary for vitellogenesis and reproduction, or lipid deposition in the liver in preparation for the winter period (Querol et al. 2002).

Regarding the hepatosomatic index, there were no significant differences between the diets. The increase in either treatment suggests a metabolic overload in the liver, which was not observed between the groups. These data differ from Urbich (2020) in a study with Nile tilapia in the finishing phase in net tanks, which reported that fish fed diets supplemented with methionine (0.4%), taurine (0.5%), and methionine with taurine (0.4% + 0.5%), with low ether extract content (2.8%), had a lower hepatosomatic index. Similarly, in studies with other species, the inclusion of taurine resulted in a lower hepatosomatic index in yellowhead catfish (*Pelteobagrus fulvidraco*) (Li et al. 2016), black carp (*Mylopharyngodon piceus*) (Zhang et al. 2018), and turbot (*Scophthalmus maximus*) (Liu et al. 2017).

Fish with higher energy reserves have a greater adaptation to the cold in order to maintain their fluidity in winter, and the most significant response to this stress is the increase in levels of unsaturated fatty acids in the bloodstream that originate from the liver, as observed by Ribeiro et al. (2012) in carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). Therefore, taurine supplementation may be a study topic to stimulate an increase in energy reserves in tilapia for the winter season. However, in this study, temperatures were maintained at optimal levels for the species, justifying once again the unsatisfactory results of taurine supplementation for juvenile tilapia under this condition.

The ratio weight: body length allows for the calculation of a parameter that determines the degree of well-being of the fish, called the condition factor (K). K is an important indicator of an individual's health status, and its value reflects recent nutritional conditions and/or the use of reserves in cyclic activities, making it possible to relate it to environmental conditions and behavioral aspects of the species (Gomiero et al. 2010). The similarity in K of the fish suggested normality in the data, regardless of supplementation or not.

Excessive concentrations of nutrients such as N and P can lead to eutrophication of the environment, compromising the water quality and support capacity of aquaculture systems

(English et al. 1993, Van Der Ploeg & Boyd 1991). Excessive nitrogenous compounds are toxic to fish and can affect the organoleptic characteristics of the meat. Among toxic compounds, ammonia is the product of protein catabolism in fish, which comprises about 80% of total nitrogen excretion by fish, and its excretion rate is influenced by various factors, such as the quantity and quality of feed (Chakraborty & Chakraborty 1998). Due to the reduced retention of nitrogen in fish, the use of taurine at the evaluated level may present a potential pollutant for open production systems, although no difference was observed in water quality variables. Furthermore, the lower retention of amino acids results in lower protein deposition and increased production and excretion costs of nitrogen, reducing fish growth (Furuya et al. 2001, Bureau & Encarnação 2006), which may be another reason for the low productive performance of tilapia fed taurine-supplemented diets.

### Animal health (hematology, immunology and experimental infection against *Aeromonas hydrophil*a)

Fish are known to be in close relationship with the environment in which they live, so blood reveals internal body condition even before there is visible clinical manifestation (Barbieri & Bondioli 2013, Musa & Omoregie 1999, Okechukwu et al. 2007). The use of hematological and immunological parameters in the evaluation of fish physiology has been increasingly recognized as a tool for assessing animal health status, with the purpose of detecting functional alterations in response to various stress conditions (Satake et al. 2009, Schutt et al. 1997, Yousefi et al. 2022), with quite reliable results (Katalog & Parlak 2004). Additionally, hematological and immunological analyses are currently used to evaluate the effect of feed additives (prebiotics, probiotics, organic acids, among others) on the health of aquatic animals (Dawoo et al. 2020, Hassaan et al. 2018, Jatobá et al. 2011, 2018, Mendoza Rodriguez et al. 2017, Moraes et al. 2018). However, there are no records of hematological analyses in tilapia supplemented with taurine.

Blood parameters are considered indicators of the healthy status and physiological conditions of fish (Kader et al. 2010). In this study, the blood parameters of the fish were within the range considered healthy for the species (Barros et al. 2009, 2015, Weiss & Wardrop 2010) and were not altered by the inclusion of taurine, except for the MCV index in the fish from the control treatment. This data, if associated with low hematocrit, could indicate the occurrence of microcytic hypochromic anemia in the animals. However, since the hematocrit level in this treatment did not show a significant difference, and the total count of erythrocytes was higher, it indicates an intensification of erythropoiesis, with a greater amount of young circulating red blood cells.

These results are consistent with Han et al. (2014) who did not observe hematological alterations in red drum (*Sciaenops ocellatus*) fed a diet supplemented with taurine, however, the authors stated that the supplementation of this amino acid promoted resistance to oxidative stress. As for Nile tilapia, taurine plays an important role during the adaptation of tilapia to high salinity water (Takeuchi et al. 2000).

As well as hematological and immunological analyses, experimental infections are commonly used to evaluate feed additives, as animal growth and health performance have a direct relationship. In this study, after experimental infection with *Aeromonas hydrophila*, the survival rate did not differ between treatments, corroborating with the hemato-immunological data and survival during cultivation, suggesting that taurine supplementation does not influence the animal health against bacterial infections.

To summarize, the inclusion of 9.7 g $kg^{-1}$ of taurine in a plant-based diet compromised the growth performance and increased production costs of Nile tilapia (*O. niloticus*) juveniles. However, it did not affect their health. Additionally, this supplementation resulted in an increased potential for pollution due to reduced nitrogen retention. Therefore, further research is needed to assess the feasibility of supplementing Nile tilapia with different levels of taurine at various life stages and under different environmental conditions, such as the BFT.

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