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Inulin Supplementation in Diets for Tropical Gar (*Atractosteus tropicus*) Larvae: Effects on Growth, Survival, and Digestive and Antioxidant Enzyme Activities

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Abstract: The effect of adding inulin to balanced diets for tropical gar (*Atractosteus tropicus*) larvae on growth, survival, digestive enzyme activity, and antioxidant activity was evaluated. The diets were supplemented with 0.5, 1.0, 1.5, 2.0, and 2.5% inulin in addition to a control diet (0% inulin). A total of 1800 larvae of *A. tropicus* distributed in 18 tanks were used; the larvae were fed five times a day (8:00, 11:00, 13:00, 15:00, and 18:00) with *Artemia* nauplii from the absorption of the yolk (from 3–7 days after hatching, DAH) up to 10 DAH, which was mixed with the experimental feeds from 8–11 DAH (co-feeding) and exclusively with the balanced diets from 12 DAH to 21 DAH. Larvae fed the control diet (0% inulin) had the highest growth in weight and length, followed by fish fed the 2.5 and 2.0% inulin inclusions. However, survival showed that the fish fed with the inclusion of 2.5% inulin had the highest percentage (34.7%) compared to the rest of the treatments. On the other hand, the highest digestive enzymatic activities (acid and alkaline proteases, amylase, and lipase) were recorded in the larvae fed with 2 and 2.5% inulin. Likewise, catalase (CAT) and superoxide dismutase (SOD) activities were higher in larvae fed the control diet with 0% inulin. Supplementation of 2.0% to 2.5% inulin in the diet is recommended for *A. tropicus* larvae as it improves survival and digestive enzyme activity during this early stage of life.

Keywords: inulin; tropical gar; prebiotic

1. Introduction

Aquaculture worldwide represents constant expansion due to its profitability and economic potential [1]. Therefore, the feeding of cultivated species is of great importance since it determines health conditions, their response to diseases, and their survival [2]. In this regard, when the feed is not appropriate and the culture conditions are poor, various infectious diseases can appear in aquatic organisms, which is a severe problem in the aquaculture industry since it increases costs and can cause significant production losses. This is why different kinds of methods have been sought to counteract this damage [3] and prebiotics and probiotics have been implemented as an alternative to improve resistance to diseases, reduce mortality, and improve survival. Prebiotics are functional carbohydrates

that are not digested by the body but are considered beneficial for health due to their biological properties [4–6]. They are considered natural food additives, and their incorporation into the diet does not require indications [7,8]. These additives can potentially increase feed efficiency, health response, and sustainability in aquaculture [9,10]. Additionally, they can be easily obtained since they are found in natural components such as milk, honey, fruits, and vegetables, among others [11,12].

One of the more promising prebiotics for aquaculture is inulin, which is an oligosaccharide formed by fructans linked to linear β (2-1) bonds which are present in a variety of plants and foods, such as garlic, onion, agave, and artichoke, among others [13–17]. This additive has been shown to positively affect growth and/or innate immune response, promote resistance to stress factors and diseases, and increase antioxidant capacity [18,19]. In this aspect, inulin and fructooligosaccharide supplemented in the diets of juveniles of great sturgeon (*Huso huso*), large yellow croaker (*Larimichthys crocea*), red pacú (*Piaractus brachypomus*) and carp (*Cyprinus carpio*) have been evaluated. Doses from 0.4% to 2% improved growth, survival, blood serum enzymes, and gut microbiota [20–23]. On the other hand, in southeastern Mexico, the tropical gar (*Atractosteus tropicus*) is one of the most important native freshwater fish species. This species has economic importance in the region [24] and it has been studied for more than 30 years, which has allowed it to close its farming cycle. Production is currently registered at the regional level. In this way, the study of this species has allowed us to know its biology, reproduction, and genetics [25,26]. Likewise, various studies have been carried out on their nutrition and aspects of digestive physiology [27–30], cannibalism during larviculture [31], and some studies related to the use of pre and probiotics in juveniles of *A. tropicus* [32–34]. However, for the larval period, only mannan oligosaccharides in balanced diets have been reported with promising results [35]. In this study, we evaluated the supplementation of inulin in diets for *A. tropicus* larvae on growth, survival, and digestive and antioxidant enzymatic activities during their culture.

2. Materials and Methods

2.1. Reproduction and Larviculture

The organisms were obtained from a spawning induced in a female *A. tropicus* through an intramuscular injection of luteinizing-releasing hormone (LHRHa 30 μg kg fish^{-1} under the pelvic fin), accompanied by 3 males for the fertilization of the eggs [34]. Once the eleutheroembryos were obtained, they were kept at the facilities of the Laboratory of Physiology in Aquatic Resources (LAFIRA) of the Academic Division of Biological Sciences of the Universidad Juárez Autónoma de Tabasco (DACBiol-UJAT), in a recirculation system with 18 circular tanks of 100 L each to start the feeding trial. Larvae hatched on the 3rd day postfertilization and feeding started from yolk absorption (3 days after hatching, DAH).

2.2. Experimental Design

Six experimental treatments were evaluated using different percentages of inulin inclusion in the microparticulate food (0.5, 1.0, 1.5, 2.0, and 2.5% inulin) and a control diet (0% inulin) carried out in triplicate. A total of 1800 *A. tropicus* larvae were distributed in 18 tanks with 100 larvae per tank. The tanks were connected to a recirculation system with a sand filter and 1 HP water pump connected to a 1500 L reservoir with a biological filter. The larvae were fed 5 times a day (8:00, 11:00, 13:00, 15:00, and 18:00 h) with *Artemia* nauplii from yolk absorption (3 DAH) until 10 DAH. The co-feeding and mixing of *Artemia* nauplii (from 20 to 50 nauplii per larvae daily) with the experimental dry feeds started from 8 DAH and they were exclusively fed with the balanced diets from 12 DAH to 21 DAH. During the experiment, the tanks were cleaned by siphoning twice a day, 1 hour after the first and last feeding. Likewise, daily monitoring of temperature (31.5 ± 0.6 °C), dissolved oxygen (4.5 ± 0.1 mg L^{-1}), and pH (7.3 ± 0.2) was carried out using an oximeter (YSI 85, USA) and a pH meter (HANNA HI 99100, Romania), respectively.

2.3. Formulation and Preparation for Experimental Diets

Diet formulation for *A. tropicus* larvae was carried out according to the protocol of Frías-Quintana et al. [29]. Some modifications were made according to the nutrients used and the percentages of inulin added by adjusting the different concentrations of inulin with sorghum flour (Table 1). The diets were prepared using an industrial mixer (CRT Global brand). Dry ingredients were sieved to the same size and added; first, the macronutrients were mixed for 10 min, then the micronutrients (vitamin, mineral, and vitamin C premix) were added, and mixing was continued for another 10 min. Afterward, the liquid ingredients (fish oil and soy lecithin) were added and they were allowed to mix for 15 min. Finally, water (approximately 400 mL kg diet^{-1}) was added. When the mixture was obtained, it was placed in a mill (TOR-REY brand) to obtain pellets with a 5 mm mesh. After the pellets were obtained, they were dried in an oven (San-Son brand) at 50 °C for 8 h [34]. When the food was prepared, it was crushed with a manual mill and sieved through a sieve according to the size of the larva's mouth.

Table 1. Experimental diet formulation with different percentages of inulin inclusion.

	Inulin (%)					
Ingredients (g kg^{-1})	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**
Fish meal [a]	297.9	297.9	297.9	297.9	297.9	297.9
Poultry meal [a]	150.0	150.0	150.0	150.0	150.0	150.0
Pork meal [a]	150.0	150.0	150.0	150.0	150.0	150.0
Starch [b]	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal [c]	100.0	100.0	100.0	100.0	100.0	100.0
Inulin [d]	0.0	5.0	10.0	15.0	20.0	25.0
Sorghum flour [c]	86.6	81.6	76.6	71.6	66.6	61.6
Fish oil [a]	40.5	40.5	40.5	40.5	40.5	40.5
soy lecithin [e]	40.0	40.0	40.0	40.0	40.0	40.0
Grenetin [f]	20.0	20.0	20.0	20.0	20.0	20.0
Mixture Vit-Min [g]	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin C [h]	5.0	5.0	5.0	5.0	5.0	5.0
Proximal composition g/100 g dry matter						
Protein	45.11	44.92	45.04	44.83	45.25	45.16
Lipid	15.21	14.93	15.10	14.82	14.93	15.07
Ash	11.94	12.35	12.01	11.99	12.29	12.19
NFE [1]	27.74	27.22	27.85	28.36	27.53	27.58

[a] Proteínas marinas y agrícolas S.A. de C.V., Guadalajara, Jalisco, México; [b] MSA Industrializadora de maíz, Guadalajara, Jalisco, México; [c] GALMEX S.A. de C.V., Villahermosa, Tabasco, México; [d] Súperfoods de Occidente S.A. de C.V., Zapopan, Jalisco, México; [e] Pronat Ultra, Yucatán, México; [f] D'gari Productos alimenticios y dietéticos relámpago, Tlalpan, EDOMEX, Mexico; [g] donated from Consorcio Súper S.A. de C.V., Guadalajara, Jalisco, México; [h] DSM Stay-C 35, Jalisco, México. [1] NFE = nitrogen-free extract: 100 − (% protein + lipids + % ash).

2.4. Growth and Survival Rates

Since the beginning of the trial, samplings were carried out to determine the growth every 7 days from 30% of the population of each tank, where the data of the individual weight (g) were recorded using an analytical balance (Ohaius HH120) and the total length (cm), respectively, by photography to be later analyzed using Image J 1.51j8 software. Additionally, at the end of the bioassay, the absolute weight gain (AWG) was calculated: final weight (g) − initial weight (g) × 100, and survival (S) (final number of fish/initial number of fish) × 100.

2.5. Sampling

At the end of the nutritional trial (21 DAH), 6 individuals per experimental unit were sampled (3 individuals for digestive and 3 individuals for antioxidant analysis), which were previously starved for a period of 24 h and euthanized with an overdose of clove oil (1 mL L^{-1}).

For enzymatic analyses, the head and tail were dissected to extract the digestive systems (stomach and intestine), which were individually preserved in an ultra-freezer at −80 °C.

2.6. Digestive Enzyme Activity

Digestive system samples were macerated in pull (n = 3 individuals per experimental unit) with 100 mM L^{-1} of glycine-HCl pH 2 for the analysis of stomach proteases and 50 mM L^{-1} of Tris-HCl + 12.5 mM L^{-1} $CaCl_2$ pH 7.5 for the analysis of intestinal proteases. Samples were centrifuged at 16,000 g at 4 °C for 15 min. The supernatant was collected and stored at −80 °C. For this study, each biological sample was measured in triplicate. Soluble protein quantification was performed using the Bradford technique [36]. For the evaluation of the activity of acid proteases (stomach) the method of Anson [37] was used, using 0.25% hemoglobin as a substrate solubilized in 100 mM L^{-1} of glycine-HCl at pH 2. Alkaline (intestinal) proteases measurement, 0.25% casein were solubilized in 50 mM L^{-1} of Tris-Cl and 10 mM L^{-1} of $CaCl_2$ pH 9.0 was used [38]. The procedure for both techniques was continued, incubating them at 37 °C and stopping the reaction with 0.5 mL of 10% trichloroacetic acid, which was then centrifuged at 16,000 g for 15 min at 4 °C. The absorbance was read at 280 nm. The lipase activity was measured with the intestinal extracts following the method of Versaw et al. [39], for which 100 mM L^{-1} of sodium taurocholate, 50 mM L^{-1} of Tris-HCl pH 7.5, and 100 mM L^{-1} of β-naphthyl acetate were used as substrates. The extract was incubated for 30 min at 37 °C and the reaction was quenched with 0.5 mL of 10% trichloroacetic acid and ethyl acetate (1:1 *v/v*); absorbance was read at 540 nm. The α-amylase activity was measured with the intestinal extracts employing the Robyt and Whelan [40] technique and the substrate used was 2% starch in a 0.05 M L^{-1} NaCl sodium citrate buffer at pH 7.5. Samples were incubated at 37 °C for 50 min and activity was quantified at 600 nm. All data obtained are shown as U mg $protein^{-1}$ according to the following equations: units by mL (U mL^{-1}) = [Δabs × final reaction volume (mL)] [ε × time (min) × extract volume (mL)]$^{-1}$; specific activity (U mg $protein^{-1}$) = U mL × mg of soluble $protein^{-1}$, where "ε" represents the molar extinction coefficient.

2.7. Antioxidant Enzyme Activities

Intestinal multienzyme extracts were used to measure catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) antioxidant activities. CAT, SOD, and GPX activities were performed using Cayman Chemical kits (Cat. 707002, Cat. 703102, and Cat. 706002, MI, USA), according to the supplier's specifications. Each measurement was performed in triplicate using the following formulas: CAT activity (nmol min^{-1} mL^{-1}) = [(μM of sample/20 min) × (sample dilution)]. SOD activity (U mL^{-1}) = [(Sample LR -y-intercept/slope) 0.23 mL × 0.01 mL^{-1}] × (sample dilution), where sample LR represent the SOD standard rate. GPX activity (nmol min^{-1} mL^{-1}) = [(Δ340 × min/0.00373 μM) − 1 × (0.19 mL/0.002 mL)] × (Sample dilution). All activities were normalized using the Bradford [37] technique for soluble protein concentration.

2.8. Statistical Analysis

A one-way ANOVA and Tukey's HSD posteriori test were performed to analyze statistical differences between treatments for survival, absolute weight gain, and digestive and antioxidant enzymes (SOD and CAT), which previously fulfilled the postulates of Normality (Kolmogorov–Smirnov) and homoscedasticity (Levene). Wet weight, total length, and GPx enzyme activity did not fulfill both postulates. Therefore, these variables were analyzed with non-parametric Kruskal–Wallis and a posteriori Nemenyi tests. A significance value of 0.05 was used to detect statistical differences. All tests were performed with Statistica V 7.0 program.

3. Results

3.1. Growth and Survival Rates

The results showed a significant difference ($p < 0.05$) in weight; the control diet with 0% inulin obtained the highest median value (±RI) of 0.078 ± 0.01 g, against treatments 1.0% and

1.5% inulin supplementation (0.069 ± 0.04 g and 0.068 ± 0.04 g, respectively (±RI)) (Figure 1a). The growth in total length behaved similarly to weight when detecting a high significance ($p < 0.05$), where the fish fed with the control diet shows similar results (2.89 ± 0.18 cm median and ±RI) to the larvae fed with the 2.5 and 2.0% inulin inclusions (2.85 ± 0.30 and 2.82 ± 0.21 cm (±RI)), respectively, while the rest of the treatments were statistically lower (Figure 1b). For weight gained, no significant differences were found ($p > 0.05$) between treatments (Control: 4.9011 ± 0.69%/day; 0.5% inulin: 4.0829 ± 0.54%/day; 1% inulin: 4.1529 ± 0.46%/day; 1.5% inulin: 4.2642 ± 0.54%/day; 2% inulin: 5.1907 ± 0.66%/day; and 2.5% inulin: 4.9442 ± 1.32%/day). However, survival did show significant differences ($p < 0.05$), where the fish fed with the inclusion of 2.5% inulin showed the highest median value (34.7 ± 5.5%) compared with larvae fed the control diet (15.1 ± 4.3%) (Figure 2).

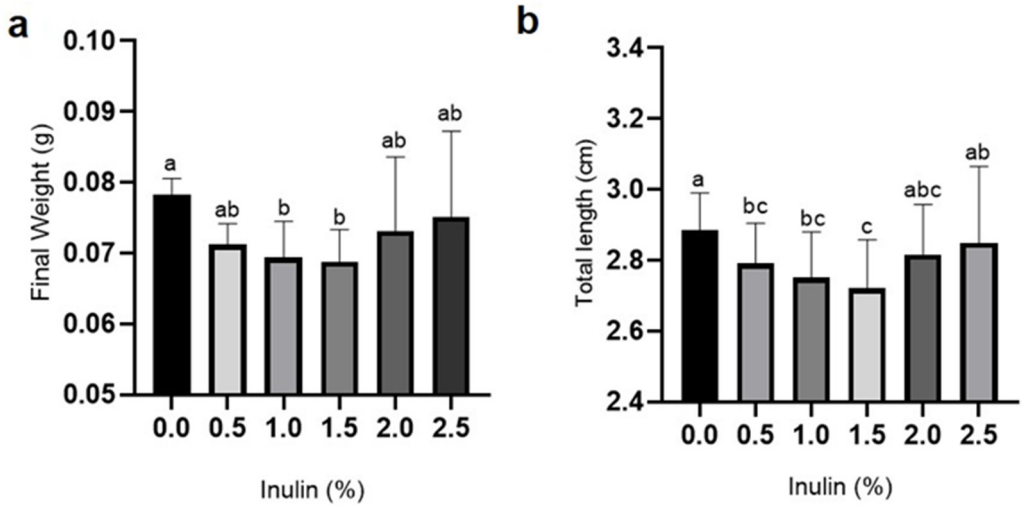

Figure 1. Final weight (**a**) and total length (**b**) of *Atractosteus tropicus* larvae fed with different percentages of inulin in the diet (0, 0.5, 1.0, 1.5, 2.0, and 2.5%). Values represent medians ± RI. Significant differences between treatments (inulin supplementation) are indicated by different letters ($p < 0.05$).

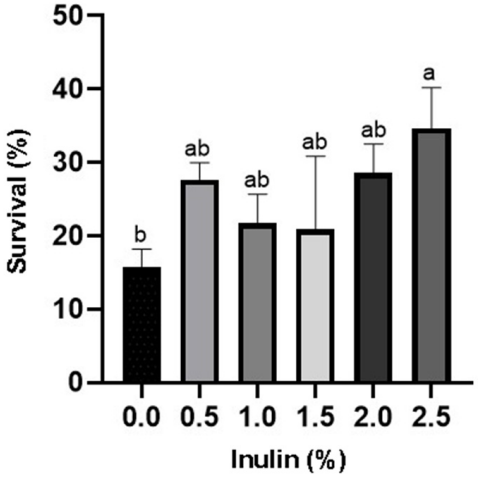

Figure 2. *Atractosteus tropicus* larvae survival (%) fed with different percentages of inulin in the diet (0, 0.5, 1.0, 1.5, 2.0, and 2.5%). Values represent mean ± SD. Significant differences between treatments (inulin supplementation) are indicated by different letters ($p < 0.05$).

3.2. Digestive Enzyme Activity

The larvae fed with the inclusion of 2.5% inulin showed the highest activities ($p < 0.05$) for acid proteases (Figure 3a) and alkaline proteases (Figure 3b), being statistically higher than the rest of the treatments. On the other hand, amylase activity (Figure 3c) was statistically higher ($p < 0.05$) for 2.0% and 2.5% of inulin supplementation than the rest of the treatments, and lipase activities (Figure 3d) were statistically higher ($p < 0.05$) for the larvae fed with 2.5% of inulin inclusion compared to the other treatments.

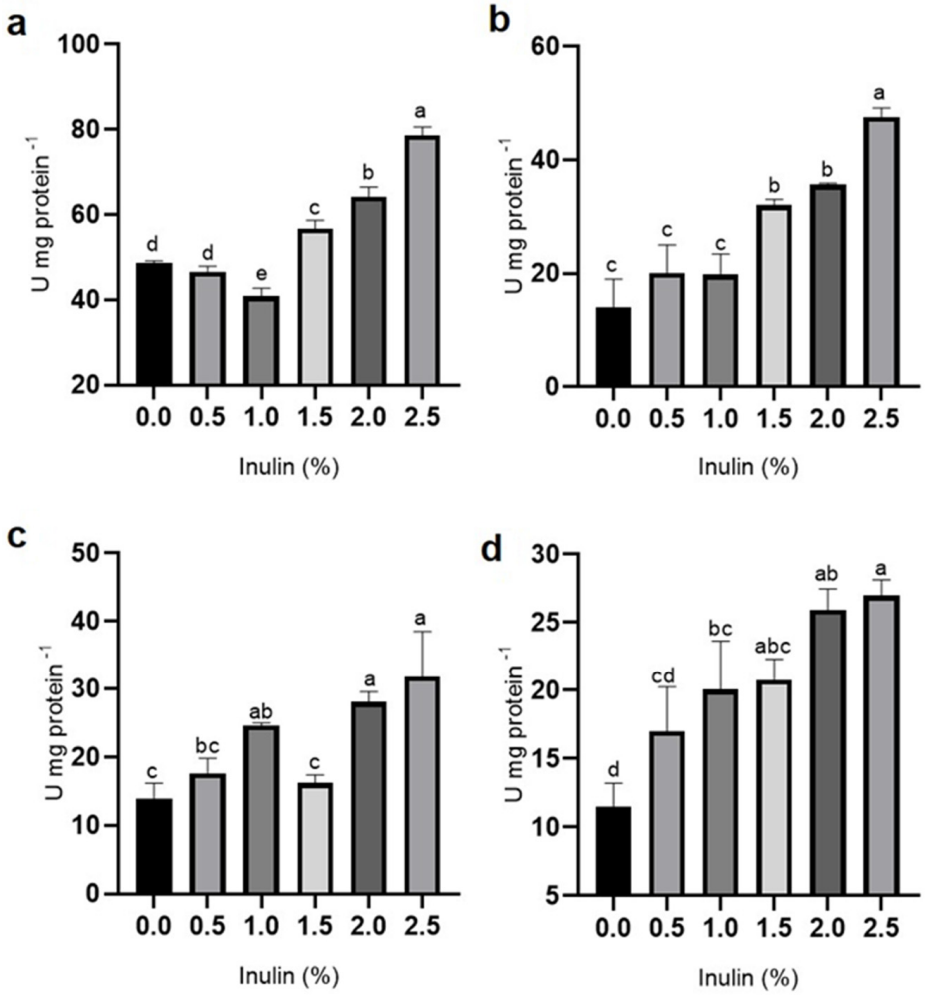

Figure 3. Acid proteases (**a**), alkaline proteases (**b**), amylase (**c**), and lipase (**d**) enzyme activities (U mg protein^{-1}) of *Atractosteus tropicus* larvae fed with different percentages of inulin in the diet (0, 0.5, 1.0, 1.5, 2.0, and 2.5%). Values represent mean ± SD. Significant differences between treatments (inulin supplementation) are indicated by different letters ($p < 0.05$).

3.3. Antioxidant Enzyme Activity

The activity of the antioxidant enzymes CAT (Figure 4a) and SOD (Figure 4b) showed the highest values ($p < 0.05$) for the larvae fed with the control diet (0% inulin) compared to the treatments supplemented with inulin. On the other hand, the GPx activity did not show significant differences ($p > 0.05$) between the treatments (Figure 4c).

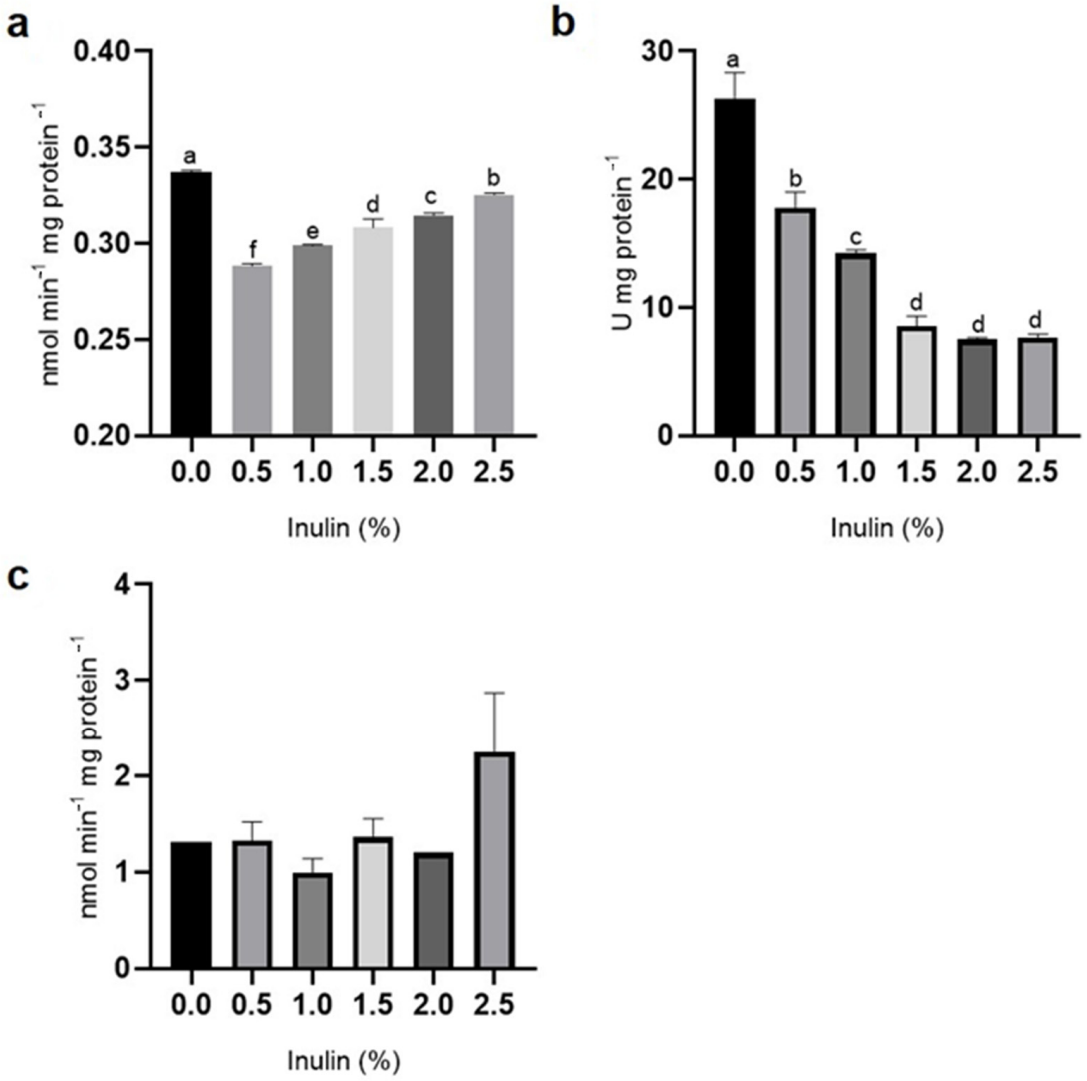

Figure 4. Catalase (**a**), superoxide dismutase (**b**), and glutathione peroxidase (**c**) antioxidant enzymes activities of *Atractosteus tropicus* larvae fed with different percentages of inulin in the diet (0, 0.5, 1.0, 1.5, 2.0, and 2.5%). Values represent mean ± SD. Significant differences between treatments (inulin supplementation) are indicated by different letters ($p < 0.05$).

4. Discussion

This experiment showed significant differences in final weight and total length in *A. tropicus* larvae fed with the control diet and the highest levels of inulin (2.0 and 2.5%) compared to the lowest levels of inulin added, which show the smallest values for weight and length. These results differ from those reported in species such as carp (*Cyprinus carpio*), Arctic char (*Salvelinus alpinus*), beluga (*Huso huso*), and rainbow trout (*Oncorhynchus mykiss*) [16,41–43] where no positive effects on growth were detected; however, other studies have revealed positive effects on growth promoted by the inclusion of inulin, such as in Nile tilapia (*Oreochromis niloticus*), roach (*Rutilus rutilus*), and *O. mykiss* [15,19,44–47]. These effects have been attributed to the fermentability of inulin by the action of the microbiome and changes in intestinal morphology, which changes as a function of development [41,48]. Likewise, some authors consider that the variations in growth, feed utilization, and health benefits when using this prebiotic depend mainly on the species, the time of application, and the dose of the supplement, as well as the type of prebiotic. Furthermore, in this case, the effect of inulin on the growth response seems to vary, so it is necessary to examine morphophysiological parameters to understand its effect [49,50].

On the other hand, the survival of *A. tropicus* larvae showed significant differences ($p < 0.05$), where the fish fed with the inclusion of 2.5% inulin showed the highest value. These results are similar to those results reported by various authors who found significant increases in the survival of various fish species [15,16,44,48,51–53]. In contrast, Akrami et al. [42] and Reza et al. [43] did not find positive effects on the survival rate of fingerlings of *H. huso* and *O. mykiss.* In addition, lower rates of food consumption, SGR, and PER were observed in the treatments with 1% and 2% inulin supplementation compared with the control diet. This suggests an inverse relationship between some of the growth indices, which leads the authors to believe that inulin is not appropriate for these species. The authors suggest that this is related to a decrease in digestive capacity by possibly exceeding the dose of the prebiotic and the low fermentability of inulin, which could affect the hydrolysis of nutrients by affecting the digestive enzymatic activity or the absorption processes of essential biomolecules.

In this regard, Olsen et al. [41] claim that the lower survival can be attributed to the inability of the intestinal microbiota to ferment the excessive levels of inulin, and the excess of inulin was detrimental to the enterocytes. Likewise, the effects of inulin on growth and survival rate vary among different species. In this regard, one of the essential roles of inulin intake is to maximize the digestibility of feed components, as previous studies suggest that this prebiotic is an excellent digestion activator since it causes a balanced growth of specific microorganisms in the posterior intestine, such as Bifidobacteria and Lacctobacilli, which contribute to the production of vitamins B1, riboflavin, B6, and K, as well as increasing the solubility and absorption of minerals such as calcium, magnesium, and iron ions. It is also known that a correct dose of the prebiotic reduces the intestinal pH and produces products derived from fermentation, such as short chains of fatty acids, which can be used as an energy source for cells [16,49,54–56].

In the case of digestive enzymes, *A. tropicus* larvae fed with the inclusion of 2.5% inulin showed the highest activities of acid, alkaline, amylase, and lipase proteases, followed by those fed with the inclusion of 2% inulin. These results are similar to those reported by Soleimani et al. [44] in *R. rutilus* larvae, where amylase, lipase, and protease activities showed a significant increase when adding 1% inulin compared with the control diet. Similarly, Hunt et al. [19] found an increase in intestinal trypsin, lipase, and amylase from fish fed 1% inulin in *O. mykiss*. However, there are studies where the inclusion of inulin did not have significant effects on trypsin-like activities, alkaline amylase phosphatase, and leucine aminopeptidase, which were carried out in juvenile Atlantic salmon (*Salmo salar*) and in *C. carpio* larvae [16,57]. This may be related to a limited synthesis of these pancreatic digestive enzymes in contrast with the high hydrolytic capacity and rapid development of the digestive system of *A. tropicus* larvae [28]. Those mentioned above can be attributed to the different digestive capacities that species have depending on their stage of development and the presence of a balanced intestinal microbiome. This is why the digestive capacities of species vary enormously; the inclusion of inulin works in a complementary way to digestion processes since the fermentation of prebiotics by the intestinal microbiome can produce short-chain fatty acids, which support the digestion of nutrients. This improves the morphology of the intestine [43,48].

In other studies, it is mentioned that the bacteria found in the intestinal tract participate in the digestion of food, which is why it contributes in a complementary way to the hydrolysis of nutrients by the action of digestive enzymes in their active function. In the same way, it is mentioned that the microbiome of the digestive tract of aquatic organisms varies according to several factors, such as the aquatic environment (temperature, pH, alkalinity, dissolved oxygen, salinity, among others), seasonal variation, diet, the stage of development of the species of fish, and the anatomy of the gastrointestinal section [58–60]. Likewise, it is mentioned that the addition of inulin in the correct proportion serves to stimulate the digestive enzyme activities by the production of a series of vitamins and enzymes. The augment in digestion efficiency leads to an increase in the intestinal mucosa [9], improving the morphology of the

gut by the fermentation of inulin which stimulates the microbiomes that participate in the digestion of food [44].

In this way, the inclusion of this prebiotic helps the nutrients in the food to be easily digested and promotes better absorption of the molecules inside the cells (enterocytes) to be transported to the tissues that require them for various metabolic processes [19].

On the other hand, the activities of the antioxidant enzymes CAT and SOD of the *A. tropicus* larvae showed the highest values for the fish fed with the control diet compared to the other treatments, which contrasts with the decrease in these activities according to the inulin ratio increases. In this sense, it is widely known that inulin improves antioxidant status through several mechanisms, such as the generation of short-chain fatty acids and antioxidant enzymes [61]. Antioxidant enzymes inhibit radical generation and prevent oxidative damage in teleost [62]. Some studies indicate that the activity of antioxidant enzymes is modulated when prebiotics, such as inulin, are included in the diet, which allows the body to prevent the formation of reactive oxidative species (ROS) [63,64]. It should be noted that cells usually produce reactive oxygen species, which is why the first antioxidant defense system is GPx, SOD, and CAT, which could indicate that our results show less antioxidant activity in treatments supplemented with inulin, unlike the control treatment. This is due to protection or inhibition of the cells to produce ROS, for which they do not need to be active, keeping the organism more stable, unlike the control treatment with the highest activity. In this sense, our results are similar to those reported by Guerreiro et al. [65], where diminished antioxidant activity in white sea bream (*Diplodus sargus*) was observed by including fructo-oligosaccharides (FOS), which are a derivative of inulin. To fully determine the antioxidant effect of inulin inclusion in the diet, further and more in-depth analyses are needed.

Contrary to our results, Hunt et al. [19] found that inulin supplementation increases the antioxidant activity of *O. mykiss*, which are essential protective enzymes that trigger various factors to prevent cell deterioration from membrane oxidation. Without the protection of antioxidant enzymes, the balance of various absorption channels, such as sodium, potassium, and calcium ions in fish, is altered, in addition to the elevation of cortisol and corticosteroids, which are essential in the regulation of the pathways involved in the mobilization of energy substrates [64]. In this regard, Olsvik et al. [66] mention that the increase in the activity of some oxidative enzymes that participate in the antioxidant response, such as SOD, CAT, and GPx, can differ in response to variations in several biotic and abiotic factors that cause stress in fish, in addition to the individual physiological variation of organisms [67]. Additionally, inulin can act effectively by changing the intestinal microbiota, which generates the production of lactic acid and other substances used as energy sources for microorganisms, which improves the absorption of nutrients in the host, generates an increased synthesis of antimicrobial peptides, promotes the growth of beneficial bacteria (probiotics), reduces luminal pH, provides an enhancement of the immune system, and prevents the adhesion of pathogens [68].

An aspect to be highlighted is the increase in the absorption capacity of nutrients which generally improves the growth of fish, for which it has been hypothesized that the fermentation process in the intestinal tract by the action of the microbiome and its association with some prebiotics, such as inulin, promotes the release of short-chain fatty acids. This is also complemented by the production of other substances, such as spermine, that stimulate the proliferation of intestinal cells [69–71]. Various authors mention that the effects on growth when adding inulin to the diet of fish, particularly in carnivores such as *A. tropicus*, are not always clear. However, an increase in digestive capacity is observed which is associated with a significant contribution of some active substances by the microbiome by inulin supplementation, such as Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), and gilthead sea bream (*Sparus aurata*) [41,57,72].

Regarding the studies carried out with prebiotics in *A. tropicus*, it can be mentioned that the growth of larvae fed with the concentration of 7.5 g Kg^{-1} of FOS increased the weight and total length, as well as an increase in the digestive lipase and amylase enzyme activities [73]. When MOS was added to the balanced feed, no significant differences were

found in the growth of the larvae in any of the treatments. However, a significant increase in lipase and alkaline protease activities was detected when supplementing with 5 g Kg^{-1} of MOS [35]. In *A. tropicus*, juveniles fed with MOS show an increase in growth when using 2 g Kg^{-1}, as well as an increase in pepsin, trypsin, lipase, and α-amylase activities [32]. The addition of FOS in the diet showed an increase in the growth of juveniles with 5 g Kg^{-1}, in addition to an increase in acid protease, chymotrypsin, leucine aminopeptidase, and lipase activities [34]. Finally, when using the prebiotic β-glucans in balanced feeds for juveniles, no differences were detected between treatments, although differences were detected in chymotrypsin activity when supplementing the diet with 10 g Kg^{-1} [33].

5. Conclusions

The incorporation of 25 g Kg^{-1} of inulin in the larvae of *A. tropicus* increases survival and improves the activities of acid and alkaline proteases, amylase, and lipase, so there is a greater bioavailability of nutrients. Likewise, an increase in the activity of the GPx antioxidant enzyme is observed, which favors the protection of cell membranes from the release of reactive oxygen species (ROS). The decrease in SOD and CAT could suggest that they play a protective role by preventing the presence of ROS; in this way, inulin supplementation can positively affect the larvae's culture during their development and the time to make food changes.

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