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*Research Article*

# Matali (*Tradescantia zebrina*) addition in diets for juveniles of two-band cichlid (*Vieja bifasciata* Steindachner, 1864): effects on growth and digestive morphology

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**ABSTRACT.** Herbaceous plants have attracted increasing attention in the aquaculture industry for their beneficial effects on the growth, health, and physiology of several fish species. Matali (*Tradescantia zebrina*) is a medicinal herb widely distributed in southeastern Mexico and recognized for its diverse pharmacological properties. This study aimed to evaluate the effects of *T. zebrina* on growth performance, cytotoxicity, and digestive morphology in the two-band cichlid (*Vieja bifasciata*), a native species with aquaculture potential. Cytotoxicity of the aqueous extract of *T. zebrina* was assessed using *Artemia* nauplii exposed for 24 h to serial dilutions ranging from 100 to 0.78 mg $mL^{-1}$. Subsequently, diets supplemented with 0% (T1), 5% (T2), 10% (T3), and 15% (T4) *T. zebrina* were evaluated for their effects on growth performance and digestive morphology in *V. bifasciata* over a 45-day feeding trial. The results demonstrated that the aqueous extract of *T. zebrina* was non-toxic, suggesting that its inclusion in fish diets is safe. The highest weight gain was recorded in T2 (2.28 ± 0.43 g), whereas the greatest total length was observed in T3 (5.07 ± 0.04 cm). Survival was highest in T4 (100%). Based on weight gain, specific growth rate, and feed conversion ratio, T2 was identified as the most effective treatment for promoting growth ($P < 0.05$). In addition, dietary supplementation with *T. zebrina* produced modifications in digestive morphology. Overall, the inclusion of 5-10% *T. zebrina* in the diet can serve as a feed supplement without adversely affecting *V. bifasciata* growth. Further studies involving gene expression, immunological responses, and enzymatic activity are recommended to validate additional potential benefits of *T. zebrina* in aquaculture.

**Keywords:** *Vieja bifasciata*; plants; cytotoxicity; growth parameters; cichlids; histology

## INTRODUCTION

The two-band cichlid, *Vieja bifasciata*, is a native fish species from southeastern Mexico that inhabits rivers, lakes, lagoons, and marshlands. This species adapts well to captivity and readily accepts commercial tilapia diets (Jiménez-Leyva et al. 2024). Its meat is characterized by a white color, a firm texture, low bone content, and a pleasant flavor (Davila-Camacho et al. 2018). Despite its desirable organoleptic characteristics and promising aquaculture potential, its consumption and commercialization remain largely limited to local markets (Sánchez-Cruz et al. 2024). In Mexico, aquaculture development has focused mainly on intro-

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duced species such as tilapia (*Oreochromis* sp.), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), and common carp (*Cyprinus carpio*) (Frías-Quintana et al. 2021). One of the main factors contributing to the successful establishment of these species has been the availability of species-specific balanced feeds, which have improved nutritional management and enhanced production efficiency (Barraza-Guardado et al. 2019). However, the rising cost of fishmeal has significantly increased feed formulation expenses (Rana et al. 2024). Consequently, considerable research efforts have focused on identifying alternative ingredients that can partially or fully replace fishmeal in aquafeeds, to reduce production costs while maintaining fish performance (González et al. 2014).

Among the most promising alternatives for aquafeed development is the use of plant-based ingredients, as several plant species provide not only appreciable amounts of protein but also a wide variety of secondary metabolites, including alkaloids, terpenes, phenolic compounds, flavonoids, and tannins (Salam et al. 2023). These bioactive compounds are distributed across various plant structures, including leaves, roots, stems, seeds, and peels (Rana et al. 2024), and have been associated with beneficial effects on fish growth, health, and physiological performance (Hassan et al. 2018). Several studies have evaluated the effects of plant-derived ingredients on fish growth performance. For instance, Mahdavi et al. (2013) evaluated the inclusion of *Aloe vera* extract in diets for *C. carpio* and reported that a 2.5% inclusion level produced the best growth response. Similarly, Giri et al. (2016) incorporated banana peel (*Musa acuminata*) into diets for rohu (*Labeo rohita*), observing significant improvements in final weight and growth rate at a 5% dietary inclusion level. Likewise, Bin Dohaish et al. (2018) supplemented *O. niloticus* diets with *Spirulina platensis* and found that a 5% inclusion level yielded the best growth performance. More recently, Abidin et al. (2022) evaluated the effects of *Azadirachta indica* extract in diets for *O. mykiss* and determined that a 7% supplementation level significantly improved growth parameters.

Matali (*Tradescantia zebrina*) is a plant native to Mexico and Central America, widely cultivated in tropical and subtropical regions. Its green leaves with violet stripes are traditionally consumed as a beverage or used in cooked and macerated preparations. This species has been associated with several medicinal properties and is traditionally used to treat kidney disorders, conjunctivitis, tuberculosis, hypertension, cough, inflammation, hemorrhoids, burns, and skin irritations (Ramos-Arcos et al. 2023). Phytochemical analyses have demonstrated that the ethanolic extract of *T. zebrina* contains phenolic compounds, whereas the aqueous extract shows a high flavonoid content (Olivo-Vidal et al. 2020). Therefore, the objective of this study was to evaluate the effects of dietary supplementation with *T. zebrina* in commercial tilapia feed on the growth performance and digestive morphology of the two-band cichlid (*V. bifasciata*), and to assess the cytotoxicity of the plant extract.

## MATERIALS AND METHODS

### Plant material

Leaves of *T. zebrina* were purchased from the Colonel Gregorio Méndez Magaña Public Market located in Villahermosa. The leaves were thoroughly washed with purified water to remove impurities and debris. Subsequently, the plant material was dried in an electric oven (Ríos Rocha S.A. No. 303554, Mexico) at 50°C for 48 h. After drying, the leaves were ground using an electric coffee grinder (A0313 Electric Grinder, China) to a particle size of 0.5-1 mm.

### Cytotoxicity test

For the cytotoxicity assay, 0.1 g of *Artemia salina* cysts were weighed and incubated in 200 mL of saline solution (38 g $L^{-1}$) to hatch. The cysts were maintained under constant aeration for 24 h at 25-29°C. The aqueous extract of matali was prepared by weighing 100 mg of dried plant material and placing it into a capped microcentrifuge tube containing 1 mL of saline solution. The mixture was allowed to stand for 48 h to facilitate extraction. A 96-well microplate was prepared using serial dilutions of the aqueous extract of *T. zebrina*, with concentrations ranging from 100 to 0.78 mg $mL^{-1}$. Subsequently, 100 µL of saline solution containing 10-15 *Artemia* nauplii was added to each well. All treatments were evaluated in triplicate. Tween®80 (Sigma P1754, St. Louis, MO, USA) was used as the positive control, whereas saline solution served as the negative control. The microplate was incubated for 24 h, after which the numbers of live and dead *Artemia* nauplii were counted in each well to calculate the mortality percentage using the following formula (Rangel-López et al. 2022):

$$\text{Mortality} = \frac{\text{Number of dead nauplii}}{\text{Initial number of nauplii}} \times 100$$

To determine the degree of cytotoxicity, the classification criteria established by Hamidi et al.

(2014) were applied. Extracts with lethal concentration ($LC_{50}$) values greater than 1.0 mg $mL^{-1}$ were considered non-toxic, values between 0.5 and 1.0 mg $mL^{-1}$ were classified as having low toxicity, values between 0.1 and 0.5 mg $mL^{-1}$ were considered moderately toxic, and values lower than 0.1 mg $mL^{-1}$ were classified as highly toxic.

**Preparation of the experimental diets**

Commercial tilapia feed (Agribrands, Purina@) in flour form was used to prepare the experimental diets. According to the manufacturer's technical specifications, the feed contained 44% crude protein, 12% lipids, 2.5% crude fiber, and 12% ash. Previously, ground matali leaves and gelatin were weighed in the proportions shown in Table 1. Subsequently, 50 mL of water was added to each mixture to obtain a homogeneous paste. The paste was then ground manually to form pellets. Finally, the pellets were dried in an electric oven (Ríos Rocha S.A. No. 303554, Mexico) at 50°C for 24 h.

**Experimental design**

The experiment was carried out at the Laboratorio de Investigación Acuícola Especializada, División Académica de Ciencias Agropecuarias, de la Universidad Juárez Autónoma de Tabasco. Experimental fish were obtained from the native species laboratory of the same institution. A total of 120 *V. bifasciata* were used, with an initial average weight of 1.2 ± 0.64 g and an average total length of 4.07 ± 0.60 cm. Fish were randomly distributed into 12 60 L tanks, with 10 fish per tank. Each treatment was evaluated in triplicate. The experimental setup consisted of a recirculating aquaculture system comprising 12 interconnected tanks, thereby allowing uniform environmental conditions across treatments. In addition, 20% of the total system water volume was renewed weekly to maintain water quality.

Four experimental treatments were established according to the inclusion level of *T. zebrina* in the diet: T1 (control), T2 (5%), T3 (10%), and T4 (20%). Each treatment was evaluated in triplicate. The feeding trial lasted 45 days. Individual biometric measurements were conducted every 15 days. Fish weight and total length were recorded using a digital balance with a precision of 0.1 g (Ohaus Scout Pro SP601, USA) and a 30 cm ichthyometer, respectively. Fish were fed ad libitum three times daily at 8:00, 13:00, and 19:00 h throughout the experimental period.

**Growth parameters**

Growth performance was evaluated using the biometric data collected every 15 days throughout the experimental period. The recorded weight and total length of the fish were used to calculate growth parameters using the formulas described by Gómez-Hernández et al. (2025).

$$\text{Survival (\%)} = \frac{\text{Final fish number}}{\text{Initial fish number}} \times 100$$

$$\text{Weight gain (g)} = \text{Final weight} - \text{initial weight}$$

$$\text{Specific growth rate} = \frac{(\ln \text{ final weight } - \ln \text{initial weight})}{\text{Days}} \times 100$$

$$\text{Feed conversion rate } = \frac{\text{Feed intake}}{\text{Fish weight gain}}$$

$$\text{Condition factor} = \frac{\text{Final weight}}{(\text{Final length})^3} \times 100$$

**Histological analysis**

Fish samples were fixed in Davidson's solution and subsequently dehydrated through a graded ethanol series (70, 80, 90, 96, and 100%). The tissues were then embedded in paraffin, sectioned at 7 µm, and stained with hematoxylin and eosin (H&E). Histological images were captured using an Axiocam ERC 5S camera (Carl Zeiss GmbH, Jena, Germany) coupled to a Zeiss Primo Star optical microscope (Primo Star, Suzhou, China). In liver sections, hepatocyte diameter (µm) was measured, whereas in intestinal sections, enterocyte height and intestinal fold length were quantified. All measurements were analyzed using ZEN 2.3 software (Carl Zeiss AG). Each histological parameter was measured 30 times per fish, with nine fish evaluated per treatment (n = 9) (Sepúlveda-Quiroz et al. 2024). Before tissue collection, fish were euthanized with clove oil at a concentration of 0.20 mL in 500 mL of water, as described by Fernandes et al. (2017).

**Statistical analysis**

The median $LC_{50}$ of the aqueous extract of *T. zebrina* was estimated using Probit analysis in SAS software version 9.0 (SAS Institute, Cary, NC, USA). Before statistical analysis, the production parameters were evaluated for normality using the Kolmogorov-Smirnov test and for homogeneity of variances using Bartlett's test. Subsequently, data were analyzed using a one-way analysis of variance (ANOVA) with $\alpha = 0.05$. When significant differences among treatments were detected, Tukey's multiple comparison test ($P < 0.05$)

**Table 1.** Composition of experimental diets with different concentrations of matali (*T. zedrina*).

| Ingredients | T1: Control | T2 : 5% | T3 : 10% | T4 : 20% |
|---|---|---|---|---|
| Tilapia commercial feed (g) | 99 | 94 | 89 | 79 |
| Matali (g) | 0 | 5 | 10 | 20 |
| Grenetine (g) | 1 | 1 | 1 | 1 |
| Total | 100 | 100 | 100 | 100 |

was applied to identify differences between treatment means. All statistical analyses were performed using the Minitab® 18 statistical software package (Minitab, LLC, USA).

## RESULTS

### Matali cytotoxicity

According to the cytotoxicity classification criteria proposed by Hamidi et al. (2014), the aqueous extract of *T. zebrina*, evaluated using the *A. salina* bioassay, showed an $LC_{50}$ value of 10.93 mg $mL^{-1}$ (Fig. 1). Based on these criteria, extracts with $LC_{50}$ values greater than 1 mg $mL^{-1}$ are considered to exhibit low or no toxicity. Therefore, the aqueous extract of *T. zebrina* can be considered safe for dietary inclusion in the *V. bifasciata*.

### Growth parameters

The growth performance results obtained for *V. bifasciata* showed no significant differences among treatments ($P > 0.05$) in final weight, final length, survival rate, or condition factor. However, treatment T2 exhibited significantly better performance ($P < 0.05$) for the following growth parameters: weight gain (1.16 ± 0.29 g), specific growth rate (1.60 ± 0.36%), and feed conversion ratio (0.78 ± 0.001) (Table 2).

### Histological effects

In the intestinal tissue, treatment T1 showed the greatest enterocyte height (20.82 ± 0.93 μm), followed by T3 (18.70 ± 1.02 μm) and T2 (18.12 ± 0.76 μm). These values were significantly higher ($P < 0.05$) than those observed in T4 (15.29 ± 0.90 μm) (Table 3; Fig. 2). Regarding intestinal fold length, the highest value was recorded in T2 (124.78 ± 29.62 μm), followed by T1 (117.65 ± 25.67 μm), T3 (112.89 ± 24.25 μm), and T4 (105.46 ± 16.21 μm). However, no significant differences were detected among treatments ($P > 0.05$) (Figure 3). In liver tissue, the largest hepatocyte area was observed in T4 (55.48 ± 2.23 $\mu m^2$), which differed significantly ($P < 0.05$) from T1 (51.98 ± 2.06 $\mu m^2$). Lower hepatocyte areas were recorded in T2 (43.42 ± 2.46 $\mu m^2$) and T3 (37.91 ± 2.65 $\mu m^2$) (Fig. 4).

## DISCUSSION

The expansion of aquaculture has increased demand for balanced feeds, placing considerable pressure on the availability of traditional raw materials used in feed production and, consequently, increasing production costs. In commercial aquaculture, feed expenses may account for 50 to 70% of total operating costs (Méndez-Martínez et al. 2018). In response to this challenge, alternative feed ingredients have been explored, including the incorporation of plant-derived additives into aquaculture diets (Toledo-Solís et al. 2021). The inclusion of medicinal and aromatic plants in fish diets has been associated with improved growth performance and enhanced immune function. For example, supplementation with oregano (*Origanum onites*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) has been reported to enhance *O. mykiss* growth (Mirghaed et al. 2020). In this context, *T. zebrina* contains secondary metabolites, including phenolic compounds and flavonoids (Cardoso et al. 2022). These compounds have been shown to reduce stress and enhance immune responses when incorporated into fish diets (Lizárraga-Velázquez et al. 2017).

In the present study, the cytotoxicity of the aqueous extract of *T. zebrina* was evaluated, yielding an $LC_{50}$ of 10.93 mg $mL^{-1}$, which, according to the criteria established by Hamidi et al. (2014), classifies the extract as non-toxic. Similar findings were reported by Rangel-López et al. (2022), who observed that the hydroalcoholic extract of *Caesalpinia coriaria* exhibited no toxicity, with an $LC_{50}$ value of 1.56 mg $mL^{-1}$. Likewise, Gómez-Hernández et al. (2025) reported that yerba mate (*Ilex paraguariensis*) showed no toxic effects, with an $LC_{50}$ of 42.42 mg $mL^{-1}$. These results support the use of the *A. salina* bioassay as a reliable method for preliminary evaluation of cytotoxicity in plant extracts intended for aquaculture applications. Furthermore, this assay provides evidence

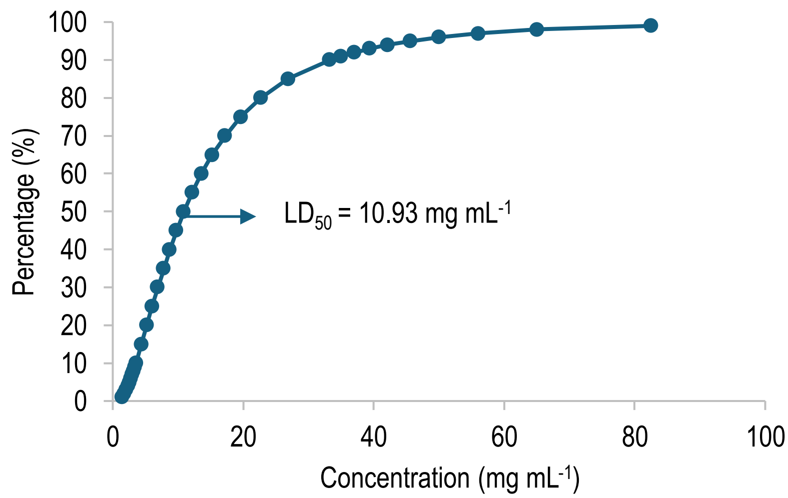


**Figure 1.** Cytotoxicity of the lethal concentration ($LD_{50}$) of the aqueous extract of matali (*T. zedrina*).

**Table 2.** Growth parameters of the two-band cichlid (*V. bifasciata*) fed with tilapia feed and supplemented with matali. (*T. zedrina*). T1, T2, T3, T4: treatments. [a,b,c]Different letters in the rows indicate statistically significant differences ($P <$ 0.05).

| Parameters | T1: Control | T2 : 5% | T3 : 10% | T4 : 20% |
|---|---|---|---|---|
| Initial weight (g) | 1.23 ± 0.03 | 1.11 ± 0.28 | 1.22 ± 0.12 | 1.24 ± 0.19 |
| Final weight (g) | 1.95 ± 0.18[a] | 2.28 ± 0.43[a] | 2.06 ± 0.44[a] | 1.66 ± 0.16[a] |
| Initial length (cm) | 4.19 ± 0.69 | 4.03 ± 0.46 | 4.08 ± 0.68 | 3.98 ± 0.57 |
| Final length (cm) | 4.87 ± 0.10[a] | 5.02 ± 0.27[a] | 5.07 ± 0.41[a] | 4.56 ± 0.15[a] |
| Survival (%) | 97.5 ± 4.33[a] | 95.0 ± 4.33[a] | 97.5 ± 4.33[a] | 100 ± 0.0[a] |
| Weight gain (g) | 0.71 ± 0.17[ab] | 1.16 ± 0.29[a] | 0.84 ± 0.32[ab] | 0.41 ± 0.23[b] |
| Specific growth rate (%) | 1.01 ± 0.20[ab] | 1.60 ± 0.36[a] | 1.14 ± 0.26[ab] | 0.65 ± 0.36[b] |
| Feed conversion rate | 0.87 ± 0.0007[c] | 0.78 ± 0.001[a] | 0.83 ± 0.001[b] | 1.00 ± 0.002[d] |
| Condition factor | 1.86 ± 0.18[a] | 1.67 ± 0.01[a] | 1.75 ± 0.20[a] | 1.95 ± 0.20[a] |

that incorporating such plant-derived ingredients into fish diets is unlikely to cause adverse effects in cultured organisms (Waghulde et al. 2019).

The growth performance of the *V. bifasciata*, showed that the treatment containing 5% inclusion of *T. zebrina* (T2) produced the highest final weight (2.28 ± 0.43 g). Similar results were reported by Méndez-Martínez et al. (2019), who evaluated the inclusion of duckweed (*Azolla filiculoides*) in tilapia fry diets and observed that a 10% inclusion level resulted in the highest final weight (6.556 g). In contrast, higher inclusion levels did not produce additional improvements. Regarding survival, the treatment containing 20% *T. zebrina* (T4) achieved 100% survival. Comparable findings were reported by Martínez et al. (2022), who observed 100% survival in Nile tilapia (*O. niloticus*) fed diets supplemented with 8% Mexican oregano essential oil (*Lippia graveolens*) and in the control group. In aquaculture studies, survival rates above 90% generally indicate that the dietary additive does not exert toxic effects on fish, supporting its safety for inclusion in experimental diets. The greatest weight gain was also observed in T2, at 1.16 ± 0.29 g. Similar responses have been documented by Hassan et al. (2018), who evaluated the effects of several medicinal plants in diets for Nile tilapia and reported that supplementation with *Curcuma longa* (191.14 g), *Rosmarinus officinalis* (194.36 g), and *Thymus vulgaris* (186.70 g) at a 1% inclusion level significantly increased weight gain compared to the control group (160.64 g). Likewise, the highest specific growth rate was obtained in T2 (1.60 ± 0.36%). In contrast, Zaman & Cho (2022) evaluated diets supplemented with *Smallanthus sonchifolius*, *Zingiber*

**Table 3.** Morphological analysis of enterocytes and hepatocyte areas in two-band cichlid (*V. bifasciata*). [a,b,c]Differences letter in the rows indicated statistically significant differences ($P < 0.05$).

| | T1: Control | T2 : 5% | T3 : 10% | T4 : 20% |
|---|---|---|---|---|
| Higth enterocyte (µm) | $20.82 \pm 0.93^a$ | $18.13 \pm 0.76^b$ | $18.70 \pm 1.02^{ab}$ | $15.30 \pm 0.90^c$ |
| Enterocyte fold (µm) | 117.65 ± 25.67 | 124.78 ± 29.62 | 112.90 ± 24.25 | 105.47 ± 16.21 |
| Hepatocyte area (µm$^2$) | $48.95 \pm 2.06^b$ | $43.43 \pm 2.46^c$ | $37.91 \pm 2.65^c$ | $55.49 \pm 2.23^c$ |

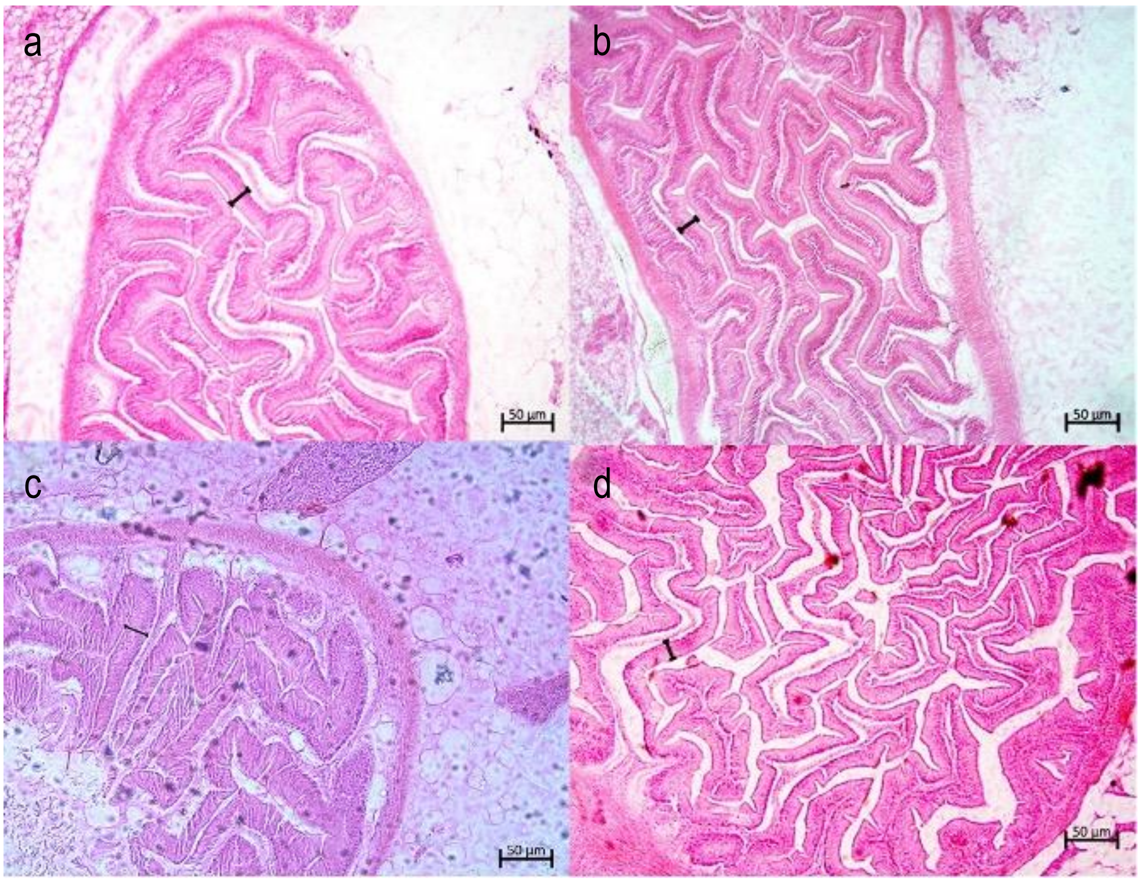


**Figure 2.** Representative histological images of the effects of *T. zedrina* on juveniles of the two-band cichlid (*V. bifasciata*). High enterocyte (µm): a) T1 (0% *T. zebrina*), b) T2 (5% *T. zebrina*), c) T3 (10% *T. zebrina*), and d) T4 (15% *T. zebrina*).

*officinale*, and *Vaccinium ashei* at a 1% inclusion level for eight weeks and found no significant differences compared with the control treatment. Regarding the feed conversion ratio, the best result was observed in T2 at 0.78 ± 0.001, indicating greater feed utilization efficiency. Conversely, Salem & Abdel-Ghany (2018) evaluated diets for Nile tilapia supplemented with orange peel (*Citrus sinensis*) at inclusion levels of 0, 1, 2, and 4 g kg$^{-1}$ and reported that the best feed conversion ratio was obtained in the control treatment, indicating that orange peel supplementation did not improve feed efficiency. Finally, the highest condition factor was recorded in T4 (1.95 ± 0.20). Similarly, Abidin et al. (2022) evaluated the inclusion of neem (*Azadirachta indica*) in diets for *O. mykiss*, at levels of 0, 5, 7, and 10%, and reported that fish fed supplemented diets exhibited a higher condition factor than those in the control group.

Mamadou et al. (2020) reported that the inclusion of plant-derived ingredients in fish diets can improve digestibility, stimulate feed intake, and consequently enhance growth performance and survival. However,

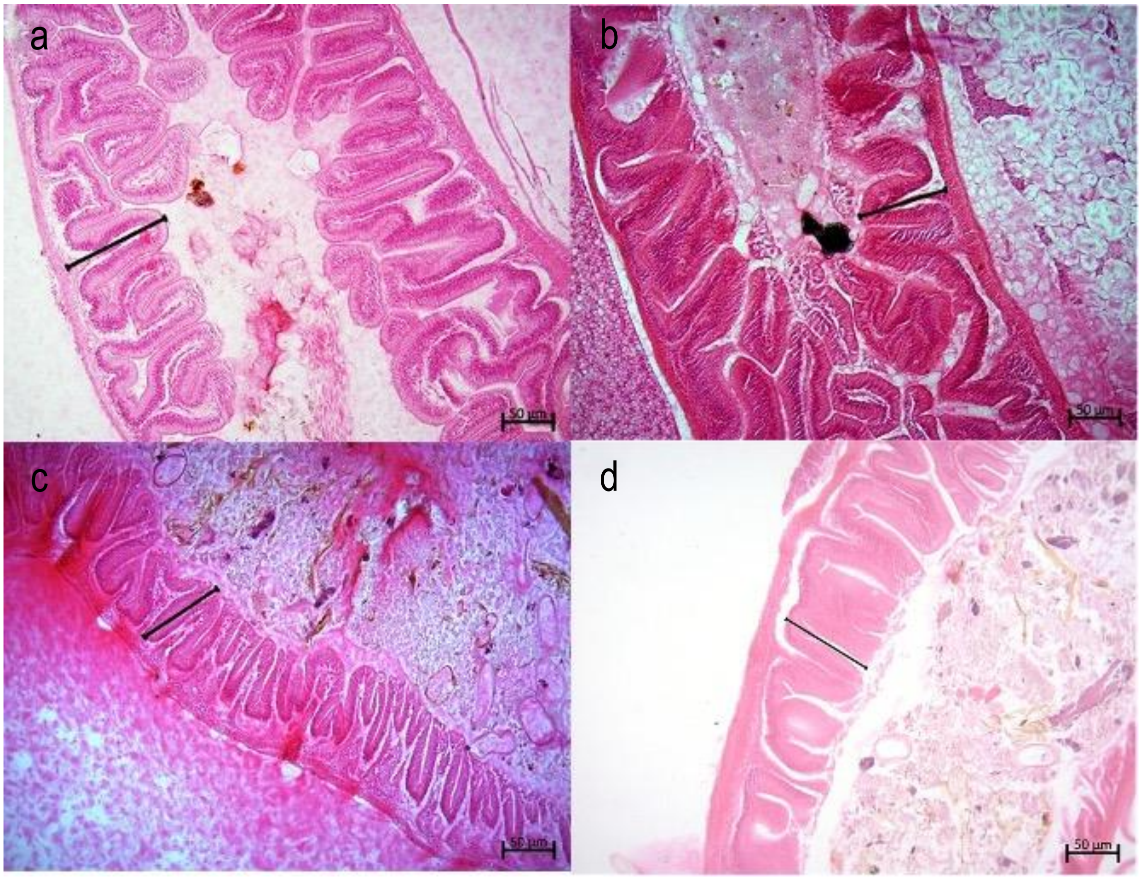


**Figure 3.** Representative histological images of the effects of *T. zedrina* on juveniles of the two-band cichlid (*V. bifasciata*). Enterocyte fold (µm): a) T1 (0% *T. zebrina*), b) T2 (5% *T. zebrina*), c) T3 (10% *T. zebrina*), and d) T4 (15% *T. zebrina*).

Vélez-Calabria et al. (2021) noted that some plants contain antinutritional compounds, deficiencies in carbohydrates and essential amino acids, and, in certain cases, may induce intestinal inflammation. Furthermore, Villasante et al. (2025) observed that plant supplementation in aquaculture diets can reduce growth performance under specific conditions. These observations are consistent with the results of the present study, which showed no significant differences among some treatments. At the same time, higher inclusion levels of *T. zebrina* were associated with negative effects on growth performance. Previous studies have demonstrated that *T. zebrina* possesses antioxidant, anti-inflammatory, cytotoxic, insecticidal, antibacterial, and antimicrobial properties (Alaba et al. 2014, Tan et al. 2014, Chan et al. 2015, Cheah et al. 2017), characteristics that have also been reported in other species belonging to the *Tradescantia* genus (Butnariu et al. 2022). Nevertheless, studies evaluating the morphological effects of herbal supplementation on fish digestive systems remain limited.

Our histological results demonstrated that enterocyte height decreased with increasing dietary inclusion of *T. zebrina*. However, intestinal fold length was greater in treatment T2, which was also associated with the best growth and size performance observed in treatments T2 and T3 compared with the control group. Previous studies have reported positive effects of herbal supplementation on digestive morphology in fish. For example, the inclusion of 1,000-1,500 mg $kg^{-1}$ of *Astragalus gallinaceus* in diets for Mozambique tilapia (*Oreochromis mossambicus*) improved intestinal villus length, crypt depth, muscular layer thickness, and the number of intestinal mucous cells (Huang et al. 2010). Similarly, Liu (2016) reported that supplementation with 1 g $kg^{-1}$ of *Eriobotrya japonica* in diets for goldfish increased intestinal villus length and muscle layer thickness. In the present study, the inclusion of 20% *T. zebrina* in diets for juvenile *V. bifasciata* significantly increased hepatocyte area. However, this treatment also resulted in the lowest values for growth, total length, and overall growth performance variables.

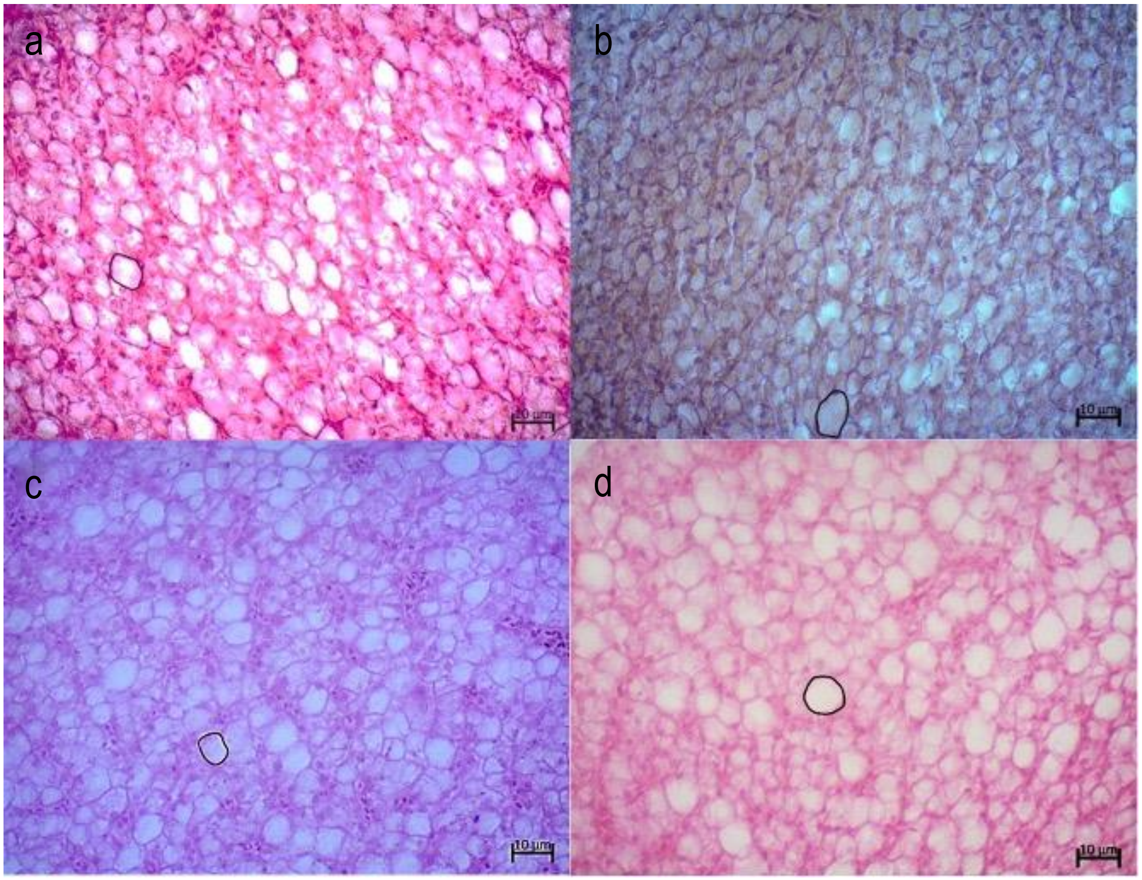


**Figure 4.** Representative histological images of the effects of *T. zedrina* on juveniles of the two-band cichlid (*V. bifasciata*). Hepatocyte area (µm): a) T1 (0% *T. zebrina*), b) T2 (5% *T. zebrina*), c) T3 (10% *T. zebrina*), and d) T4 (15% *T. zebrina*).

Previous studies have shown that supplementation with herbs such as green tea (*Camellia sinensis*), cinnamon (*Cinnamomum zeylanicum*), and American ginseng (*Panax quinquefolium*) can improve growth performance in Nile tilapia (*O. niloticus*) (Abdel-Tawwab et al. 2010, Ahmad et al. 2011, Abdel-Tawwab 2012). Likewise, certain herbal additives have been reported to enhance digestive enzyme activity and improve feed conversion efficiency, thereby improving growth performance (Radhakrishnan et al. 2014). Although the beneficial effects of herbal components in aquaculture diets have been widely described, incorporating histological analyses would strengthen the interpretation of these effects and provide stronger evidence of the physiological benefits associated with herbal supplementation.

## CONCLUSION

The cytotoxicity results indicate that the evaluated plant does not exhibit toxic effects, allowing it to be safely incorporated into fish feed. Minimal differences were observed among treatments in growth parameters, with T2 showing the best performance. It is suggested that additional research be conducted using different protein levels in diets containing 5% plant material to evaluate their nutritional and physiological effects. Likewise, it is recommended to explore other application methods for the plant to determine its efficacy under different culture conditions.

### Credit autor contibution

J.A. Castillo-Mayo: methodology and analysis; L. Rangel-López: methodology, analysis and original draft preparation; Y.J. Trejo-Sánchez: methodology; M.A. Perera-García: results analysis; M.N. Vite-García: results analysis; G.M. Pérez-Jiménez: methodology and results analysis; C.A. Álvarez-González: review and editing; C.A. Sepúlveda-Quiroz: methodology, analysis and original draft preparation. All authors have read and agreed to the published version of the manuscript.

**Conflic of interest**

The authors declare no conflict of interest

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