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ADDITION OF YERBA MATE (*Ilex paraguariensis* A. St. Hil.) IN TILAPIA FEED FOR THE GROWTH OF THE MAYAN CICHLID (*Cichlasoma uropthalmus* Günther)

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ABSTRACT

Native species with aquatic potential can be found in Southeastern Mexico, such as the Mayan cichlid (*Cichlasoma uropthalmus* Günther), which is commercially valuable and adaptable to farming conditions. The aim of this study was to evaluate the cytotoxicity and the effect of yerba mate (*Ilex paraguariensis* A. St. Hil.) on the growth parameters of the Mayan cichlid when added to a balanced tilapia feed. Dried, lightly toasted, and crumbled yerba mate from the ROSAMONTE brand was used. The cytotoxicity test was carried out using a bioassay with *Artemia salina* L. Serial dilutions from the yerba mate aqueous extract (0.78–100 mg mL^{-1}) were used to determine the mortality of the nauplii after 24 h of exposure and to calculate the lethal dose (DL50). The fish were distributed at random in nine interconnected fish tanks within a recirculation system, with 10 fish per unit. For 45 d, the fish were fed with a commercial tilapia diet (PURINA), homogenously mixed with non-pelletized yerba mate. Three replicates were established per treatment, corresponding to diets with 0, 1, and 2 % yerba mate (treatments T1 to T3). The feed was offered until the fish were satiated three times a day. A probit analysis was carried out to evaluate DL50. The productive parameters were evaluated with normality and homogeneity tests and arcsine transformation for the percentage data, followed by a one-way analysis of variance (ANOVA) and a Tukey test ($p < 0.05$). The DL50 obtained was 42.42 mg mL^{-1}, indicating a lack of toxicity. No significant differences were found in the productive parameters ($p > 0.05$). The best values were recorded in T3 for final weight (0.53 ± 0.04 g), food conversion rate (2.69 ± 0.06), and condition factor (1.86 ± 0.43), and in T2 for weight gain (0.43 ± 0.06 g) and specific growth rate (4.16 ± 0.89 %). The highest survival rate was obtained in T1 (93.3 ± 11.51 %) and T3 (93.3 ± 5.77 %). Yerba mate is not toxic and can be added to the diets of *C. urophthalmus*, although it does not significantly improve their growth.

Keywords: feed additive, cytotoxicity, growth parameters.

INTRODUCTION

The Mayan cichlid (*Cichlasoma uropthalmus* Günther) is a fish from the cichlid family. Its distribution ranges from the Coatzacoalcos River to Isla Mujeres in Mexico and includes Belize, Guatemala, Honduras, and Nicaragua (Villarreal *et al*., 2011). The species is promoted (Calzada-Ruiz *et al*., 2019) and introduced into aquaculture programs (Jiménez-Martínez *et al*., 2009), based on research on its reproduction, fecundity, nutrition, and population density (Jiménez-Martínez *et al*., 2012).

Yerba mate (*Ilex paraguariensis* A. St. Hil.) is consumed on a daily basis as an infusion in South American countries such as Argentina, Paraguay, Brazil, and Uruguay (Avena-Álvarez *et al*., 2019). This plant contains several active compounds, such as xanthines, from which caffeine, theophylline, and theobromine are derived (Messina *et al*., 2015). Additionally, it contains polyphenols, caffeoyl derivatives, saponins, triterpenes, and basic metabolic minerals, all considered beneficial due to their antioxidant and hypolipidemic effects, with benefits to the central nervous system (Cuesta *et al*., 2018). It also contains tannins, which increase the efficiency of food intake and are also considered beneficial for health (Ramírez *et al*., 2022). On the other hand, Pozebon *et al*. (2015) determined the presence of other elements such as aluminum (Al), barium (Ba), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorous (P), strontium (Sr), and zinc (Zn).

Kuropka *et al*. (2021) administered a yerba mate hydroalcoholic extract to mice at a concentration of 10 mg kg^{-1} of body weight per day and found that it has a kidney protective effect and reduces high cholesterol. In turn, Lobo *et al*. (2020) fed lambs a 2 % yerba mate extract, favoring the intake of dry matter and reducing fat thickness, as well as producing an increase in the final weight and in white blood cells and lymphocytes. Barbato *et al*. (2019) obtained a difference of 1.79 L in milk produced in cows supplemented with 500 g of yerba mate a day in comparison with the cows fed normally.

The addition of plants rich in active compounds to the feed of farmed fish can favor their growth parameters, as shown with *Ulva lactuca* L. and *Lemna gibba* L., obtaining the greatest weight in young tilapia with the addition of 20 % of these plants (Aguilera-Morales *et al*., 2022). Hassan *et al*. (2018) found that by adding 1 % *Rosmarinus officinalis* Spenn., they obtained a greater growth rate in tilapia offspring. Salem and Abdel-Ghany (2018) obtained better growth results when they gave 2 g kg^{-1} of *Citrus sinensis* rind (L.) Osbeck. Cruz-Velázquez *et al*. (2014) obtained better growth in pacu (*Piaractus brachypomus*) and tilapia (*Oreochromis niloticus*) when they added 15 % *Lemna minor* L. and *Azolla filiculoides* Lam. to their feed. Therefore, the aim of this study was to evaluate the cytotoxicity and effect of yerba mate added to the tilapia feed on the growth of the Mayan cichlid.

MATERIALS AND METHODS

Plant material

Yerba mate (*Ilex paraguariensis* A. St. Hil.) from ROSAMONTE (Misiones, Argentina) was used. According to the product specifications, the leaves are dried, lightly toasted, crumbled, and packaged in paper bags. The nutritional information reports 4.7 g of carbohydrates, 0.6 g of proteins, 0 g of total and trans fats, 0 g of sodium, and an energy content of 21 kcal. Prior to analysis, the plant material underwent additional grinding to achieve a particle size of 0.35 mm, resulting in a flour-like texture.

Cytotoxicity test

For the cytotoxicity test, 0.1 g of artemia cysts (*Artemia salina* L.) were weighed and placed in a salt solution (38 g L^{-1}) to hatch. They were constantly ventilated for 24 h at temperatures from 25 to 29 °C. Subsequently, 100 mg of the yerba mate were weighed and placed in a microcentrifuge tube with a cover and 1 ml of the salt solution and left to stand for 48 h. In a 96-well plate, a serial microdilution was performed, where 100 µl of the yerba mate aqueous extract was placed, obtaining concentrations between 100 and 0.78 mg mL^{-1}. Straight afterwards, 10 to 15 artemia were added, with 100 µL of the salt solution. Tween 80 (Sigma P1754, St. Louis, MO, USA) was used as a positive control and salt solution as a negative control. The plate was incubated for 24 h, and the test was evaluated in triplicate. After this time period, a reading was taken from every well, where the living and dead artemia were counted to determine the percentage of mortality using the following formula (Rangel-López *et al*., 2022):

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

In order to establish the level of toxicity, the criteria defined by Mentor *et al*. (2014) were applied, in which the values of over 1 mg mL^{-1} indicate the absence of toxic effects, values between 0.5 and 1 mg mL^{-1} indicate low toxicity, those between 0.1 and 0.5 mg mL^{-1} indicate medium toxicity, and values that do not surpass 0.1 mg mL^{-1} indicate high toxicity.

Preparation of the experimental feed

According to the manufacturer's information, PURINA (Mexico) commercial tilapia feed (0.35 mm) was used, with a protein content of 44 %, 12 % raw fat, 2.5 % raw fiber, 12 % humidity, and 12 % ash. The food was uniformly mixed with yerba mate (Table 1) and not pelletized.

Experimental design

The experiment was established in the aquaculture laboratory of the Academic Division of Farming Science at Juárez Autonomous University in Tabasco, Mexico (17° 78″ 59′ N, 92° 95″ 50′ W). A total of 120 fish were used, which were weighed

Table 1. Commercial feed and yerba mate (*Ilex paraguariensis* A. St. Hil.) content per treatment for Mayan cichlid consumption (*Cichlasoma uropthalmus* Günther).

Ingredients	T1: Control	T2: 1 % yerba mate	T3: 2 % yerba mate
Feed	100 g	99 g	98 g
Yerba mate	0 g	1 g	2 g
Total	100 g	100 g	100 g

and measured individually, for an average weight of 0.15 ± 0.11 g. The fish were distributed at random in nine tanks with a capacity of 80 L each, and 10 fish were placed in each tank. To maintain water quality in all treatments (water temperature of 28°C, dissolved oxygen of 5 ppm, pH of 8, and total ammonium below 0.1 mg L^{-1}), the tanks were linked together in a recirculation system. All treatments were carried out in triplicate. The feed for each corresponding treatment was given three times per day, at 8:00, 11:00, and 14:00, until satiation. The experimental period was 45 d long.

Growth parameters

Using an ichthiometer (mm) and a digital scale with an accuracy of 0.1 g (Ohaus Scout Pro SP601, Mexico), the initial biometrics for total length and weight were measured every 15 d. To determine the growth parameters, the formulas proposed by Arellano-Carrasco *et al*. (2023) were used:

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

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

$$\text{Specific growth rate} = \frac{(\ln \text{Final weight} - \ln \text{Initial weight}}{\text{Days fed}} \times 100$$

$$\text{Feed conversion rate} = \frac{\text{Feed provided}}{\text{Weight increase}}$$

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

Statistical analysis

The lethal dose values (DL50) of the yerba mate aqueous extract were analyzed with a probit analysis using the SAS version 9.0 software (SAS Institute, Cary, NC, USA). From the data obtained on the productive parameters, Kolmogórov-Smirnov normality tests and Bartlett's homogeneity tests were performed. For the parameters expressed as percentages, an arcsine square root transformation was applied. Subsequently, a one-way analysis of variance (ANOVA) was applied ($\alpha = 0.05$), as well as Tukey's test with a significance level of $p < 0.05$ to determine significant differences between treatments, using the package Minitab 18.

RESULTS AND DISCUSSION

Cytotoxicity of yerba mate

According to the criteria established for interpreting the results of the aqueous extract (Figure 1), yerba mate had a toxicity value of 42.42 mg mL^{-1}, indicating that it is nontoxic. Therefore, the use of this plant added to the tilapia feed is considered safe for fish intake. In comparison, Waghulde *et al*. (2019), who evaluated the aqueous extracts of *Annona reticulatatuvo* L. (18.92 mg mL^{-1}), *Allium fisolisum* L. (1846.55 mg mL^{-1}), and *Brassica oleraceaae* L. (64.83 mg mL^{-1}), determined that each species has a degree of toxicity. In turn, Alawi *et al*. (2018) determined that the aqueous *Acacia nilotica* (L.) Delile extract presents no toxicity at concentrations of 500 ppm.

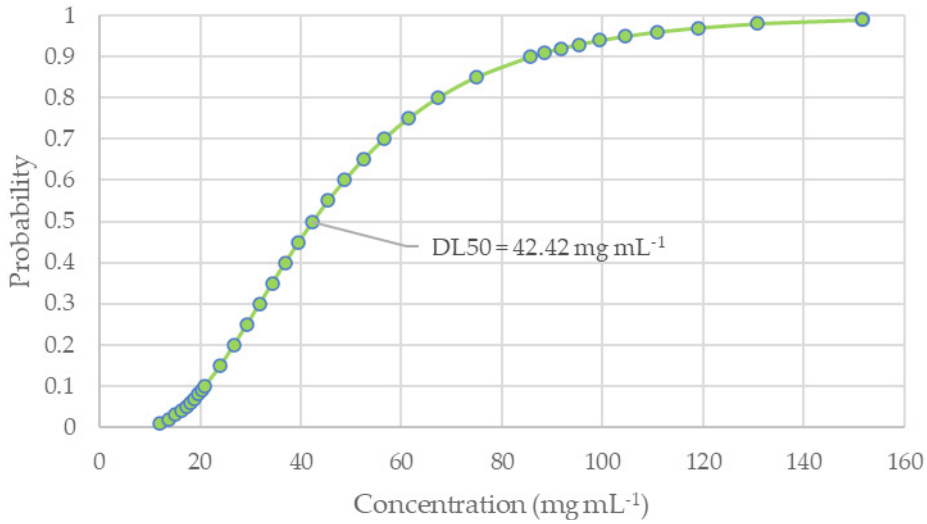

Figure 1. Cytotoxicity of the lethal dose (DL50) of the yerba mate (*Ilex paraguariensis* A. St. Hil.) aqueous extract.

Growth parameters

Experiments have been conducted on the incorporation or addition of plants to fish diets, including seed, leaf, and plant meals (Zetina-Córdoba *et al*., 2010), which may have immunostimulant properties and enhance fish growth (Akrami *et al*., 2015). The results of the growth parameters (Table 2) indicate that no significant differences were observed ($p > 0.05$) between treatments. However, in terms of final weight, treatment T3 (0.53 ± 0.04 g) stood out in comparison to the control. In contrast, Botello-León *et al*. (2011) substituted fish meal with sugarcane by-products, obtaining the best results in the treatments with 14 and 26 % protein.

Table 2. Growth parameters of the Mayan cichlid (*Cichlasoma uropthalmus* Günther) in the treatments evaluated.

Growth parameters	T1: control	T2: 1 %	T3: 2 %
Initial weight (g)	0.15 ± 0.11	0.15 ± 0.11	0.15 ± 0.11
Final weight (g)	0.46 ± 0.04^{A}	0.52 ± 0.04^{A}	0.53 ± 0.04^{A}
Survival (%)	93.3 ± 11.55^{A}	90.0 ± 10.0^{A}	93.3 ± 5.77^{A}
Weight gain (g)	0.31 ± 0.04^{A}	0.40 ± 0.09^{A}	0.38 ± 0.04^{A}
Specific growth rate (%)	2.52 ± 0.19^{A}	3.43 ± 1.35^{A}	2.80 ± 0.18^{A}
Feed conversion rate	3.07 ± 0.17^{A}	2.88 ± 0.48^{A}	2.69 ± 0.06^{A}
Condition factor	1.68 ± 0.16^{A}	1.75 ± 0.14^{A}	1.86 ± 0.43^{A}
Initial length (cm)	1.88 ± 0.07	1.86 ± 0.23	1.89 ± 0.05
Final length (cm)	3.08 ± 0.04^{A}	3.09 ± 0.05^{A}	3.07 ± 0.24^{A}

T1, T2, T3: treatments with yerba mate (*Ilex paraguariensis* A. St. Hil.) included in the diet. ^{ABC} Different letters in the rows indicate significant statistical differences ($p \leq 0.05$).

In terms of survival, the best results were obtained for treatments T1 and T3 (93.3 ± 11.55 and 93.3 ± 5.77 %). On the other hand, Kristiana *et al.* (2020) added noni (*Morinda citrifolia* L.) fruit to commercial tilapia feed with 40 % protein in various concentrations and obtained a survival rate of 100 % in all treatments. Villarreal *et al*. (2011) added soybean protein and wheat gluten to the feed and found that the treatments with higher percentages showed higher mortality. It is worth noting that the Mayan cichlid is considered to be aggressive and territorial, which increases its survival rate.

For weight gain, T2 (0.43 ± 0.06 g) produced the best yields. Emshaw *et al*. (2023) used *Pontederia crassipes* (Mart.) Solms fermentation in young tilapia and found that adding 10 % of the fermentation improved weight gain; however, their control group had similar results. Abidin *et al.* (2022) added neem (*Azadirachta indica* A. Juss.) extract to feed rainbow trout (*Oncorhynchus mykiss*) and found a greater weight gain in fish fed with a 7 % extract. In this study, the best feed conversion rate was obtained in T3 (2.69 ± 0.06). Dohaish *et al*. (2018) reported a feed conversion rate higher than that of this study when adding 5 % *Spirulina platensis* in the diets of *Oreochromis niloticus*.

The specific growth rate was best represented by T2 (4.16 ± 0.89). In comparison, Galeana-López *et al*. (2020) provided maize (*Zea mays* L.) ear leaf extract in the diets of *O. niloticus*, obtaining the best specific growth rate with the 200 mg kg^{-1} feed treatment. Specifically, no comparisons could be made with other authors, due to a total lack of investigations on fish using yerba mate. Regarding the condition factor, the best result was observed in treatment T (1.86 ± 0.43), which is similar to reports by Martínez-Santiago *et al*. (2022), who used oregano essential oil (*Lippia graveolens* Kunth) in tilapia feed.

Yerba mate has 9.5 % protein, 6.3 % lipids, and 7.04 % ash content (Berté *et al*., 2011; Cogoi *et al*., 2013; Frizon *et al*., 2013), as well as secondary metabolites such as methylxanthines, caffeine, theobromine, phenolic acids, chlorogenic acid (Valduga *et al*., 2016), saponins, triterpenes, flavonoids, and xanthines (García-Lázaro, 2023), tannins, carotenoids, and polyphenols (Najman *et al*., 2024). Lizárraga-Velázquez *et al*. (2018) mention that when polyphenols are added to or present in carnivorous fish feed, they can improve immune and antioxidant defenses. Ayutunde *et al*. (2016) mention that diets with leaf meals containing phenols, tannins, and saponins may hinder fish growth. Therefore, yerba mate could benefit fish by enhancing their immune system. However, it can affect the growth parameters of their offspring (Lobo *et al*., 2020).

CONCLUSIONS

Based on the cytotoxicity results with *Artemia salina*, it was determined that the plant can be included in the diet of *Cichlasoma uropthalmus* since it does not exhibit toxicity and therefore does not have a negative effect on its feeding. No variations were found in the growth parameters. Further research that includes a higher proportion of yerba mate in the diet and in pellet form is recommended, as well as studies to determine whether it has an impact on the immune system of the fish.

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