Effect of dietary tannins on productive parameters and diesel toxicity in juvenile Nile tilapia (*Oreochromis niloticus*)

Running title: Effect of dietary tannins on Nile tilapia
Section: Original Research Articles
doi: 10.22201/fmvz.24486760e.2026.1420

Julio César Cruz-Valdez[1]
0000-0003-2832-7745

Oscar Daniel García Pérez[1*]
0000-0002-1642-935X

Rodrigo Gallardo-Morales[1]

Jorge Ramsy Kawas-Garza[2]
0000-0003-3543-4506

Denisse Garza-Hernández[1]
0000-0002-2317-1043

Alicia Guadalupe Marroquín-Cardona[1]
0000-0002-3973-7128

[1] Universidad Autónoma de Nuevo León. Facultad de Medicina Veterinaria y Zootecnia. General Escobedo, Nuevo León, México.

[2] Universidad Autónoma de Nuevo León. Facultad de Agronomía. General Escobedo, Nuevo León, México.

* Corresponding author: oscar_garcia83@hotmail.com; oscar.garciapr@uanl.edu.mx

Dates:
Submitted: 2024-10-02
Accepted: 2025-10-27
Published: 2026-02-26

Cite this as:

Cruz-Valdez JC, García Pérez OD, Gallardo-Morales R, Kawas-Garza JR, Garza-Hernández D, Marroquín-Cardona AG. Effect of dietary tannins on productive parameters and diesel toxicity in juvenile Nile tilapia (*Oreochromis niloticus*). Veterinaria México OA. 2026;13. doi: 10.22201/fmvz.24486760e.2026.1420.

Effect of dietary tannins on productive parameters and diesel toxicity in juvenile Nile tilapia (*Oreochromis niloticus*)

Abstract

Nile tilapia is a highly nutritious source of protein, and its production through aquaculture practices leads to a lower carbon footprint when compared to the production of terrestrial species. However, environmental contamination poses a significant risk in aquaculture, necessitating strategies to mitigate its impacts on fish. This study evaluated the effects of experimental diets supplemented with tannins on performance parameters of *Oreochromis niloticus* over a 60-day feeding trial. At the end of the experiment, their potential hepatoprotective role was assessed in fish exposed to diesel-induced toxicity. The fish were fed a control diet (T1) or a diet supplemented with either 2 % (T2) or 4 % tannins (T3). Fourteen fish per tank were allocated across three tanks per treatment. After 60 days, seven fish from each treatment group were exposed to an acute diesel exposure to either an acute dose of diesel (50 µg/g) or fish oil (control). Performance parameters were then compared, and liver samples were obtained and used to determine alkaline phosphatase (ALP), carboxylesterase (CaE), and glutathione S-transferase (GST) enzymatic activity. Diesel exposure led to increased ALP activity, decreased CaE, and GST activities. However, no significant alterations in enzyme activities were observed in fish maintained under dietary treatments T2 and T3 following diesel exposure compared to the control group. Moreover, performance parameters remained unaffected by the inclusion of tannins in diets. These findings suggest that dietary tannins may provide a protective effect against diesel-induced physiological disturbances.

Keywords: Tannins; Diet; Diesel toxicity; Tilapia; Enzyme activity.

Study contribution

Aquaculture is among the world's fastest-growing industries, and tilapia is one of the most farmed fish. Feed represents a high percentage of the production cost, so diets are continually designed to improve animal nutrition. Another problem is the increase of xenobiotics in aquatic environments and water sources used, causing negative effects on cultured organisms. The present study evaluated the effect of including condensed tannins in diets for tilapia exposed to diesel. The inclusion of tannins in the tilapia diet did not cause changes in performance parameters; however, when the tilapia were exposed to diesel, it assisted in lowering the physiological stress effect on the liver, observing a minor alteration in the activity of hepatic detoxification enzymes. Therefore, the inclusion of tannins in tilapia diets can reduce the physiological stress caused by this type of xenobiotic.

Introduction

Aquaculture production is among the most profitable activities contributing to the global economy. It provides access to affordable, high-quality protein sources and is the world´s fastest-growing industry, producing approximately 80 million tons annually. This growth necessitates ongoing research and technical innovations.(1) Anthropogenic activities have led to contamination in aquaculture environments, as most pollutants end up in water bodies.(2, 3) Aquifer contamination, along with abrupt temperature changes and low dissolved oxygen levels, is are common threat in the aquaculture industry, adversely affecting animal production.(4) Petroleum derivatives, particularly diesel oil, are significant pollutants in aquatic ecosystems and are highly toxic to aquatic organisms.(5, 6)

In the presence of a xenobiotic, such as diesel, the physiological system of organisms initiates specific chemical reactions catalyzed by detoxification enzymes, primarily produced by the liver.(7) Carboxylesterase (CaE), one of the phase I enzymes, transforms xenobiotics into more polar compounds by modifying their functional groups,(8) thereby enabling phase II enzymes, such as GST, to conjugate these products and facilitate their excretion. Alkaline phosphatase (ALP) is an inducible enzyme whose activity increases in response to hepatic injury, tissue regeneration processes, or contaminant-induced stress, observed across both aquatic organisms and mammals. These enzymes have been extensively studied and are used as exposure biomarkers in aquatic organisms; furthermore, they are highly relevant in toxicology due to their ability to exhibit significant variations within minutes, hours, or days following exposure.(9)

Incorporating alternative plant ingredients into diets for aquatic organisms is a common strategy to enhance antioxidant capacity.(10) Tannins, a group of phenolic compounds, have garnered significant attention for their potential benefits. They are prevalent in many higher plants and can be classified based on their chemical structure into condensed and hydrolysable tannins.(11) The anticarcinogenic and antimutagenic properties of tannins are attributed to their antioxidative capabilities, particularly in inhibiting the generation of free radicals, thereby protecting against cellular damage and reducing the effects of physiological stress on the liver.(12) Therefore, the objective of the present study was to evaluate the protective effect of experimental diets containing 2 % and 4 % of a commercial tannin blend-comprising hydrolysable tannins (HT) and condensed tannins (CT)- on the performance of Nile tilapia (*O. niloticus*) during a 60-day bioassay. At the end of the trial, fish were exposed to hydrocarbon-induced stress to measure the enzymatic activity of CaE, GST, and ALP as indicators of hepatic protection.

Materials and methods

Ethical statement

The animal study protocol was approved by the Ethics Committee of Veterinary Medicine School at Universidad Autónoma de Nuevo León (Animal research protocols 36/2022, 52/2022).

Experimental diets

Formulated diets for juvenile tilapia were prepared using corn, soybean meal, and wheat as the main ingredients to ensure optimal growth. A control diet (CD) containing 36 % crude protein and 7.5 % crude fat was prepared and used as a baseline for creating diets with 2 % (T2) and 4 % (T3) tannin inclusions, substituting for portions of corn, soybean meal, and wheat flour (**Table 1**). The commercial product Sylvafeed® ByPro (Silvateam, Peru), derived from chestnut (*Castanea sativa*) and quebracho (*Schinopsis* spp.) trees, was the tannin source. This commercial blend, in powder form, is known to have a composition of 0.70 g/g (tannic acid equivalent) from chestnut and 0.16 g/g CT (leucocyanidin equivalent) from quebracho.(13)

Therefore, the T2 diet (2 % tannins) contained 14 g HT and 6 g CT per kg of feed, while the T3 diet (4 % tannins) had 28 g HT and 12 g CT per kg. The ingredients were ground, mixed, and the final feed was processed using an Optima Single Screw Wenger X-165 extruder (Sabetha, KS, USA) to produce 4 mm diameter pellets.

Table 1. Composition and proximate analysis of experimental diets for Nile tilapia

	Tannins (%)		
Ingredients (%)	**0**	**2**	**4**
Corn, ground	19	18.9	17.5
Soybean meal	54	53	52.5
Wheat meal	17.33	16.8	16.7
BHT[1]	0.02	0.02	0.02
Soybean oil	4.4	4.4	4.39
Monocalcium phosphate	3.6	3.4	3.4
Premix[2]	0.5	0.5	0.5
Arginate	0.5	0.5	0.5
Vitamin C	0.05	0.05	0.05
Sodium chloride	0.5	0.5	0.5
Tannins[3]	0	2	4
Total	100	100	100
Chemical analyses			
Crude protein (g/kg)	358	349	356
Crude lipids (g/kg)	75	77	74
Gross energy (Kcal/g)	3.8	3.8	3.9

[1] BHT = butylhydroxytoluene, antioxidant.

[2] Premix = Mineral and vitamin premix.

[3] Tannins = tannins of chestnut (*Castanea sativa*) and quebracho (*Schinopsis* spp.) trees.

Study design and rearing system

Nile tilapia was provided by a commercial hatchery in Soto La Marina, Tamaulipas, Mexico. The fish were acclimated (4 days) to glass tanks under the following water conditions: temperature 30 ±0.7 °C, pH 8.5 ±0.2, total ammonia nitrogen (0.08 ±0.05 mg/L), nitrate (below detection limits), nitrites (11.3 ±3.9 mg/L), and saturated dissolved oxygen. A natural photoperiod provided a 12:12-h light-to-dark ratio. After the acclimation period, one hundred and twenty-six juvenile fish with an initial mean wet weight of 0.186 ±0.001 g were randomly allocated into one of the three dietary treatments. Each treatment was done in triplicate glass tanks, with 14 animals per tank. The tanks, equipped with integrated biological filters and aeration pumps, were filled up to an operational volume of 120 L, and water was replaced every three days. The initial feeding ratio was based on a 10 % total biomass per tank and was provided at three feeding times (8:00, 12:00, and 17:00 h). The feces and uneaten feed were siphoned out of the tank daily in the morning before the feeding. Feeding rations were adjusted according to fish weight gain and the total number of animals in the tanks.

Performance parameters

At the end of the study (day 60), the weight of each fish was individually recorded using a precision analytical scale (A&D, Precision FZ-i/FX-i Series). Excess water was removed from the organisms with a cotton cloth before weighing. Survival and feed consumption were recorded daily. Growth rate per tank was calculated as the difference between the average final weight and the average initial weight. Feed intake was estimated daily based on the feed added to the tank, the feed remaining the next day, and the number of fish in the tank. Total feed consumption was estimated for the entire study period. The feed conversion ratio was calculated based on the weight of feed consumed per unit of weight gain. The survival rate was calculated for each tank.

Acute diesel exposure challenge

To assess whether any of the experimental diets provided to Nile tilapia could help maintain biomarker activity at levels like those observed in the control group despite xenobiotic exposure, fourteen fish per treatment were maintained at the end of the feeding period (60 days). Seven fish were exposed to diesel in one aquarium, while the remaining seven were kept in a separate aquarium as the control group. For diesel exposure, fish were intraperitoneally injected with 0.2 mL of menhaden fish oil laboratory-grade (Sigma Aldrich-F8020) containing a dose of 50 µg/g of petroleum diesel. The control fish received an injection of 0.2 mL of menhaden oil without diesel. To ensure minimal contamination and proper asepsis, new syringes were used for each fish during the intraperitoneal application.

The exposure period, method of application, and dosage were selected based on previous research.(5, 9, 14) After intraperitoneal injection, fish were kept in their respective tanks for 72 hours, and after that, they were euthanized by hypothermia. The sampling time was determined based on subsequent studies in which a positive biomarker response was observed.(5, 9, 14) These procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine at Universidad Autónoma de Nuevo León (Animal research protocols 36/2022, 52/2022). Liver samples were then collected and stored in a deep freezer at -70 °C for subsequent enzymatic activity analysis. The treatment groups for analyzing enzymatic activity were established as follows: OT1, fish oil exposed and fed with CD (no tannins); OT2, fish oil exposed and fed the 2 % tannins diet; OT3, fish oil exposed and fed the 4 % tannins diet; DT1, diesel exposed and fed CD; DT2, diesel exposed and fed 2 % tannins diet; and DT3, diesel exposed and fed 4 % tannins diet.

Enzymatic activity biomarkers

Liver samples from fish in each tank (n = 7) were utilized to measure ALP, CaE, and GST levels. These samples were homogenized in double-distilled water using a 1:10 ratio (sample weight to water, m/v) with a mortar and pestle for four minutes. The homogenized samples were then centrifuged at 15 300 *g* for 30 minutes at 4 °C. The supernatant was separated from the superior lipid layer and from the precipitate and was aliquoted into tubes (0.1 mL) and stored at -150 °C until further use. The protein content of the extracts was quantified using the Bradford method, with bovine serum albumin as the standard for the calibration curve.(15)

CaE activity was determined in microplates following the methodology described by Aguilera-González et al.(9) The reaction mixture consisted of 200 µL of 50 mM tris-HCl buffer (pH 7.1), 10 µL of extract, and 100 µL of 2 mM p-nitrophenyl acetate. Absorbance was measured at 405 nm for 120-second intervals over a period of 10 min using an EPOCH microplate reader (Biotek, Vermont, USA). Each sample had three analytical replications, and a buffer was used as a control, accordingly. The linearity of the reaction was verified, and enzymatic activity was expressed in µmol/min/mg/protein using the molar extinction coefficient of 18.5 mM/cm for p-nitrophenol.

ALP activity was determined using p-nitrophenyl phosphatase substrate.(16) The reaction consisted of 200 µL of diethanolamine buffer (1.0 M) with 50 mM $MgCl_2$ (pH 9.8), 10 µL of the enzymatic extract, and 10 µL of substrate to achieve a final concentration of 0.4 mM. Absorbance was measured at 405 nm under the same conditions as for CaE, and enzymatic activity was expressed in µmol/min/mg/protein, using the molar extinction coefficient of 18.5 mM/cm for p-nitrophenol. GST activity was analyzed using a method adapted to microplates.(17) The substrate mixture contained 300 µL of reduced L-glutathione (200 mM) and 1-chloro-2, 4-dinitrobenzene (CDNB;100 mM) in Dulbecco's phosphate-buffered saline as well as 10 µL of enzymatic extract as a reaction initiator. Absorbance was measured every minute for 10 minutes at a wavelength of 340 nm. GST activity was expressed in µmol/min/mg/protein, with a molar extinction coefficient of 5.3 mM/cm for CDNB.(18)

Statistical analysis

SPSS statistical program was used for data analyses (SPSS 16.0, 2007; SPSS Inc., Chicago, Illinois). Recorded final weight, feed intake, growth rate, feed conversion ratio, and survival rate were analyzed with one-way ANOVA. Before analysis, data were tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively, and both assumptions were met. Tukey's multiple range test was used to detect differences among experimental diets. To assess the effects of tannin supplementation and diesel exposure on enzymatic biomarkers, a two-way factorial ANOVA was conducted (2 tannin levels × 2 exposure type), followed by Tukey's test for multiple comparisons. The significance level was set at $\alpha = 0.05$.

Results

Dietary effects on growth

The growth performance of the fish, in terms of individual final weight, feed consumption, feed conversion rate, and percentage of weight gain, is shown in **Table 2**. At the end of the 60-day study, there were no significant effects ($P > 0.05$) of dietary tannin on the growth performance of the fish at any of the inclusion levels. An overall fish survival rate of 91 % was recorded through the study, and no statistical differences ($P > 0.05$) were detected among treatments.

Table 2. Performance parameters of Nile tilapia fed for 60 days with experimental diets

Experimental diet*	Mean weight (g)	Feed intake (g/fish)	Growth rate (%)	Feed conversion ratio
CD	26.7 ±6.6	32.3 ±12	1435 ±600	3.9 ±2.4
T2	23.3 ±7.3	31.1 ±16	1483 ±503	4.7 ±3.5
T3	27.8 ±7.0	33.9 ±17	1556 ±506	3.5 ±2.2
Probability	0.6999	0.8340	0.9755	0.9970

* CD, Control Diet; T2, 2 % tannins diet; T3, 4 % tannins diet.

Data are presented as means ±standard deviation (n = 3). Data were analyzed with One-way ANOVA.

Enzymatic activity

Overall, fish in the DT1 group (exposed to diesel and fed a control diet with 0 % tannins) exhibited lower enzymatic activity of CaE ($P = 0.0060$) and GST ($P = 0.0010$) compared to those in the OT1 group (fed fish oil and a control diet with 0 % tannins). Conversely, for ALP activity, DT1 group animals displayed the highest enzymatic activity among all dietary groups ($P < 0.0010$). In terms of GST activity, diesel-exposed fish from the DT1 (0 % tannins) and DT2 (2 % tannins) groups showed the lowest enzymatic activity. However, animals in the DT3 group (4 % tannins) had GST activity levels comparable to those in treatments without diesel exposure (**Table 3**). Regarding CaE activity, animals in the DT2 group showed no significant differences compared to fish that consumed the control diet DT1. Interestingly, fish in the DT3 treatment (4 % tannins) exhibited similar CaE activity levels ($P > 0.05$) to those in the control group without diesel exposure (**Table 3**).

Table 3. Enzymatic activity of alkaline phosphatase, carboxylesterase, and glutathione S-transferase in Nile tilapia fed for 60 days with tannins and exposed to diesel

Treatment groups ç	**ALP†**	**CaE†**	**GST†**
OT1	1.07 ±0.3^{a}	1.63 ±0.34^{b}	4.89 ±0.28^{b}
OT2	1.93 ±1.4^{ab}	1.33 ±0.43^{ab}	4.75 ±0.42^{b}
OT3	1.64 ±0.4^{a}	1.33 ±0.2^{ab}	4.68 ±0.32^{b}
DT1	3.27 ±2.0^{b}	1.1 ±0.43^{a}	4.16 ±0.69^{a}
DT2	0.79 ±0.1^{a}	1.29 ±0.56^{ab}	4.49 ±0.26^{ab}
DT3	1.36 ±0.3^{a}	1.56 ±0.27^{b}	4.79 ±0.26^{b}
One-way ANOVA Probability	0.001**	0.006**	0.001**
Factorial probability			
Diet (% tannins)	0.076	0.538	0.0214*
Exposure (Oil vs Diesel)	0.409	0.180	0.003
Diet × Exposure interaction	0.001**	0.004**	0.003**

ALP: alkaline phosphatase.

CaE: carboxylesterase.

GST: glutathione S-transferase.

ç OT1: fish oil + CD; OT2: fish oil + 2 % tannins; OT3: fish oil + 4 % tannins; DT1: diesel + CD; DT2: diesel + 2 % tannins; DT3: diesel + 4 % tannins.

† Enzymatic activity is expressed as μmol/min/mg/protein (mean ± standard deviation, n = 7).

*Significant probability ($P < 0.05$).

**Highly significant probability ($P < 0.01$). Different letters in the same column indicate significant differences among treatments with Tukey mean comparisons ($P < 0.05$).

Discussion

Our results demonstrated that feeding Nile tilapia (*Oreochromis niloticus*) diets containing 2 % and 4 % tannins did not alter performance parameters such as mean weight, feed intake, growth rate, and feed conversion ratio compared to fish fed a control diet (CD) for 60 days. The performance of *O. niloticus* fed diets supplemented with tannins aligns with previous findings. For example, feeding Nile tilapia with three different levels of condensed tannins (5, 15, and 25 g/kg) did not affect feed intake, body weight gain, final body weight, or feed conversion ratio over an 80-day trial.(19) However, the same study reported a negative impact on these parameters when hydrolysable tannins were used at inclusion levels of 15 and 25 g/kg (i.e., 1.5 and 2.5 %, respectively). Previous research studies conducted on *Lateolabrax japonicus* indicated that a diet supplemented with 0.01 % condensed tannins improved growth and reduced serum glucose levels.(20)

Interestingly, the current study found that a diet with 4 % quebracho tannins was well-tolerated by *O. niloticus*, as no significant differences were observed in performance parameters between fish consuming the control diet and those ingesting the tannins (T2, T3). For a long time, fish have been used as indicators of water quality because they are extremely sensitive to environmental contaminants that cause changes in growth and other physiological responses.(21) The analysis of key enzymes involved in the biotransformation of xenobiotics can serve as biomarkers of contaminant exposure and may help assess the protective effects of dietary supplements in organisms subjected to environmental stressors.(5)

Exposure to xenobiotics can induce changes in the activity of phase I and II hepatic enzymes associated with the detoxification pathway, and their evaluation provides valuable insight into the physiological impact of such stressors on the liver.(14) In the present study, the intraperitoneal route was selected to ensure a controlled and effective delivery of the xenobiotic. This Approach was based on a protocol described by Aguilera-González et al.,(9) who evaluated three different modes of xenobiotic exposure in fish—including dietary, waterborne, and intraperitoneal administration. Their findings demonstrated that intraperitoneal injection at a specific dose allows for precise application that consistently induces significant changes in hepatic detoxification enzyme activity without causing mortality, thereby providing a reliable model for assessing sublethal physiological responses.

In the acute diesel exposure challenge, fish from DT1 (0 % + diesel exposure) exhibited significantly higher ($P < 0.0010$) ALP activity and lower CaE ($P < 0.0060$) and GST ($P < 0.0010$) activity compared to fish from OT1 (0 % tannins). The decrease in CaE and GST activities in diesel-exposed fish is expected, as similar studies with *Atractosteus spatula* exposed to β-naphthoflavone and *Lepisosteus oculatus*, exposed to hexachlorobenzene, and hexachlorobutadiene showed reduced liver GST and CaE activities compared to control animals.(9, 22) Similarly, *Oreochromis niloticus* and southern sailfin catfish (*Pterygoplichthys anisitsi*) exposed to diesel, biodiesel, or their mixtures demonstrated inhibition of GST activity along with an increase in superoxide dismutase (SOD) activity.(5, 14)

The increased GST activity observed in Nile tilapia following diesel exposure is consistent with previous findings in other aquatic species considered more resistant(5) and potentially suitable as sentinels of hydrocarbon contamination, such as catfish(14) and other aquaculture species.(23–25) However, reduced GST activity has also been reported in fish exposed to diesel or other aromatic hydrocarbons, and is often associated with hepatic damage(23–26), suggesting that the direction of GST modulation may depend on the severity of exposure. SOD enzymes, known for their antioxidant properties that reduce free radicals(27, 28), showed increased activity in the mentioned studies, correlating directly with enhanced protection against oxidative stress.

Meanwhile, the reduced GST activity of fish exposed to diesel or other aromatic hydrocarbons is also linked to liver damage and oxidative stress caused by these types of compounds.(23–26) Regarding the increased ALP activity observed in fish from the DT1 group, this finding is like other studies conducted on guppy fish (*Poecilia reticulata*)(29) that were exposed to tannery effluents exhibited elevated ALP levels in their gills ($P < 0.05$), although not so in other organs such as liver and muscle. Conversely, decreased ALP activity has been noted in marsh frog (*Rana ridibunda*), where animals exposed to 7,12-dimethylbenz(a)anthracene showed lower liver ALP activities compared to control animals.(30) In addition to being used as an indicator of tissular damage, ALP activity has been used as a biomarker of hepatotoxicity.(31)

Therefore, the high ALP levels in DT1 fish suggest liver damage, further supported by the low GST activity and CaE levels measured in this treatment group.

Interestingly, the ALP, CaE, and GST activities measured in fish from DT2 (diesel-exposed and fed with a 2 % tannin diet) and DT3 (diesel-exposed and fed with a 4 % tannin diet) groups were similar ($P > 0.5350$) to those in the OT1, OT2, and OT3 groups, which were not exposed to diesel. A possible explanation for these findings is the beneficial effects of dietary condensed tannins, likely due to their well-documented antioxidant properties and their ability to prevent liver damage or biological alterations caused by diesel-derived hydrocarbons.(23–25)

Although condensed tannins are well-known for their antioxidant activity,(32) their inclusion in diets for sea bass (*Lateolabrax japonicus*) significantly increased the antioxidant effects. This increase in antioxidant capacity can reduce the damage caused by free radicals generated during exposure to xenobiotics, thereby reducing injury to organs such as the liver.(33) This may explain why the addition of tannins in the DT2 and DT3 groups reduced hepatotoxicity related to diesel exposure, as no significant differences were observed in the activity levels of the enzymatic biomarkers ALP, CaE, and GST between diesel-exposed fish and control animals without exposure. This is further supported by the insignificant differences observed in the ALP, CaE, and GST enzymatic activities between diesel exposed animals and fish oil-injected, control animals.

One caveat of adding tannins to diets is their potential antinutritional effects.(34) These phenolic compounds can be categorized into hydrolysable and condensed tannins. Hydrolysable tannins are polyesters of phenolic acid, such as gallic acid, hexahydroxydiphenic acid, and/or their derivatives, combined with D-glucose or quinic acid. They are usually present in low amounts in plants and are considered Generally Recognized as Safe additives by the FDA.(35) Condensed tannins, on the other hand, are polymers of flavan-3-ols or flavan-4-diols and related flavanol residues linked via carbon-carbon bonds, lacking a carbohydrate core as found in hydrolysable tannins.(11) The use of condensed tannins in animal feed is considered safer, as they do not interfere with nutrient absorption, and no significant deleterious effects have been reported from their inclusion in feed for aquaculture species(20) or other domestic animals.(36)

In the US, chestnut and quebracho extract tannins are classified by FDA as 'Natural flavoring substances and natural substances used in conjunction with flavors', while European regulations categorize them as 'Natural products–botanically defined'.(37) However, recent reports suggest that feeding condensed tannins derived from grape seeds to other aquaculture species, such as Chinese seabass (*Lateolabrax maculatus*) may increase intestinal permeability and negatively affect the microbiota.(38) In our study, however, we observed no evident deleterious effects of tannin supplementation in Nile tilapia. On the contrary, tannin inclusion resulted in no significant differences in performance parameters and appeared to mitigate the negative effects of diesel exposure by preventing alterations in the activity of hepatic biomarker enzymes.

Differences in efficacy and safety of condensed tannins may be attributed to variations in tannin sources, doses, and species evaluated. For instance, while dietary grape seed tannins at 2 g/kg are harmful for Chinese bass,(38) quebracho tannins at 1 g/kg appear safe for poultry over 42 days (39) and even for goats at inclusion levels of up to 5 % in the diet.(40) In our study, dietary supplementation with a mixture of chestnut and quebracho tannins at 2 % and 4 % did not adversely affect the performance parameters of Nile tilapia over a 60-day feeding period. On the contrary, tannin inclusion appeared to attenuate diesel-induced alterations in hepatic enzyme activity (ALP, CaE, and GST), suggesting a potential role. These findings highlight the relevance of species-specific response and tannin composition when evaluating their use in aquafeeds, and support further research into their application as dietary additives for mitigating environmental stress in aquaculture.

Data availability

All relevant data are within the manuscript and its supporting information files.

Acknowledgments

The authors thank MNA de México for the formulation of diets and feed provided. The authors also thank Aqua Laboratorios for performing the feed chemical analyses of the diets.

Funding statement

This research was funded by PAICyT CT1482-20 UANL research program.

Conflicts of interest

The authors have no conflict of interest to declare regarding this publication.

Author contributions

Conceptualization: OD García-Pérez, JC Cruz-Valdez.

Data curation: OD García-Pérez, JC Cruz-Valdez.

Formal analysis: D. Garza-Hernández, AG Marroquín-Cardona.

Funding acquisition: JR Kawas-Garza, OD García-Pérez, D. Garza-Hernández.

Investigation: OD García-Pérez, JC Cruz-Valdez.

Methodology: R Gallardo-Morales, OD García-Pérez, JC Cruz-Valdez.

Project administration: JR Kawas-Garza, OD García-Pérez.

Resources: OD García-Pérez, JC Cruz-Valdez, JR Kawas-Garza.

Supervision: OD García-Pérez, JC Cruz-Valdez, R Gallardo-Morales.

Validation: OD García-Pérez, JC Cruz-Valdez.

Visualization: OD García-Pérez, JC Cruz-Valdez, R Gallardo-Morales, JR Kawas-Garza, AG Marroquín-Cardona

Writing – original draft: OD García-Pérez, JC Cruz-Valdez.

Writing – review and editing: AG Marroquín-Cardona, JR Kawas-Garza.

References

1. Olusola SE, Emikpe BO, Olaifa FE. The potentials of medicinal plant extracts as bio-antimicrobials in aquaculture. International Journal of Medicinal and Aromatic Plants. 2013;3(3):404–412.
2. Meijide FJ, da Cuna RH, Prieto JP, Dorelle LS, Babay PA, Lo Nostro FL. Effects of waterborne exposure to the antidepressant fluoxetine on swimming, shoaling, and anxiety behaviours of the mosquitofish *Gambusia holbrooki*. Ecotoxicology and Environmental Safety. 2018;163:646–655. doi: 10.1016/j.ecoenv.2018.07.085.
3. Zhu S, Zhang Z, Zagar D. Mercury transport and fate models in aquatic systems: a review and synthesis. Science of the Total Environment. 2018;639:538–549. doi: 10.1016/j.scitotenv.2018.04.397.
4. Bastardo A, Ravelo C, Castro N, Calheiros J, Romalde JL. Effectiveness of bivalent vaccines against *Aeromonas hydrophila* and *Lactococcus garvieae* infections in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Shellfish Immunology. 2012;32(5):756–761. doi: 10.1016/j.fsi.2012.01.028.
5. Nogueira L, Madeira-Sanchez AL, da Silva DGH, Cid-Ferrizi V, Benedito-Moreira A, Alves de Almeida E. Biochemical biomarkers in Nile tilapia (*Oreochromis niloticus*) after short-term exposure to diesel oil, pure biodiesel and biodiesel blends. Chemosphere. 2011;85:97–105. doi: 10.1016/j.chemosphere.2011.05.037.
6. Mohammadi M, Mirza-Alizadeh A, Mollakhalili-Meybodi N. Off-Flavors in fish: a review of potential development mechanisms, identification and prevention methods. Journal of Human Environment and Health Promotion. 2021;7(3):120–128. doi: 10.52547/jhehp.7.3.120.

7. Shukla G. A review on liver enzymes as a useful biomarker to evaluate the effects of pesticides on freshwater fish. World Journal of Biology Pharmacy and Health Sciences. 2024;19:171–176.

8. Wheelock CE, Miller JL, Miller MJ, Phillips BM, Huntley SA, Gee SJ, Tjeerdema RS, Hammock BD. Use of carboxylesterase activity to remove pyrethroid-associated toxicity to *Ceriodaphnia dubia* and *Hyalella azteca* in toxicity identification evaluations. Environmental Toxicology and Chemistry. 2006;25(4):973–984.

9. Aguilera-González C, Cruz J, Mendoza-Alfaro R. Physiological response of alligator gar juveniles (*Atractosteus spatula*) exposed to sub-lethal doses of pollutants. Fish Physiology and Biochemistry. 2015;41:1015–1027. doi: 10.1007/s10695-015-0066-5.

10. Abdel-Latif HM, Abdel-Daim MM, Shukry M, Nowosad J, Kucharczyk D. Benefits and applications of *Moringa oleifera* as a plant protein source in Aquafeed: a review. Aquaculture. 2022;547:737369. doi: 10.1016/j.aquaculture.2021.737369.

11. Prusty AK, Sahu NP, Pal AK, Reddy AK, Kumar S. Effect of dietary tannin on growth and haemato-immunological parameters of *Labeo rohita* (Hamilton) fingerling. Animal Feed Science and Technology. 2007;136:96–108. doi: 10.1016/j.anifeedsci.2006.08.023.

12. Muniyandi K, George E, Sathyanarayanan S, George BP, Abrahamse H, Thamburaj S, Thangaraj P. Phenolics, tannins, flavonoids, and anthocyanins contents influenced antioxidant and anticancer activities of *Rubus fruits* from Western Ghats, India. Food Science and Human Wellness. 2019;8(1):73–81. doi: 10.1016/j.fshw.2019.03.005.

13. Adejoro FA, Hassen A, Akanmu AM. Effect of lipid-encapsulated acacia tannin extract on feed intake, nutrient digestibility, and methane emission in sheep. Animals. 2019;9(11):863. doi: 10.3390/ani9110863.
14. Nogueira L, Humberto da Silva DGH, Kikuchi-Oliveira TY, Correa da Rosa JM, Arantes-Felicio A, Alves de Almeida E. Biochemical responses in armored catfish (*Pterygoplichthys anisitsi*) after short-term exposure to diesel oil, pure biodiesel and biodiesel blends. Chemosphere. 2013;93:311–319. doi: 10.1016/j.chemosphere.2013.04.083.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72(1–2):248–254. doi: 10.1016/0003-2697(76)90527-3.
16. Mazorra M, Rubio J, Blasco J. Acid and alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. Comparative Biochemistry and Physiology Part B: Biochemical and Molecular Biology. 2002;131:241–249. doi: 10.1016/S1096-4959(01)00502-4.
17. Wilce MC, Parker MW. Structure and function of glutathione S-transferases. Biochimica et Biophysica Acta (BBA) –Protein Structure and Molecular Enzymology. 1994;1205 (1):1–18. doi: 10.1016/0167-4838(94)90086-8.
18. Brodeur JC, Suarez RP, Natale GS, Ronco AE, Zaccagnini ME. Reduced body condition and enzymatic alterations in frogs inhabiting intensive crop production areas. Ecotoxicology and Environmental Safety. 2011;74:1370–1380. doi: 10.1016/j. ecoenv.2011.04.024.

19. Buyukcapar HM, Atalay Aİ, Kamalak A. Growth performance of Nile tilapia (*Oreochromis niloticus*) fed with diets containing different levels of hydrolysable and condensed Tannin. Journal of Agricultural Science and Technology. 2011;13:1045–1051.
20. Peng K, Wang G, Zhao H, Wang Y, Mo W, Wu H, Huang Y. Effect of high level of carbohydrate and supplementation of condensed tannins on growth performance, serum metabolites, antioxidant and immune response, and hepatic glycometabolism gene expression of *Lateolabrax japonicus*. Aquaculture Reports. 2020b, Rep 18: 100515. doi: 10.1016/j.aqrep.2020.100515.
21. Yancheva V, Stoyanova S, Velcheva I, Georgieva E. Fish as indicators for environmental monitoring and health risk assessment regarding aquatic contamination with pesticides. International Journal of Zoology and Animal Biology. 2020;3:1–4. doi: 10.23880/izab-16000210.
22. Huang TL, Obih PO, Jaiswal R, Hartley WR, Thiyagarajah A. Evaluation of liver and brain esterases in the spotted gar fish (*Lepisosteus oculatus*) as biomarkers of effect in the lower Mississippi River basin. Bulletin of Environmental Contamination and Toxicology. 1997;58:688–695. doi: 10.1007/s001289900388.
23. Simonato JD, Guedes CLB, Martinez CBR. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicology and Environmental Safety. 2008;69:112–120. doi: 10.1016/j.ecoenv.2007.01.012.
24. Zhang JF, Wang XR, Guo HY, Wu JC, Xue YQ. Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, *Carassius auratus*.

Ecotoxicology Environmental Safety. 2004;58:110–116. doi: 10.1016/j.ecoenv.2003.08.025.

25. Van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology. 2003;13:57–149.
26. Zhang JF, Shen H, Xu TL, Wang XR, Li WM, Gu YF. Effects of long-term exposure to low-level diesel oil on the antioxidant defense system of fish. Bulletin of Environmental Contamination and Toxicology. 2003;71:234–239. doi: 10.1007/s00128-003-0155-5.
27. Kopecka-Pilarczyk J, Correia AD. Biochemical response in gilthead seabream (*Sparus aurata*) to *in vivo* exposure to a mix of selected PAHs. Ecotoxicology Environmental Safety. 2009;72:1296–1302. doi: 10.1016/j.ecoenv.2008.12.003.
28. Almeida EA, Bainy ACD, Loureiro APM, Martinez GR, Miyamoto S, Onuki J, Barbosa LF, Garcia CCM, Prado FM, Ronsein GE, Sigolo CA, Brochini CB, Martins AMG, Medeiros MHG, Mascio P. Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: antioxidants, lipid peroxidation, and DNA damage. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology. 2007;146:588–600. doi: 10.1016/j.cbpa.2006.02.040.
29. Aich A, Goswami AR, Roy US, Mukhopadhyay SK. Ecotoxicological assessment of tannery effluent using guppy fish (*Poecilia reticulata*) as an experimental model: a biomarker study. Journal of Toxicology and Environmental Health Part A. 2015;78(4): 278–286. doi: 10.1080/15287394.2014.960045.

30. Al-Attar AM. The influence of dietary grapeseed oil on DMBA-induced liver enzymes disturbance in the frog, *Rana ridibunda*. Pakistan Journal of Nutrition. 2004;3(5):304–309. doi: 10.3923/pjn.2004.304.309.

31. Firat O, Cogun HY, Yuzereroglu TA, Go˙k G, Kargin F, Kotemen Y. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to (*sic.*) serum biochemistry of Nile tilapia, *Oreochromis niloticus*. Fish Physiology and Biochemistry. 2011;37(3):657–666. doi: 10.1007/s10695-011-9466-3.

32. Huang Q, Liu X, Zhao G, Hu T, Wang Y. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal Nutrition and Feed Technology. 2018;4(2):137–150. doi: 10.1016/j.aninu.2017.09.004.

33. Peng K, Wang G, Wang Y, Chen B, Sun Y, Mo W, Li G, Huang Y. Condensed tannins enhanced antioxidant capacity and hypoxic stress survivability but not growth performance and fatty acid profile of juvenile Japanese seabass (*Lateolabrax japonicus*). Animal Feed Science and Technology. 2020a;269:114671. doi: 10.1016/j.anifeedsci.2020.114671.

34. Hajra A, Mazumder A, Verma A, Ganguly DP, Mohanty BP, Sharma AP. Antinutritional factors in plant-origin fish feed ingredients: the problems and probable remedies. In: UC Goswami, editor. Advances in Fish Research. Delhi, India: Narendra Publishing House; 2013 pp.193–202.

35. Code of Federal Regulations. Select Committee on GRAS Substances opinion: tannic acid (hydrolyzable gallotannins). 1977. Title 21(3): 21CFR184. 1097. FDA. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=184.1097.

36. Jeronimo E, Pinheiro C, Lamy E, Dentinho MT, Sales-Baptista E, Lopes O, Capela e Silva F. Chapter: 5. Tannins in ruminant nutrition: impact on animal performance and quality of edible products. In: CA Combs, editor. Tannins: Biochemistry, Food Sources and Nutritional Properties. Biochemistry Research Trends, Series. New York, US: Nova Science Publishers; 2016.
37. Caprarulo V, Giromini C, Rossi L. Review: chestnut and quebracho tannins in pig nutrition, the effects on performance and intestinal health. Animal. 2021;15(1):100064. doi: 10.1016/j.animal.2020.100064.
38. Chen B, Qiu J, Wang Y, Huang W, Zhao H, Zhu X, Peng K. Condensed tannins increased intestinal permeability of Chinese seabass (*Lateolabrax maculatus*) based on microbiome-metabolomics analysis. Aquaculture. 2022;560:738615. doi: 10.1016/j.aquaculture.2022.738615.
39. Redondo EA, Redondo LM, Bruzzone OA, Diaz-Carrasco JM, Cabral C, Garces VM, Liñeiro MM, Fernandez-Miyakawa ME. Effects of a blend of chestnut and quebracho tannins on gut health and performance of broiler chickens. PLoS ONE. 2022;17(1):e0254679. doi: 10.1371/journal.pone.0254679.
40. Paolini V, Bergeaud JP, Grisez C, Prevot F, Dorchies P, Hoste H. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. Veterinary Parasitology. 2003;113(3-4):253–261. doi: 10.1016/S0304-4017(03)00064-5.