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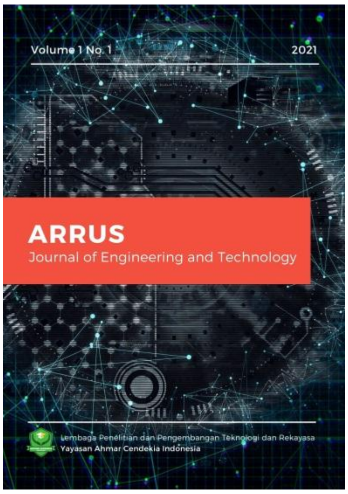

RESEARCH ARTICLE

Green Feed Innovation with Goat Milk Yogurt Casein to Mitigate Dioxin Accumulation in Nile Tilapia

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Abstract: Dioxins are toxic and persistent organochlorine pollutants that bioaccumulate in fish tissues, posing health risks to consumers. This study evaluated the potential of casein isolated from goat milk yogurt as a bioprotective agent to reduce dioxin bioaccumulation in Nile tilapia (Oreochromis sp.). A Completely Randomized Design (CRD) was employed with three groups: a negative control (no exposure), a positive control (dioxin exposure), and a therapy group (dioxin exposure plus dietary casein supplementation). Fish were maintained for 30 days, consisting of 15 days of dioxin exposure followed by 15 days of casein therapy. Survival rate was highest in the negative control (100%), intermediate in the therapy group (67%), and lowest in the positive control (17%). Gas Chromatography–Mass Spectrometry (GC–MS) analysis showed that casein supplementation reduced dioxin concentration in muscle tissue threefold compared with the positive control (0.02% vs. 0.06%; $p < 0.001$). Histopathological analysis further indicated that casein attenuated hepatic damage, with a lower damage score in the therapy group (10) compared to the positive control (16). These findings suggest that casein from goat milk yogurt has potential as a functional feed ingredient to suppress dioxin accumulation and mitigate toxic effects in fish, although full physiological recovery was not achieved.

Keywords: Bioaccumulation, Aquatic Toxicology, Organoclorine Pollutant, Nutraceuticals, Feed Innovation

1. Introduction

Dioxins are a group of organochlorine pollutants that continue to be a significant concern in global contamination issues. These compounds are toxic, persistent, and lipophilic, making them difficult to degrade and allowing them to persist in the environment for extended periods. The primary sources of dioxins are anthropogenic, including waste incineration, chemical industry processes, and the disposal of medical waste. Due to their chemical stability, dioxins can readily disperse through air, water, and soil, subsequently entering the food chain and accumulating in the tissues of living organisms. Such accumulation poses widespread health risks, as dioxins are known to be carcinogenic, immunotoxic, and capable of disrupting the endocrine system (Alcock and Jones 1996; Kaur and Maru 2024; Mathew et al. 2025; Suhartawan et al. 2023; WHO 2023).

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The primary route of dioxin exposure in humans is through the consumption of animal-based foods. According to the WHO (2019), over 90% of dioxin exposure originates from the consumption of meat, fish, and dairy products. Fish are particularly vulnerable because aquatic environments often serve as deposition sites for dioxins, whether from atmospheric deposition or industrial and domestic effluents. These compounds accumulate in sediments and undergo bioaccumulation and biomagnification, transferring into fish tissues via gills, dietary intake, or direct contact with contaminated substrates. The risk of contamination is particularly high in aquaculture systems, which are often situated near industrial zones or residential areas with limited water circulation. Such conditions accelerate dioxin accumulation in sediments and culture media. Additionally, the relatively higher lipid content in farmed fish further increases the potential for dioxin accumulation in fish tissues (Foran et al. 2005a, 2005b; Frakes, Zeeman, and Mower 1993; Jensen et al. 2020; Mikołajczyk et al. 2020).

In Indonesia, Nile tilapia (Oreochromis sp.) is one of the main aquaculture commodities due to its affordable price, preferred taste, and adaptability to various environmental conditions (Melen Febrianti 2023; Nasution 2025; Turua, Subyantoro, and Ratang 2014). However, the potential for dioxin contamination in tilapia tissues poses health risks to consumers. Chronic dioxin exposure has been associated with metabolic disorders, hormonal dysfunction, reduced immunity, and an increased risk of degenerative diseases (WHO 2023). Therefore, effective mitigation strategies are required to reduce dioxin accumulation while ensuring the safety of the food supply.

Mitigation efforts in fish aquaculture have so far focused primarily on external control measures, such as filtration systems, aeration, or microorganism-based bioremediation (Baron, Børresen, and Jacobsen 2005; Ding et al. 2023; Nicula et al. 2022; Setianingrum and Haskito 2021). Although these methods can reduce toxin concentrations in the culture environment, their effectiveness is limited when facing chronic, cumulative exposure. This underscores the need for alternative approaches, such as functional feed interventions that act directly within the digestive tract, thereby inhibiting dioxin absorption before it is distributed to fish tissues.

Casein derived from fermented goat milk has potential as a bioprotective agent for mitigating dioxin accumulation. Casein, the primary protein in milk, comprises αs1-, αs2-, β-, and ϰ-casein fractions, arranged in micellar structures with a high affinity for lipophilic compounds. Fermentation produces bioactive peptides and lactic acid, which not only enhance the binding capacity for toxic compounds but also support gut microbiota balance and improve fish growth performance (Aeni, Srihardyastutie, and Mahdi 2020; Sarode et al. 2016). Utilizing this local protein source also aligns with sustainability and circular economy principles by optimizing the use of goat milk that has not been fully absorbed by the market.

The novelty of this study lies in the application of a functional feed based on fermented casein as an internal mitigation strategy to reduce dioxin bioaccumulation in Nile tilapia. This approach differs from conventional methods, which primarily focus on external control of the cultural environment. Therefore, this research aims to evaluate the effectiveness of fermented goat milk casein in reducing dioxin accumulation in tilapia tissues while also assessing its contribution to fish health and growth within a sustainable aquaculture system. The research question is: To what extent can the inclusion of fermented goat milk casein in feed reduce dioxin bioaccumulation in Nile tilapia (Oreochromis sp.) tissues and support fish health and growth in a sustainable aquaculture system?

2. Research Method and Materials

The entire experimental series was designed in accordance with animal research ethics, applying the 3R principles (replacement, reduction, refinement) to minimize stress, pain, and discomfort in the test fish.

2.1 Materials and Tools

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The materials used included uniform-sized Nile tilapia (Oreochromis sp.) fingerlings, commercial fish pellets, fresh goat milk, lactic acid bacteria culture, physiological NaCl solution, 10% formalin, and dioxin standard solution. The equipment consisted of individual tarpaulin tanks, aeration and filtration systems, a low-temperature drying oven, a 60-mesh sieve, muslin cloth, a sprayer, a digital balance, water quality measuring instruments (pH meter, DO meter, thermometer), and a Gas Chromatography-Mass Spectrometry (GC-MS) instrument.

Short details on calibration: Prior to analysis, the GC–MS instrument was calibrated using a multi-point external calibration curve prepared from certified dioxin standard solutions, and instrument performance was verified through daily checks of retention time stability and peak response consistency.

2.2 Methods

2.2.1 Preparation of Experimental Feed

(1). Feed 1 (Dioxin-exposed feed)

Feed 1 consisted of standard feed treated with a dioxin solution to reach the concentrations specified in the study design. This feed aimed to induce oxidative stress as a model for toxicity testing. Feed 1 was administered to the positive control and treatment groups during the first 15 days of the fish rearing period.

(2). Feed 2 (Casein-supplemented feed)

Feed 2 was formulated by incorporating casein isolated from fermented goat milk yogurt into the base feed. The casein served as a bioactive compound with the potential to bind and reduce dioxin accumulation in fish tissues. This feed was given specifically to the treatment group following the dioxin exposure phase, from day 16 to day 30 of rearing.

(3). Feed 3 (Control/standard feed)

Feed 3 was the standard commercial feed without any additives or special treatment, used as a control to represent normal fish conditions without intervention. It was provided as follows:

(a). Negative control group: for the full 30-day rearing period.
(b). Positive control group: after the end of the dioxin exposure phase (day 16 to day 30).

2.2.2 Experimental Design and Fish Rearing

This study employed an experimental method with a completely randomized design (CRD). Statistical considerations were based on the criterion $p(n-1) \geq 15$, where p represents the number of treatment groups and n the number of replicates. A total of 18 fish were used as test animals and divided into three treatment groups, each with six replicates. The treatment groups were: (1) negative control (NC), fish fed without dioxin or casein; (2) treatment group (TG), fish fed with both dioxin exposure and casein; and (3) positive control (PC), fish fed with dioxin exposure but without casein (see Figure 1).

Dioxin exposure was administered through feed at a concentration of 50 ng/mL per kilogram of feed, while casein was added at 10% of the total feed weight, as described by Subandiyah et al. (2014). Fish were reared in individual tarpaulin tanks equipped with aeration and filtration systems, and water quality (temperature, pH, and dissolved oxygen) was monitored daily to ensure stability across tanks. All tanks were supplied with the same water source and maintained under identical aeration and filtration settings to minimize variation in environmental parameters.

The treatment lasted 30 days, divided into two phases: a 15-day phase of dioxin exposure followed by a 15-day phase of casein therapy. Feed was provided twice daily ad libitum, with

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consumption recorded to monitor physiological responses. Survival rate (SR) was calculated using the formula:

$$SR = \frac{\text{Number of Surviving Fish}}{\text{Initial Number of Fish}} \times 100\% \text{ (Eq. 1)}$$

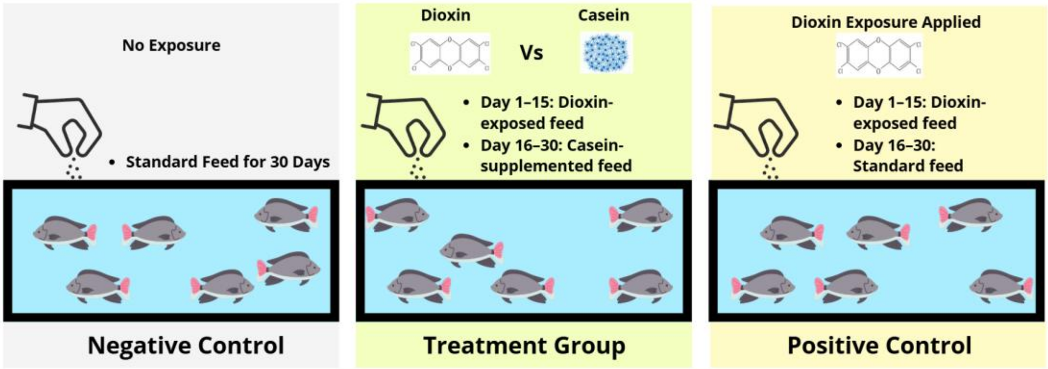

Figure 1. Experimental Design

2.2.3 Sample Collection and Laboratory Analysis

After the treatment period, muscle samples were collected to determine dioxin concentrations using Gas Chromatography–Mass Spectrometry (GC-MS), while liver samples were subjected to histopathological examination to evaluate morphological alterations induced by dioxin exposure and casein treatment.

2.2.4 Data Analysis

Data, including dioxin concentration, survival rate, and other supporting parameters, were statistically analyzed using an independent t-test. Histopathological observations were evaluated using a scoring system.

3 Results And Discussion

3.1 Protective Effect of Casein Against Dioxin Accumulation

The treatment of test fish was applied consistently over a 30-day period, as outlined in the experimental design. Aquatic environmental conditions were maintained within optimal ranges through monitoring of temperature, pH, and dissolved oxygen levels, and feed was administered according to each group's specific treatment. This approach enabled the controlled and systematic observation of fish physiological responses, allowing for the direct attribution of differences between groups to the treatments applied. Daily observations are presented in Figure 2, while survival rates are summarized in Table 1.

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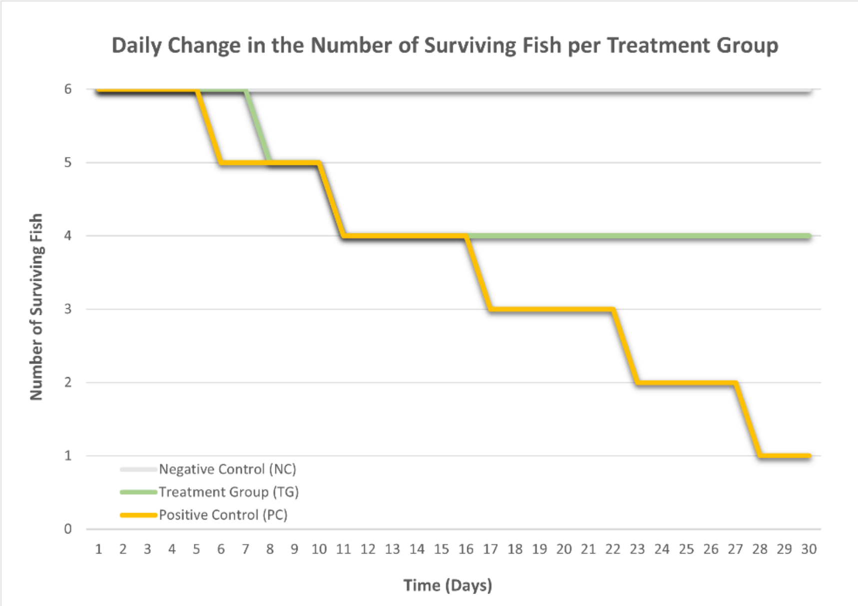

Figure 2. Survival Curves of Fish According to Treatment Groups

Observations indicated clear differences in survival among the experimental groups. The negative control group maintained high survival throughout, reflecting optimal rearing conditions. The positive control group showed a marked decline in survival during the mid-treatment period, demonstrating the toxic effects of dioxin on fish physiology. The casein-supplemented group exhibited higher survival than the positive control, though not as stable as the negative control. These results suggest that casein may offer protective effects against oxidative stress and organ damage induced by dioxin exposure.

Survival rate (SR) calculations based on Equation 1 revealed clear differences among the groups. The positive control group, exposed to dioxin without treatment, exhibited the lowest SR at 17%. The casein-supplemented treatment group showed an increased SR of 67%, while the negative control group, not exposed to dioxin, reached the highest SR of 100%.

Table 1. Survival Rate of Fish Across Different Treatment Groups

Group		Number of Survived Fish	Number of Dead Fish	Survival Rate
Positive Control	Dioxin-exposed	1	5	17%
Treatment	Dioxin-exposed + therapy	4	1	67%
Negative Control	No exposure	6	0	100%

These results suggest that casein supplementation enhances the physiological resilience of fish against dioxin toxicity. This finding is consistent with previous reports stating that milk proteins, particularly casein, can bind lipophilic toxic compounds, thereby reducing bioaccumulation and increasing the tolerance of aquatic organisms to contaminants (Allbban 2021; Subandiyah et al. 2014). Conversely, dioxin exposure is known to decrease survival through mechanisms involving oxidative stress, metabolic disruption, and histopathological damage to vital organs such as the liver, kidneys, and digestive tract (SCF 2000; Senthilkumar et al. 2002).

The previously described survival results are complemented by an analysis of dioxin concentrations in fish muscle tissue, presented as relative estimates based on retention time (RT) in GC-MS. The positive control group, exposed to dioxin without supplementation, exhibited the highest dioxin levels (≈0.06%) along with high mortality (D). In contrast, the casein-supplemented treatment group showed a significant reduction in dioxin concentration

(≈0.02%) with most fish surviving (S). The negative control group, not exposed to dioxin, showed no toxin accumulation, and all fish survived; therefore, it was excluded from the statistical analysis. Detailed estimates of dioxin concentrations for each sample, along with the results of statistical tests, are presented in Table 2.

Table 2. Dioxin Concentration in Fish Muscle and Survival Status Across Experimental Groups

Group	Fish Sample Dioxin Concentration (%) (Survival Status)	Average Dioxin Level (%)	Group Comparison	t	df	p-value (two-tailed)	Significance
Positive Control	0.08 (S), 0.05 (D), 0.06 (D), 0.07 (D), 0.04 (D), 0.06 (D)	0.06	Positive Control vs Treatment	5.861	10	<0.001	Significant
Treatment (Dioxin + Kasein)	0.03 (S), 0.02 (S), 0.02 (S), 0.01 (S), 0.02 (D), 0.03 (D)	0.02					
Negative Control	0.00 (S) × 6	0.00	Not included in statistical analysis				

Note: *S = Survived, D = Died*

The statistical analysis, showing a significant difference between the positive control group and the treatment group in dioxin accumulation in fish muscle after casein supplementation ($p < 0.001$) with equal variance assumption (Levene's test $F = 1.190$, $p = 0.301$), provides strong evidence of casein's potential to reduce the bioaccumulation of harmful lipophilic compounds. This reduction in dioxin concentration is likely due to casein's binding mechanism, which decreases the bioavailability of the toxin.

3.2 Macroscopic and Histopathological Analysis

Macroscopic examination of the fish liver revealed distinct differences among treatment groups. Figure 3 illustrates the visual appearance of the liver in each group. The liver of fish in the negative control (NC) group appeared bright brown, indicating a physiologically normal organ without pathological disturbances. In contrast, the positive control (PC) group exhibited a dark black coloration, a classical indication of severe tissue damage caused by toxin accumulation, particularly lipophilic compounds such as dioxins. In the therapy group (TG), the liver appeared slightly darkened but not as intense as in the PC group, suggesting moderate damage and indicating that casein supplementation was able to reduce the extent of injury induced by dioxin exposure.

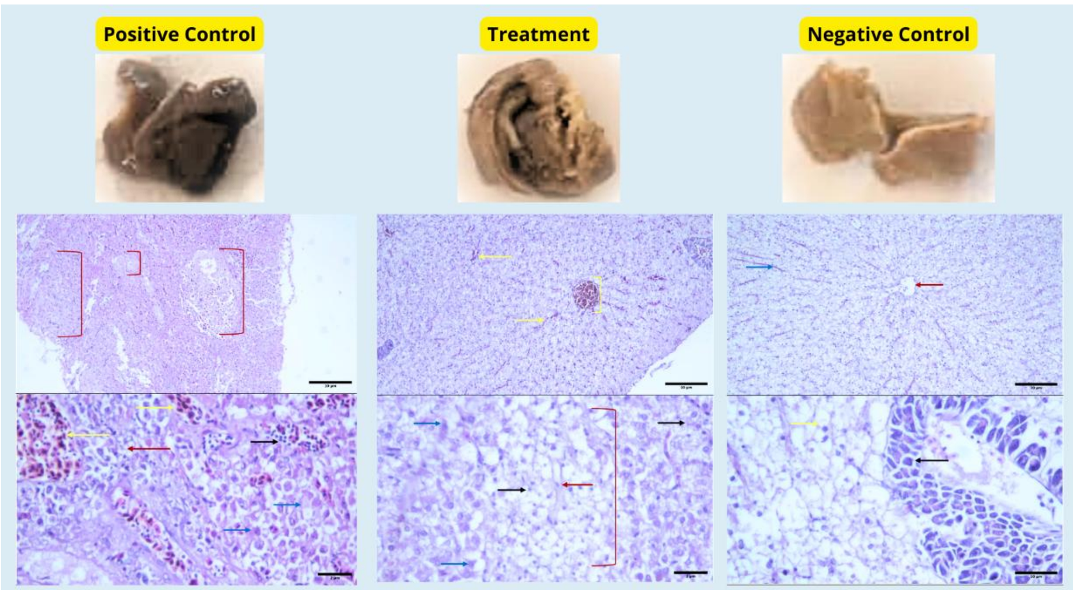

Figure 3. Comparison of Macroscopic and Histopathological Features of Fish Liver in Positive Control, Treatment, and Negative Control Groups

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These macroscopic findings were supported by histopathological observations summarized in Table 3. In the NC group, the hepatocyte structure remained well-preserved, with no signs of necrosis, degeneration, congestion, fibrosis, or inflammatory cell infiltration. This condition reflects the stable physiological status of fish in this group. Conversely, the PC group showed extensive liver damage, with a total histopathological score of 16. Massive hepatocyte necrosis was observed, accompanied by degenerative changes characterized by nuclear shrinkage and altered cell morphology. Severe congestion was evident in the central vein and sinusoids, indicating impaired blood flow. In addition, mild inflammatory cell infiltration was present, representing an inflammatory response to dioxin toxicity.

Table 3. Macroscopic and Histopathological Characteristics of Fish Liver Across Experimental Groups

Observed Parameter	NC (Negative Control)	PC (Positive Control)	TG (Therapy Group)	Explanation
Liver Color (macroscopic)	Normal (light brown)	Darkest	Slightly darkened, still close to normal	Liver color reflects health status: a healthy liver is light brown; darker coloration indicates greater damage
Focal Necrosis	Not observed	Severe necrosis (extensive tissue damage)	Moderate necrosis (moderate tissue damage)	Necrosis: death of liver cells. The greater the necrosis, the more severe the liver damage
Hepatocyte Degeneration	Absent	Severe degeneration, nuclear shrinkage	Moderate degeneration	Degeneration: weakening/damage of hepatocytes, marked by changes in nuclear morphology
Central Vein & Sinusoidal Congestion	None, only mild	Evident congestion (extensive blood pooling)	Moderate congestion	Congestion: blood accumulation in hepatic vessels, indicating impaired blood flow
Fibrosis/Connective Tissue Proliferation	Not observed	Not observed	Not observed	Fibrosis: permanent scar tissue formation. Absence indicates that damage has not become permanent
Inflammatory Cell Infiltration	Not observed	Present, but not severe	Present, but not severe	Infiltration: immune cells entering the liver, indicating an active response to tissue damage
General Hepatocyte Condition	Normal	Severely impaired, liver cell structure destroyed	Moderate impairment, some areas still normal	Hepatocytes: the main functional liver cells. Overall condition reflects healthy (NC), severely damaged (PC), or moderately damaged (TG)

In the TG group, the liver damage score was 10, lower than that of the PC group. Necrosis and hepatocyte degeneration were still observed, but only at moderate levels. Congestion was present, although less severe than in the PC group, and inflammatory cell infiltration remained mild. Interestingly, some areas of liver tissue retained a relatively normal structure, indicating that casein supplementation effectively mitigated the damage, although it did not completely restore the liver to its normal state.

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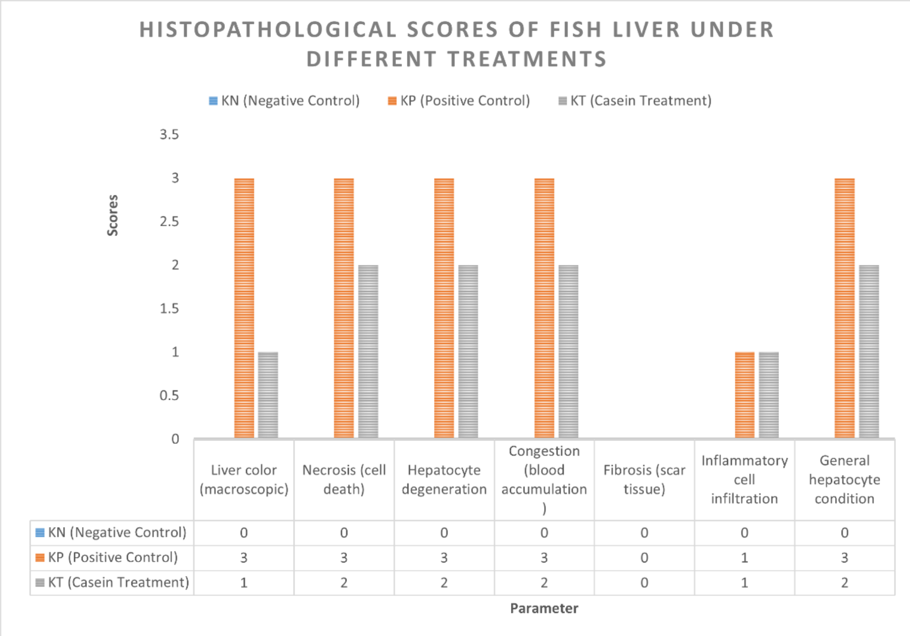

Figure 4. Histopathological Scores of Fish Liver under Different Treatments

The differences in damage severity among groups were further emphasized in Figure 4, which presents a quantitative comparison of histopathological scores. The PC group recorded the highest score, representing the most severe damage, whereas the NC group consistently remained at zero. The TG group occupied an intermediate position.

In summary, the study demonstrates that diosignificantly increases survival and toxinival rates, increases toxin accumulation in muscle tissue, and induces histological damage in the fish liver. Casein supplementation provided a protective effect by lowering dioxin concentrations in tissues and improving histopathological outcomes, although full physiological recovery was not achieved. These findings underscore the potential of casein as a nutraceutical agent for mitigating the bioaccumulation and toxic effects of lipophilic compounds in aquatic organisms. Moreover, the demonstrated efficacy of casein highlights its potential for commercialization as a functional feed additive for aquaculture industries seeking strategies to reduce contaminant burdens. Additionally, incorporating casein-based supplementation into feed formulations may contribute to more sustainable aquaculture practices by enhancing fish health, improving feed safety, and reducing reliance on environmentally harmful chemical interventions

4. Conclusions

This study demonstrates that dioxin exposure exerts severe impacts on Nile tilapia (Oreochromis sp.), as indicated by the low survival rate (17%), high toxin accumulation in muscle tissue (0.06%), and extensive liver damage at both macroscopic and histopathological levels. These findings underscore the toxic nature of dioxins, which disrupt metabolism, impair vital organs, and ultimately cause fish mortality.

Dietary supplementation with casein isolated from goat milk yogurt was shown to provide protective effects. The therapy group exhibited a 67% increased survival rate and a threefold reduction in muscle dioxin concentration compared with the positive control group (0.02% vs. 0.06%; $p < 0.001$). Histopathological analysis revealed only moderate hepatic damage in the therapy group, with some areas of normal tissue remaining, suggesting that casein reduced toxic effects, although complete physiological recovery was not achieved.

Therefore, the incorporation of casein into fish feed can be regarded as an effective internal mitigation strategy to suppress dioxin bioaccumulation and improve organ condition in Nile

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tilapia. Moreover, the utilization of casein derived from goat milk yogurt aligns with the principles of sustainability in aquaculture while contributing to food safety assurance for consumers of fish products.

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