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Toxicity of Low-Level Multiple-Mycotoxin Mixture in Nile Tilapia (*Oreochromis niloticus*) Is Prevented with Organically Modified Clinoptilolite Feed Additive

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Abstract: Organically modified clinoptilolite (member of the zeolite family of minerals; MinazelPlus®) feed additive, with an average weight of 30 ± 2 g, was used to prevent mycotoxicosis in Nile tilapia (*Oreochromis niloticus*) through its supplementation for 42 days to the diet contaminated with multiple mycotoxins: aflatoxin B1 (40 µg/kg), fumonisin B1 and B2 (600 µg/kg), zearalenone (50 µg/kg), and deoxynivalenol (150 µg/kg). The fish were divided randomly into four experimental groups (basal diet control—C; fed 2 g/kg MinazelPlus®—MZ; fed multiple mycotoxins—MT; and fed a combination of MinazelPlus® and multiple mycotoxins—MZ + MT). Each group consisted of triplicate aquarium setups, with six fish in each replicate. Sampling was performed in weeks 2, 4, and 6. The lymphocyte count was significantly higher in the MZ group compared with the MT group and the MT + MZ group in week 6. An overall decrease in the neutrophil count was observed in the experimental groups. Histopathological analysis was performed in weeks 2 and 6, revealing significant changes in the liver, intestines, kidney, and spleen of fish from the MT group, while the MT + MZ and MZ groups were similar to the control. The addition of 2 g/kg MinazelPlus® has the ability to prevent and reduce the adverse effects of chronic exposure to low concentrations of multiple mycotoxins in juvenile Nile tilapia.

Keywords: tilapia's health; mycotoxins; zeolite; modified clinoptilolite MinazelPlus®

Key Contribution: The administration of MinazelPlus® significantly influenced lymphocyte and neutrophil counts in the experimental groups and prevented the occurrence of significant histopathological changes in the liver, intestines, kidney, and spleen of fish exposed to mycotoxins.

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1. Introduction

Aquaculture production is the fastest-growing industry in the global food economy. In terms of total production, aquaculture has recently overtaken capture fisheries and now supports over half of all seafood production worldwide. Asia is the leader in aquatic animal production, accounting for 91.4 percent of the total aquaculture production [1]. The continued growth of aquaculture is driven by increases in human consumption demands, resulting in increased need for animal feed to support aquatic animal production.

According to the FAO [1], Nile tilapia (*Oreochromis niloticus*) was the second most produced fish worldwide in 2022, with an estimated production of 5.3 million tons [1]. It is expected that tilapia's role in meeting seafood protein demands will be met with continued

increases in its production, especially in the developing regions of the world [2]. Therefore, the need for farmed tilapia feed will also increase, posing a problem to be solved with two-fold approach: growth of feed production and reduction in feed wastage, which was mentioned as a high-profile goal in the FAO's 2022 edition of The State of World Fisheries and Aquaculture (SOFIA) [3]. In 2017, the amount of feed provided for tilapia was recorded at 9.2 million tons, whereas a projected estimate for the year 2025 indicates an increase to 13.25 million tons, a 45% increase in less than 10 years [4].

A major raw material source for animal feed production is represented by plants, including ingredients used for the production of cultured aquatic animal feed (mainly used in finfish and shrimp aquaculture). However, an inherent risk of plant-based protein sources (e.g., soybeans, wheat, maize/corn, and others) lies in the introduction of mycotoxin contamination from raw ingredients into aquafeed formulations [5,6]. The majority of aquaculture finished feed samples and plant-based meal samples, such as soybean meal, wheat, and maize, analyzed across multiple studies were found to be contaminated with more than one mycotoxin [7–9].

Among over 300 known mycotoxins, the dominant toxins are aflatoxins, zearalenone, fumonisin, ochratoxin, and deoxynivalenol. Furthermore, these few mycotoxins have the highest economic impact, due to their prevalence in feedstuff and negative effects on livestock production efficiency [10]. The European Commission guidelines recommend that the maximum levels and guidance values for aflatoxin B1, deoxynivalenol, zearalenone, and fumonisin in animal feedstuffs be established, with acceptable levels of 20 μg/kg, 5000 μg/kg, 2000 μg/kg, and 10,000 μg/kg, respectively [11,12]. However, there are no respective values for mycotoxin contamination specifically developed for fish feed, with exception of fumonisins (FB1 + FB2), with a maximum acceptable level of 10,000 μg/kg [11]. It is known that the simultaneous presence of multiple mycotoxins below regulatory limits can lead to cumulative and/or synergistic toxicity effects [13].

Currently, a number of strategies are being employed to prevent feed contamination with mycotoxins and reduce mycotoxin absorption in the gastro-intestinal tract. A rationale for the usage of mineral-based mycotoxin absorbers as feed additives, such as natural zeolites (clinoptilolite), has been confirmed by multiple studies [14,15]. More recently, Tomašević-Čanović [14] and Daković et al. [15] reported that clinoptilolite can effectively adsorb various types of mycotoxins, even when their chemical composition varies. Clinoptilolite's binding properties are due to the unique physiochemical structure and characteristics of this mineral. This particular zeolite is made out of very fine polycrystalline lamellas, forming the basis of its rigid structure. At the same time, free spaces in its "skeleton" and its superb ion exchange properties make it an effective mycotoxin binder. It is important to note that clinoptilolites have been evidenced to display a plethora of positive effects on animals when feed is supplemented with them, among which we can name hemostatic, anti-inflammatory, antimicrobial, antitumor, and immunostimulant activities [16,17].

It has also been noted that naturally occurring zeolites, including clinoptilolites, have limited effectiveness in the adsorption of the full spectrum of mycotoxins, which has prompted further studies focusing on surface modifications of mineral powder by the addition of different chemical substituents [10]. MinazelPlus® is a proprietary patented result of such studies, where natural clinoptilolite adsorption and selective properties are improved through a two-step modification process. Tribochemical surface modification, during which organic cations are added, is followed by a three-part physio-chemical modification, resulting in a partial change in the clinoptilolite's surface polarity through the attachment of long-chained cations. Such modification makes the adsorption of the less polar and non-polar mycotoxins possible, thus improving the overall efficacy of the feed additive. The effectiveness of MinazelPlus® in the adsorption of mycotoxins in an aquaculture environment is supported by several studies conducted on Gilthead sea bream (*Sparus aurata*), Nile tilapia (*Oreochromis niloticus*), or Rainbow trout (*Oncorhynchus mykiss*) [18–20].

The exposure of fish to mycotoxin-contaminated feed can result in acute and chronic pathologies, such as liver and kidney diseases, carcinogenesis, gastro-intestinal disturbances, reproductive disorders, and immune system suppression [21,22]. The low-level presence of mycotoxins in fish diet can lead to reduced growth rate and decreased feed intake, causing significant economic impacts on aquaculture production [5,8,22]. Another layer of this problem's complexity is the varying level of contamination related to geographical and climatic factors. In the analysis performed by Gonçalves et al. [8], the areas of Southeast Asia are mentioned to be the most affected by this problem due to the general climate conditions, such as the occurrence of seasons of heavy tropical rain. Furthermore, previous studies by Gonçalves et al. [8] noted that 84% of the feed samples were contaminated with more than one mycotoxin in Asian countries and samples from Europe tested positive for more than one mycotoxin in about 50% of cases. This clearly demonstrates the need to perform multi-mycotoxin studies to reflect real aquaculture conditions.

As mentioned above, due to the growth, production volume, and distribution of Nile tilapia aquaculture in tropical and sub-tropical areas and its overlapping with areas related to significant feed contamination with multiple mycotoxins, it is the fish species with the highest risk of being exposed to multiple mycotoxins in routine aquaculture operations. Furthermore, as tilapia is the most produced fish in Thailand aquaculture [23] and is one of the most important sources of animal protein for populations throughout Southeast Asia, it is imperative to understand the potential for multiple low-concentration mycotoxins effects on farmed Nile tilapia's health and the possible ways to prevent them. In order to prevent health consequences, different risk and toxicity mitigation strategies are employed, including the addition of mycotoxin absorbers such as clinoptilolites (zeolites) with high ion exchange capacity to commercial feed. The adsorption of mycotoxins can effectively reduce their toxic potential and further assist in disease prevention, support optimal growth rates, improve nutrient absorption, and increase the overall survival rate of aquatic animals in aquaculture [20,24].

This study aims to determine the potential of organically modified clinoptilolite (MinazelPlus®) feed additive to safely protect the health of Nile tilapia exposed to a field-relevant mixture of mycotoxins at concentrations equaling or below the regulatory minimum. We examined if the addition of this broad-spectrum mycotoxin adsorber to fish feed contaminated with low levels of multiple mycotoxins similar to an actual field situation prevented the negative effects on the overall health and condition of fish during chronic dietary exposure.

2. Materials and Methods

2.1. Animals and Experimental Conditions

A total of 72 Nile tilapia (*Oreochromis niloticus*) with an average body weight of 30 ± 2 g were divided into four experimental groups (Table 1), and 6 fish were randomly distributed in each of the three 50 L glass tanks per group. The tanks were equipped with a continuous single-pass flow-through system sourced from conditioned water. The fish were fed twice daily for a period of 42 days, with the exception of the day before the planned sampling. We performed daily measurements of water quality parameters, such as temperature, pH, and nitrite. Weekly water parameter measurements were conducted for total ammonia (TAN), nitrate, dissolved oxygen, and carbonate hardness. The temperature was constantly kept at 27 ± 0.5 °C, the pH level ranged between 7.6 and 8, the nitrite concentration was <0.02 mg/L, the total ammonia (TAN) level consistently remained below 0.05 mg/L, the nitrite concentration was 0.9 ± 0.08 mg/L, the dissolved oxygen level was 7.89 ± 0.15 mg/L, and the carbonate hardness was 14.19 ± 1.92 KH. The water quality parameters were measured and regulated to provide stable environmental conditions [25].

Table 1. Description of experimental groups.

Experimental Group	Diet
Control (C)	Basal diet
Mycotoxins (MT)	Basal diet + multiple mycotoxins *
MinazelPlus® (MZ)	Basal diet + 2 g/kg MinazelPlus® **
MinazelPlus® and Mycotoxins (MZ + MT)	Basal diet + 2 g/kg MinazelPlus® ** + multiple mycotoxins *

* Multiple mycotoxins: a mixture of low concentrations of mycotoxins. Aflatoxin B1 (AFB1) at 40 µg/kg, fumonisin (FUM) at 600 µg/kg, zearalenone (ZEN) at 50 µg/kg, and deoxynivalenol (DON) at 150 µg/kg. ** MinazelPlus® feed additive is an organically modified clinoptilolite, commercially used as an absorber of both polar and non-polar mycotoxins in various animal feed types.

The fish were kept under a 12 h light and 12 h dark photoperiod by using indoor lights. The studies were conducted at Chair for Fish Diseases and Fisheries Biology, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Germany, as approved by the German Animal Welfare Experimental Animal Ordinance for animal husbandry and welfare from the Ethical Committee for Animal Experiments—Regierung von Oberbayern, Maximilianstr. 39, 80534 München (Ref. No. ROB-55.2-2532.Vet_02-20-142). Before the experimental trial, the fish were acclimated for a period of two weeks. Observation of general fish behavior and clinical signs before and during feeding was performed twice per day as part of the general laboratory animal routine check that was recorded in the animal facility logbook. The animals were kept in accordance with the guidelines of the European Union directives for animal welfare [26].

2.2. Experimental Diets

The experimental diets for Nile tilapia were prepared by using commercial feed. The analysis of the feed composition was performed at the Department of Animal Nutrition and Feed Science, Wrocław University of Environmental and Life Sciences (Wrocław, Poland), according to the AOAC protocol [27] (dry matter, 91.9%; ash, 9.24%; crude protein, 39.81%; fat, 13.3%; fiber, 5.24%; energy, 19.3 MJ/kg), confirming that the nutritional requirements of the fish were met [28,29]. Organically modified clinoptilolite (MinazelPlus®; Patent Co., Mišićevo, Serbia) was added to the basal diet according to the safety and efficacy study protocols [18,30]. Predetermined amounts of mycotoxins (in the form of contaminated corn meal) were added to the basal diet with the inclusion of and without MinazelPlus®. The mycotoxin levels used in this experiment were based on the analysis of mycotoxins in animal feed from Southeast Asia, as reported by Gruber-Dorninger et al. [9]. Mycotoxin-contaminated corn meal was provided by Patent Co. The feed mixtures were applied to four experimental groups according to the schedule in Table 1.

The experimental feed was prepared according to Royes and Chapman [31] and Gonzalez and Allan [32] by using commercial pelleted feed, ground and mixed with multiple-mycotoxin-contaminated corn meal, with or without 2 g/kg MinazelPlus®. The mycotoxin concentration levels in the experimental diets were analyzed by Patent Co. to confirm that the concentrations of multiple mycotoxins in the experimental diets were close to the desired levels (Supplementary File: Figure S1). Corn meal without mycotoxin contamination was used in the control diet. Following the homogenization of the feed, water and oil were added, thoroughly mixed into a pliable dough, and extruded through a 2 mm grinder disc. Subsequently, dough pellets were air-dried in a chemical cabinet for 42 h, followed by grading through a 1.8–2.2 mm sieve. The experimental diets were vacuum-packed and stored at −30 °C until usage.

2.3. Sample Collection

On days 14, 28 *, and 42 (weeks 2, 4, and 6) with respect to the start of the experiment, six fish were randomly selected from each experimental group. On day 28, only 12 fish in total were sampled for the required health check. The fish were starved for 24 h before sampling and euthanized with an overdose of neutrally buffered and aerated tricaine

methanesulfonate solution (MS-222; final concentration of 300 mg/mL) followed by a blow to the head. Fish were weighed and their length was measured to calculate their growth performance, followed by the collection of blood samples for hematological and biochemical parameters (Sections 2.5 and 2.6). Fish were then subjected to pathology examination via dissection, and internal organs were collected for histopathology (Section 2.7).

2.4. Growth Determination

Growth performance was calculated according to the following:

$$\text{Weight gain (WG; g)} = \text{final body weight} - \text{initial body weight}$$

$$\text{Average daily weight gain (g/day)} = (\text{final body weight} - \text{initial body weight})/\text{t (days)}$$

$$\text{Specific growth rate (SGR; \% day}^{-1}\text{)} = 100 \times [\ln(\text{final body weight}) - \ln(\text{initial body weight})]/\text{t (days)}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake}/\text{weight gain}$$

$$\text{Body weight increase (BWI; \%)} = 100 \times (\text{weight gain}/\text{initial body weight})$$

2.5. Blood Sampling and Hematological Parameters

Blood samples were collected with a heparinized syringe from a caudal vein, according to Witeska et al. [33]. Blood smears were stained with Diff-Quick® (CellaVision, Lund, Sweden) according to the method described by Meyers [34], and differential leukocyte counts were performed (400–1000×) according to Salkova et al.'s [35] method.

2.6. Biochemical Parameters

Blood samples were kept on ice for 1 h, which was followed by plasma separation (centrifugation at 7000× *g* for 15 min). Plasma was stored at −80 °C for further analysis [36,37] of the levels of total protein, albumin, and globulin, as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Biochemical parameter analysis was performed by the Clinic of Small Animal Internal Medicine, Centre for Clinical Veterinary Medicine, Ludwig-Maximilian-University Munich, Germany, by using the Cobas Integra 400 plus biochemical analyzer (Roche, Basel, Switzerland).

2.7. Histopathological Examination

On days 14 and 42, six fish from each group were euthanized as described above, and necropsies were performed. The tissue samples from the liver, kidney, intestine, and spleen were collected and fixed for 24 h in 10% ice-cold neutral buffered formalin solution (NBF) solution [38] and stored at 4 °C before being transferred into 70% ethanol. Tissue samples were processed for histopathology, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E) at the Institute of Pathology [34,39]. Histopathological samples were then evaluated with the Delta Optical ProteOne (400–1000×) and photographed with the microscope-mounted camera DLT-Cam PRO 5 (Delta Optical, Warsaw, Poland).

2.8. Statistical Analysis

The statistical analysis was performed by using RStudio statistical software (version 4.2.2). The normality of the collected data was analyzed by using the Shapiro–Wilk test, while the homogeneity of variance was checked with Bartlett's test. A one-way analysis of variance (ANOVA) test was performed, using Tukey's HSD test to analyze the differences among the experimental diets. Additionally, the Kruskal–Wallis test was performed, followed by Dunn's test with the same purpose. A significant level of 95% was considered for all parameters ($p \leq 0.05$), and the ±standard errors of means are presented.

3. Results

3.1. Growth Performance

The growth performance of Nile tilapia after 42 days of exposure to multiple mycotoxins, both with and without the addition of organically modified clinoptilolite feed additive (MinazelPlus®), is presented in Table 2. The results show that there were no statistically significant differences ($p > 0.05$) among the groups on day 14 (Supplementary File: Table S1) and day 42 (Table 2). However, the presented results show a positive trend for all growth performance parameters in fish treated with MinazelPlus® in comparison with the other studied groups. Nile tilapia fed the diet containing 2 g/kg MinazelPlus® showed the highest increase in weight gain, percentage weight gain, daily weight gain, and specific growth rate. In addition, Nile tilapia that were fed MinazelPlus® showed an improvement in the feed conversion ratio.

Table 2. Growth performance of Nile tilapia after 42 days.

	Treatment			
	C	**MT**	**MZ**	**MZ + MT**
Weight gain (g)	28.20 ± 4.31	22.21 ± 2.19	36.38 ± 5.41	26.48 ± 3.76
Percentage weight gain (%)	92.66 ± 14.16	72.98 ± 7.21	119.50 ± 17.79	87.00 ± 12.35
Daily weight gain (g/day)	0.67 ± 0.10	0.53 ± 0.05	0.87 ± 0.13	0.63 ± 0.09
Specific growth rate (%/day)	1.53 ± 0.19	1.29 ± 0.10	1.83 ± 0.20	1.47 ± 0.15
Feed conversion ratio	1.38 ± 0.32	1.58 ± 0.18	1.04 ± 0.19	1.38 ± 0.17

Data are presented as means ± SEMs, with n = 6. C (control group), MT (mycotoxin group), MZ (MinazelPlus® group), and MZ + MT (MinazelPlus® and mycotoxin group).

3.2. Hematological Parameters

Differential blood cell counts (Figure 1) showed a statistically significant increase in lymphocyte counts in the MinazelPlus® group on day 42 compared with both the MT and MZ-MT groups. There were no significant differences compared with the fish from the control group (Figure 1b) or among groups on day 14 (Supplementary data Figure S2). The group treated with MinazelPlus® showed a statistically significant decrease in neutrophil counts compared with the other groups on day 42 (Figure 1c). Additionally, no significant differences among the groups in terms of total leukocyte counts and monocyte counts were observed. Nevertheless, Nile tilapia treated with MinazelPlus® showed a higher number of total leukocytes in comparison with the other groups (Figure 1a,d).

3.3. Biochemical Parameters

The plasma biochemical parameter analysis of Nile tilapia after 42 days of exposure to multiple mycotoxins, with (MZ + MT) and without (MT) the addition of organically modified clinoptilolite feed additive (MinazelPlus®), is shown in Table 3. The results show no statistically significant differences ($p > 0.05$) among the groups on day 42. Notably, an increasing trend for the levels of total protein, albumin, and globulin in the MinazelPlus® additive group was observed. Furthermore, the levels of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were reduced in fish that received MinazelPlus® compared with other groups. The results indicate that feeding Nile tilapia a diet supplemented with 2 g/kg organically modified clinoptilolite feed additive (MinazelPlus®) had positive effects, resulting in increasing trends for total protein, albumin, globulin, ALT, and AST levels. The results of 14 days of treatment do not show significant differences in the studied parameters (Supplementary File: Table S2).

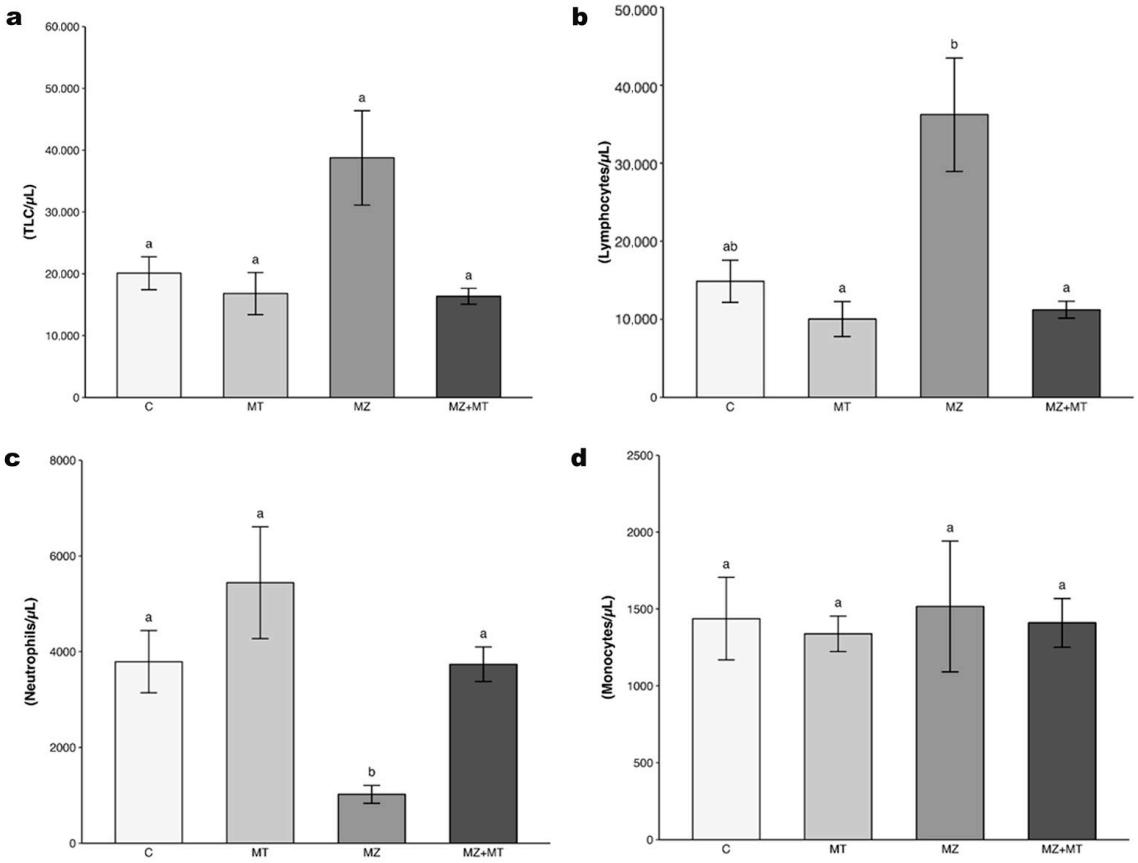

Figure 1. Hematological parameters of Nile tilapia after 42 days; values are means ± SEMs, with n = 6. Different letters designate statistical differences among groups ($p \leq 0.05$). (**a**) Total leukocyte counts, (**b**) lymphocyte counts, (**c**) neutrophil counts, and (**d**) monocyte counts.

Table 3. Biochemical parameters of Nile tilapia.

	Treatment			
	C	**MT**	**MZ**	**MZ + MT**
Total protein (g/L)	27.90 ± 0.92	25.48 ± 1.06	29.07 ± 2.79	27.45 ± 1.16
Albumin (g/L)	9.55 ± 0.45	8.42 ± 0.49	9.97 ± 0.73	8.67 ± 0.55
Globulin (g/L)	18.35 ± 0.64	17.07 ± 0.61	19.11 ± 2.73	18.78 ± 0.76
ALT (U/L)	23.67 ± 3.33	29.83 ± 5.71	18.50 ± 2.28	26.33 ± 3.46
AST (U/L)	40.67 ± 2.99	50.83 ± 3.47	35.33 ± 5.61	41.83 ± 5.49

Data are presented as means ± SEMs, with n = 6. C (control group), MT (mycotoxin group), MZ (MinazelPlus® group), MZ + MT (MinazelPlus® and mycotoxin group), ALT (alanine aminotransferase), and AST (aspartate aminotransferase).

3.4. Clinical Signs

The Nile tilapia group fed the diet contaminated with multiple mycotoxins for 42 days showed clinical signs consistent with the observed histopathological changes in the fish, as displayed in Figure 2. The fish showed symptoms such as yellow skin and fin rot (Figure 2a), along with congested and moderately inflamed intestines (Figure 2b). Additionally, the presence of fluid in the abdominal cavity (ascites) was observed (Figure 2c). Furthermore, a pale appearance of the liver was also observed, indicating a possible pathology (Figure 2d). Pictures representative of the other groups are in the supplementary file (Figure S3).

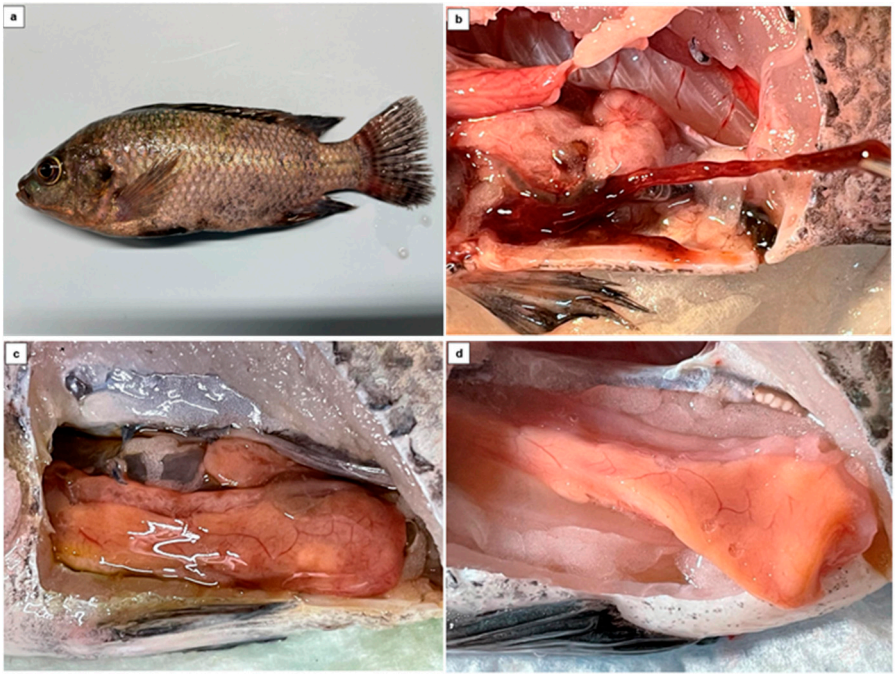

Figure 2. Clinical signs of Nile tilapia exposed to multiple mycotoxins for 42 days. (**a**) Yellow skin and fin rot. (**b**) Congested and moderately inflamed intestines. (**c**) Ascites—the presence of fluid in the abdominal cavity. (**d**) Pale liver indicating possible pathology.

3.5. Histopathological Examination

3.5.1. Histopathological Changes in Liver of Nile Tilapia

The histopathological changes in the liver of Nile tilapia after 42 days of exposure to multiple mycotoxins, both with and without the addition of organically modified clinoptilolite feed additive (MinazelPlus®), are shown in Figure 3. Nile tilapia fed the diet containing 2 g/kg MinazelPlus® showed intact hepatic lobular architecture with normal hepatocytes (Figure 3b). Normal hepatic architecture with mild fatty change in hepatocytes, as well as mild vacuolation of some hepatocytes and slight degeneration of hepatocytes, was observed in fish from the control group (Figure 3a). The group of fish exposed to mycotoxins showed several pathological signs, particularly severe hepatocyte deformation, vacuolar degeneration of hepatocytes, disarrangement of cells, and hepatocyte cloudiness. In addition, congestion of the blood sinusoids and pyknosis and coagulation necrosis of the hepatocytes were also identified. The hepatopancreas showed the symptoms of necrosis with lymphocytic infiltration and necrosis of pancreatic acini (Figure 3c).

The group of fish fed the mycotoxin diet supplemented with 2 g/kg MinazelPlus® showed pyknosis, blood sinusoid, and marked degenerative changes in hepatocytes compared with the control group. Furthermore, there were moderate vacuolation of hepatocytes, moderate fatty changes in hepatocytes, and mild necrosis of the hepatopancreas with lymphocytic infiltration (Figure 3d). For the purpose of direct comparison, Figure S4, representing the liver tissue of the mycotoxin and MinazelPlus® and the mycotoxins groups, is provided in the supplementary file.

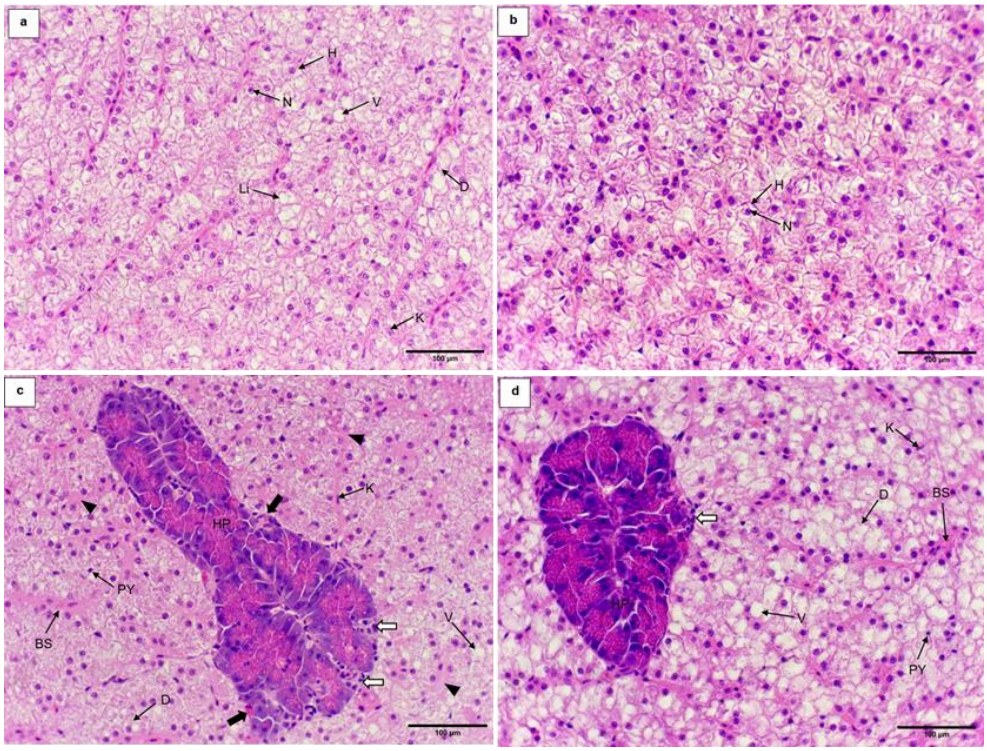

Figure 3. Liver histopathology of Nile tilapia, stained with hematoxylin and eosin, where bar = 100 µm. BS = blood sinusoid; D = degeneration of hepatocytes; H = hepatocyte; HP = hepatopancreas; K = Kupffer cell; Li = lipid; N = nucleus; PY = pyknosis; V = vacuolation degeneration. (**a**) Control group showed normal hepatic architecture with mild fatty change in hepatocytes, mild vacuolation of some hepatocytes, and slight degeneration of hepatocytes. (**b**) MinazelPlus® group showed intact hepatic lobular architecture with normal hepatocytes. (**c**) Mycotoxin group showed severe hepatocyte deformation, vacuolar degeneration of hepatocytes, disarrangement of cells, hepatocyte cloudiness, congestion of blood sinusoids, pyknosis, and coagulation necrosis of hepatocytes (arrowheads). The hepatopancreas showed necrosis with lymphocytic infiltration (white arrows) and necrosis of pancreatic acini (black arrows). (**d**) Mycotoxin and MinazelPlus® group showed pyknosis, blood sinusoid, and moderate degeneration of hepatocytes, as well as visible moderate vacuolation of hepatocytes, moderate fatty changes in hepatocytes, and mild necrosis of hepatopancreas with lymphocytic infiltration (white arrow).

3.5.2. Histopathological Changes in Intestines of Nile Tilapia

Figure 4 presents the histopathological changes in the intestine of Nile tilapia after 42 days of exposure to multiple mycotoxins, both with and without the addition of organically modified clinoptilolite feed additive (MinazelPlus®). The control group and group of fish treated with 2 g/kg MinazelPlus® showed a normal intestinal structure consisting of the mucosa, submucosa, muscle layer, and serosa. Furthermore, goblet cells, lamina propria, and normal villi were intact with normal enterocytes, as shown in Figure 4a,b. The group of fish exposed to mycotoxins showed various pathological indications, such as severe necrosis of the enterocytes along with lymphocytic infiltration, infiltration of mononuclear cells in the lamina propria, edemas in the lamina propria, and infiltration of neutrophils, in addition to hyperplasia of goblet cells, muscle layer degeneration, severe necrosis, degeneration, and cell lysis in the villi of the intestine. Additionally, shorter intestinal villi were observed compared with the other group (Figure 4c). The group of fish fed the mycotoxin-contaminated diet supplemented with 2 g/kg MinazelPlus® showed edemas in the lamina propria and goblet cell hyperplasia in some villi. Also, there were signs of moderate necrosis, degeneration, and cell lysis in the villi of the intestine (Figure 4d).

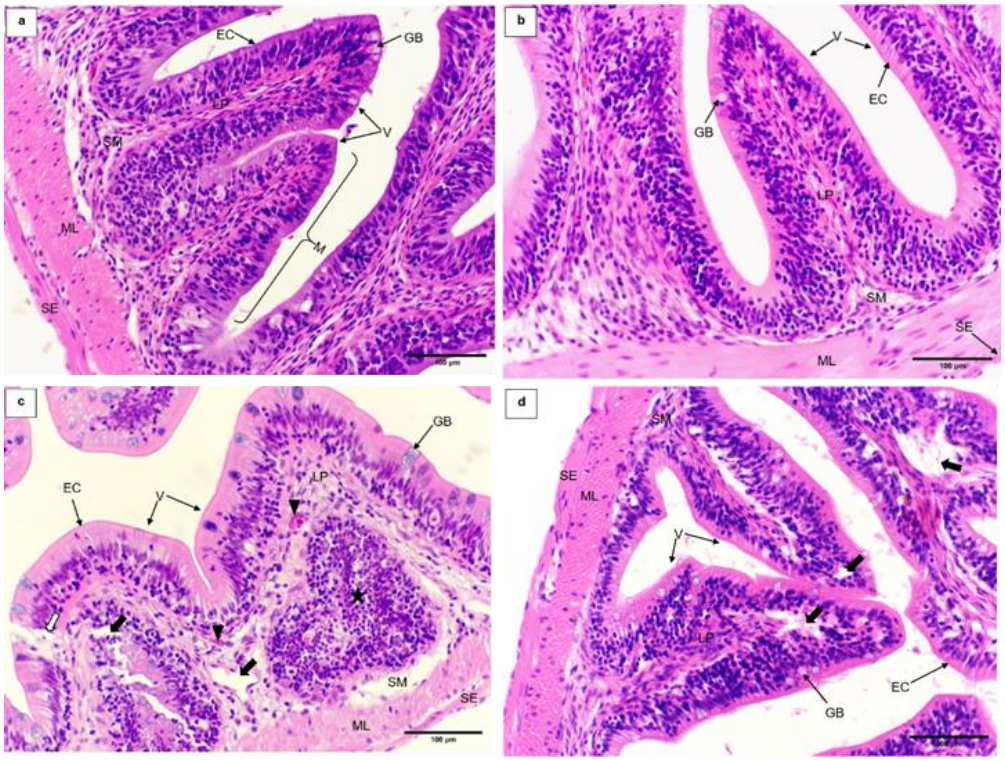

Figure 4. Intestine histopathology of Nile tilapia, stained with hematoxylin and eosin, where bar = 100 µm. EC = enterocyte; GB = Goblet cell; LP = lamina propria; M = mucosa; ML = muscle layer; SE = serosa; SM = submucosa; V = villi. (**a**) Control group showed normal structure of intestine, consisting of mucosa, submucosa, muscle layer, serosa, normal villi with normal enterocytes, goblet cells, and lamina propria. (**b**) MinazelPlus® group showed normal structure of intestine, consisting of mucosa, submucosa, muscle layer, serosa, normal villi with normal enterocytes, goblet cells, and lamina propria. (**c**) Mycotoxin group showed severe necrosis of enterocytes (white arrow) with lymphocytic infiltration, infiltration of mononuclear cells in lamina propria (star), edemas in lamina propria (black arrows), heterophil infiltration (arrowheads), hyperplasia of goblet cells, muscle layer degeneration, severe necrosis, degeneration, cell lysis in villi of intestine, and shorter intestinal villi. (**d**) Mycotoxin and MinazelPlus® group showed edemas in lamina propria (black arrows), goblet cells hyperplasia in some villi, moderate necrosis, degeneration, and cell lysis in villi of intestine.

3.5.3. Histopathological Changes in Posterior Kidney of Nile Tilapia

The histopathological changes in the posterior kidney of Nile tilapia after 42 days of exposure to multiple mycotoxins, both with and without the addition of organically modified clinoptilolite feed additive (MinazelPlus®), are shown in Figure 5. The control group showed a normal kidney structure consisting of a normal glomerulus with Bowman's space, as well as renal tubules (Figure 5a). The group of fish treated with 2 g/kg MinazelPlus® showed a normal structure of the renal tubules, as shown in Figure 5b. The group of fish exposed to mycotoxins revealed increased tubular lumen, mononuclear cell infiltration, intertubular hemorrhages, and perivascular fibrosis accompanied by mononuclear infiltration. Fish in this group also showed cystic tubular and glomerular expansion with the absence of Bowman's space. Additionally, severe necrosis of some renal tubules and moderate vacuolation degenerations of the renal tubular epithelium were observed (Figure 5c). The fish group that was fed mycotoxin-contaminated diet supplemented with 2 g/kg MinazelPlus® showed an increase in the tubular lumen, infiltration of mononuclear cells, intertubular hemorrhages, and cystic tubular. Furthermore, glomerular expansion with the absence of Bowman's space, along with mild necrosis of some renal tubules and mild vacuolation degeneration of the renal tubular epithelium were observed in this group (Figure 5d).

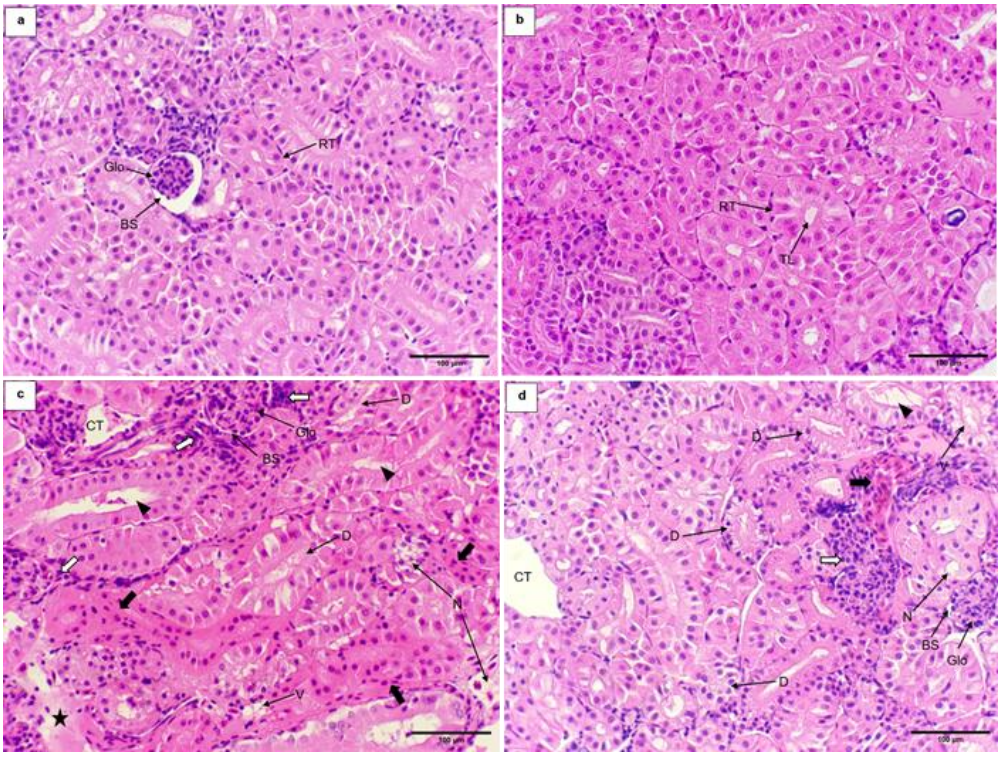

Figure 5. Kidney histopathology of Nile tilapia, stained with hematoxylin and eosin, where bar = 100 µm. BS = Bowman's space; CT = cystic lumen; D = degeneration; Glo = glomerulus; N = necrosis; RT = renal tubules; TL = tubular lumen; V = vacuolation. (**a**) Control group showed normal histological structure, normal glomerulus (Glo) with Bowman's space (BS), and renal tubules (RTs). (**b**) MinazelPlus® group showed normal architecture of renal tubules (RTs). (**c**) Mycotoxin group showed increase in tubular lumen (arrowhead), mononuclear cell infiltration (white arrows), intertubular hemorrhages (black arrows), perivascular fibrosis accompanied by mononuclear infiltration (star), cystic tubular (CT), glomerular expansion with absence of Bowman's space, severe necrosis of some renal tubules, and moderate vacuolation degeneration of renal tubular epithelium. (**d**) Mycotoxin and MinazelPlus® group showed increase in tubular lumen (arrowhead), mononuclear cell infiltration (white arrows), intertubular hemorrhages (black arrows), cystic tubular (CT), glomerular expansion with absence of Bowman's space, mild necrosis of some renal tubules, and mild vacuolation degeneration of renal tubular epithelium.

3.5.4. Histopathological Changes in Spleen of Nile Tilapia

Figure 6 illustrates the histopathological changes in the spleen of Nile tilapia after 42 days of exposure to multiple mycotoxins, both with and without the addition of organically modified clinoptilolite feed additive (MinazelPlus®). Both the control group and the group of fish treated with 2 g/kg MinazelPlus® showed a normal structure of the spleen, consisting of red pulp and white pulp, as shown in Figure 6a, b. The group of fish exposed to mycotoxins showed marked lymphoid depletion, along with melanomacrophage centers (Figure 6c). However, the group of fish that consumed contaminated mycotoxins supplemented with 2 g/kg MinazelPlus® showed a mild degree of lymphoid depletion and a low density of melanomacrophage centers compared with the group of fish that consumed only mycotoxins (Figure 6d).

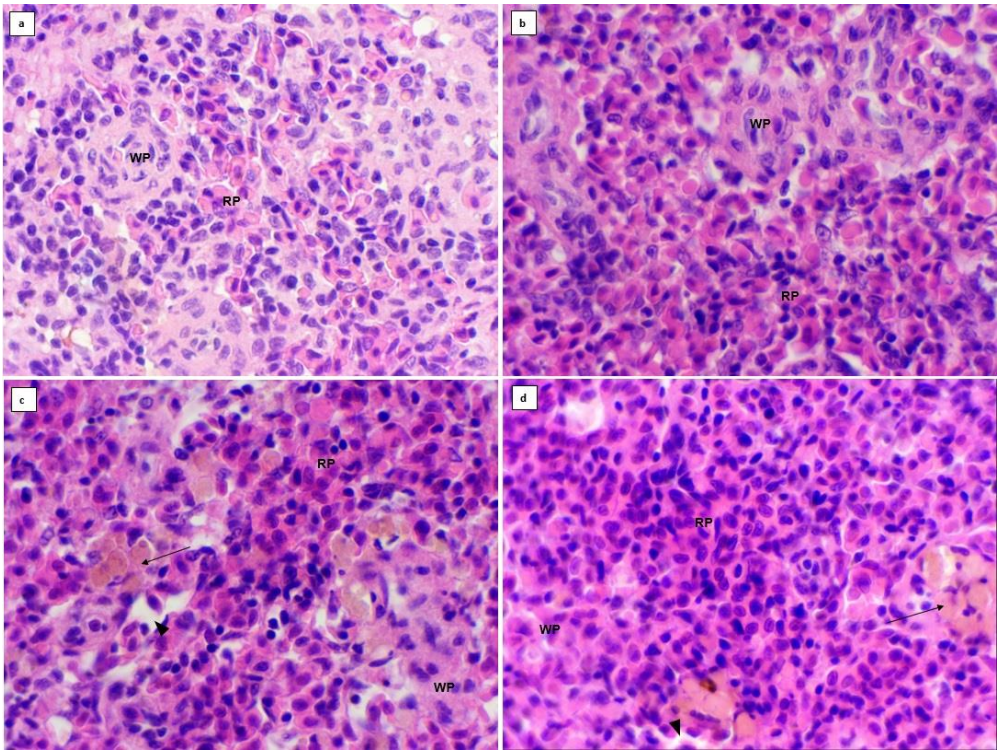

Figure 6. Spleen histopathology of Nile tilapia, stained with hematoxylin and eosin, where bar = 100 µm. RP = red pulp; WP = white pulp. (**a**) Control group showed normal structure of red pulp (RP) and white pulp (WP). (**b**) MinazelPlus® group showed normal structure of red pulp (RP) and white pulp (WP). (**c**) Mycotoxin group showed marked lymphoid depletion (arrowheads) with melanomacrophage centers (arrows). (**d**) Mycotoxin and MinazelPlus® group showed mild degree of lymphoid depletion (arrowheads) and low density of melanomacrophage centers (arrow).

4. Discussion

The samples collected from fish fed plant-based raw materials and animal finished feed were contaminated with multiple mycotoxins [40–42] and presented with multiple symptoms, such as damage to the internal organs, particularly the liver, kidneys, and intestines (Figures 2–6) after 6 weeks of dietary exposure. Among direct toxic effects, the literature supports that mycotoxin-related pathologies includes carcinogenesis, damage to neurons and blood cells, and immune system interference [22,43–45]. It is well established that chronic exposure to mycotoxins at low doses can interfere with human and animal health [5,46].

Studies of dietary mycotoxin effects mostly focus on individual mycotoxins, particularly aflatoxin B1 [47–49]. There is limited information available regarding the effects on fish that have simultaneously consumed multiple mycotoxins [21,50], and no such information is available to us regarding multiple-mycotoxin exposure at levels below regulatory limits. In this study, we fed Nile tilapia low (at or below regulatory limits) concentrations of multiple mycotoxins (40 µg/kg AFB_1, 600 µg/kg FB, 50 µg/kg ZEN, and 150 µg/kg DON) to mimic the frequently detected levels of mycotoxins found in animal feed [9].

To investigate the role of mycotoxin absorbers in mycotoxicosis prevention and control, contaminated feed was supplemented with 2 g/kg organically modified clinoptilolite feed additive (MinazelPlus®) for 42 days. Several studies have shown that modified clinoptilolite has the ability to adsorb different mycotoxins, with the large internal surface area and cation exchange binding polar mycotoxins and the organic chains added on the surface binding non-polar mycotoxins. Therefore, such modifications provide the capability of performing the simultaneous and selective adsorption of multiple mycotoxins and do not engage in the adsorption of other macro- or micro-nutrients [10,14,15].

The most common sign of mycotoxicosis in aquacultured fish presents as stunned growth rate and poor growth performance [50,51]. In the present study, there were no significant differences ($p > 0.05$) in growth performance among the groups; therefore, it is possible that the applied concentrations and duration of multiple-mycotoxin exposure did not cause a detectable effect on growth performance [17,52–54]. However, Nile tilapia fed the 2 g/kg MinazelPlus®-supplemented diet (MZ group) showed positive trends regarding weight gain, percentage weight gain, daily weight gain, and specific growth rate (Table 2). In addition, fish fed MinazelPlus® showed an improvement in the feed conversion ratio in comparison with the other groups (Table 2). Simultaneously, fish exposed to multiple mycotoxins without the dietary supplement MinazelPlus® (MT group) showed decreased growth performance compared with fish exposed to multiple mycotoxins with the addition of 2 g/kg MinazelPlus® (MZ + MT group) and the control group (Table 2), suggesting that the addition of organically modified clinoptilolite feed additive (MinazelPlus®) to the diet can potentially improve growth performance.

Similar results regarding production parameters have been reported in other studies. Zahran et al. [20] found that there were no significant differences in growth performance among the experimental groups of Nile tilapia that received 16 µg/kg AFBs for 8 weeks. However, the experimental groups that received a diet supplemented with modified zeolite (clinoptilolite) showed a significant increase in growth performance. Hassaan et al. [24] reported that tilapia exposed to AFB_1 had decreased growth performance. However, adding 5 g/kg or 10 g/kg nanozeolite to the diet improved the growth performance compared with feed contaminated with AFB_1 but without nanozeolite supplementation. According to Alinezhad et al. [55], trout (*Oncorhynchus mykiss*) fed 5 mg of aflatoxins B_1 and supplemented with 0.5–1% nanostructured zeolite for 52 days showed no difference in growth performance compared with the other experimental groups. Some research has shown that the negative effects of mycotoxins could become visible after prolonged exposure to high levels of mycotoxins in feed. Marijani et al. [56] found that there was no growth difference between the control group and groups with different concentrations of AFB_1 in the first 2 months. However, after 3 months, the weight of fish exposed to 200 µg/kg and 2000 µg/kg AFB_1 diets was lower compared with the control and 20 µg/kg AFB_1 diets.

Hematological parameters are important indicators often used to assess the health and well-being of fish in aquaculture. White blood cell count (WBC) is commonly utilized as an indication of the immunological potential of fish [57–69]. We found a statistically significant decrease ($p \leq 0.05$) in the lymphocyte count in fish from the MT and MZ + MT groups compared with the MZ group. Nevertheless, there were no significant differences compared with the control group (Figure 1b). In the MZ group, a statistically significant decrease in the neutrophil count was observed in comparison with the other group (Figure 1c). On the contrary, Saei et al. [60] discovered no significant difference in lymphocytes, neutrophils, and monocytes in fingerlings of rainbow trout (*Oncorhynchus mykiss*) when fed diets contaminated with 1 mg/kg aflatoxin for 60 days. Our research findings agree with those of Zahran et al. [17], who observed a reduction in neutrophil counts and an increase in lymphocyte counts in Nile tilapia fed a diet contaminated with 16 µg/kg aflatoxin compared with fish fed a diet contaminated with aflatoxins with MinazelPlus® supplementation. The enhanced efficacy shown in our research may be attributable to the ability of MinazelPlus® to decrease the transfer of mycotoxins from the digestive tract into the bloodstream by binding them and expelling them with feces [20,54,61]. This also indicates that MinazelPlus® successfully prevented the immunosuppressive impact of mycotoxins and protected the immune system of Nile tilapia.

Plasma biochemical parameters, including total protein, albumin, globulin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), showed no significant differences ($p > 0.05$) in Nile tilapia in our study. These findings corroborate with the research studies conducted by Saei et al. [60] and Tola et al. [62], who stated that trout (*Oncorhynchus mykiss*) showed no changes in ALT, AST, total serum protein, albumin,

and globulin levels when fed diets contaminated with 1 mg/kg aflatoxin for 60 days. Nevertheless, the finding showed a decreasing trend in total protein, albumin, and globulin but an increase in ALT and AST activity in fish exposed to multiple mycotoxins without the dietary supplement MinazelPlus® (MT group) compared with the other groups (Table 3).

Our study indicates that Nile tilapia in the MZ group had positive outcomes, resulting in improvements in total protein, albumin, globulin, ALT, and AST levels compared with the MZ + MT group. The fish in the MZ + MT group showed a more effective increase in plasma total protein, albumin, and globulin levels compared with the other groups, demonstrating protective potential. Other studies by Naiel et al. [63] found that AFB_1 caused a significant reduction in serum total, albumin, and globulin levels, along with decreased ALT and AST in Nile tilapia fed 2.5 mg of AFB. Deng et al. [64] found that AFB_1 concentrations above 638 μg/kg led to decreases in the levels of total protein and albumin, as well as reduced ALT and AST in the plasma of Nile Tilapia. Similar reductions were seen in Nile tilapia that were given 200 μg/kg AFB_1 [65]. Our study results may vary from those of other studies due to the differences in concentrations, duration of exposure, and combined effects of multiple mycotoxins in the diet.

After 42 days of exposure to low-level concentrations of multiple mycotoxins, Nile tilapia showed clinical signs and histopathological alterations in the liver, intestines, kidney, and spleen attributable to mycotoxin toxicity. Our findings are consistent with research conducted by Naiel et al. [63] and Fornari et al. [21], who determined several pathological alterations in Nile tilapia, such as coagulation necrosis of hepatocytes, severe hepatocyte deformation, necrosis with lymphocytic infiltration, and necrosis of pancreatic acini (Figure 3c). Naiel et al. [63], Shahafve et al. [66], Hassaan et al. [24], and Abu-Hassan et al. [67] observed similar liver histopathological signs in Nile tilapia and Common carp fed AFB_1-contaminated feed, including severe hepatocyte deformation, vacuolar degeneration, cell disarrangement, hepatocyte cloudiness, blood sinusoid congestion, and pyknosis.

We found pathological changes in the intestines of fish from the MT group consistent with reported mycotoxin-related pathologies. The infiltration of mononuclear cells in the lamina propria, edemas in the lamina propria, infiltration of neutrophils, and shorter intestinal villi were noted (Figure 4c). Our research findings are similar to those of Shahafve et al. [66], who observed that fish fed a diet contaminated with AFB_1 showed hyperplasia of goblet cells and severe necrosis of the enterocytes, along with lymphocytic infiltration, muscle layer degeneration, severe necrosis, degeneration, and cell lysis in the villi of the intestine. This, however, is in contrast with the findings of Huang et al. [68] and Tuan et al. [69], who observed no histological lesions in the intestines of Prussian carp (*Carassius gibelio*) administered different concentrations of AFB_1 or in the intestines of Nile tilapia fed 1 mg of AFB_1.

Consuming multiple mycotoxins for six weeks without any additional protective substances also caused histological alterations in the kidney and spleen (Figures 5c and 6c). The fish from the MT group showed increased tubular lumen mononuclear cell infiltration, intertubular hemorrhages, and perivascular fibrosis accompanied by mononuclear infiltration. Fish in this group also showed cystic tubular and glomerular expansion with the absence of Bowman's space. Severe necrosis of some renal tubules and moderate vacuolation degeneration of the renal tubular epithelium were seen in the kidney. Similar observations of histopathological changes were noted in common carp kidneys after consuming diets contaminated with 0.5, 0.7, and 1.4 mg/kg AFB for 21 days [70], whereas Nile tilapia showed similar changes after consuming diets contaminated with 0.6 ppm fumonisin B_1 [67]. In addition, common carp showed histological alterations in the spleen marked by lymphoid depletion, and melanomacrophage necrosis was observed in fish exposed to mycotoxins. The findings of Abu-Hassan et al. [67] on common carp exposed to fumonisin B_1 also support our observations. The findings of Jain [71] indicate that the effects of clinoptilolite were heavily time-dependent and the differences observed in the short-term were significantly smaller compared with the long-term group. Therefore, the

duration of application can be considered one of the key factors in supplementation of fish feed with clinoptilolites such as MinazelPlus®.

5. Conclusions

Supplementation with 2 g/kg organically modified clinoptilolite of diets with low-dose multiple-mycotoxin contamination has the potential to improve growth performance and preserve the biochemical and immunological responses of Nile tilapia. Simultaneous exposure to multiple mycotoxins in feed at concentrations near or below regulatory limits may affect tilapia's health. The addition of a broad-spectrum mycotoxin absorber (MinazelPlus®) to the diet prevented mycotoxin-related health consequences, supporting its use in mycotoxicosis prevention and control programs in aquaculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9110449/s1. Table S1: Growth performance of Nile tilapia in week 2, Table S2: Biochemical parameters of Nile tilapia blood in week 2, Figure S1: Results of feed analysis, representing diets which were utilized in experiments, Figure S2: Hematological parameters of Nile tilapia, Figure S3: Clinical signs of Nile tilapia exposed to various diets utilized in experiments during 42 days of exposure, Figure S4: A. Mycotoxin group showed severe hepatocyte deformation. B. Mycotoxin and MinazelPlus® group showed PY—pyknosis.

Author Contributions: W.H. designed the study, conducted the trials, analyzed the data, and wrote the original draft of the manuscript. D.P. designed the study, supervised the study, revised and edited the manuscript, and approved the manuscript to be published. K.W. and P.C. revised and edited the manuscript and performed the experiments. J.R. supervised the study. N.T. performed mycotoxin analysis and discussed the results. W.T. and N.S. performed the experiments. F.B. supervised the study and revised the histopathological examination part. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in this article.

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Conflicts of Interest: Author Nemanja Todorović was employed by Patent Co. DOO. The remaining authors declare that the research study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

List of Abbreviations

AFB_1	aflatoxin B1
FB	fumonisin B_1 and B_2
ZEN	zearalenone
DON	deoxynivalenol

C	basal diet control
MZ	diet containing addition of 2 g/kg MinazelPlus®
MT	diet with multiple mycotoxins
MZ + MT	combination of MinazelPlus® and multiple mycotoxins

References

1. FAO. *The State of World Fisheries and Aquaculture 2024. Blue Transformation in Action*; FAO: Rome, Italy, 2024. [CrossRef]
2. El-Sayed, A.F.M.; Fitzsimmons, K. From Africa to the world—The journey of Nile tilapia. *Rev. Aquac.* **2023**, *15*, 6–21. [CrossRef]
3. FAO. *The State of World Fisheries and Aquaculture 2022. Sustainability in Action*; FAO: Rome, Italy, 2022. [CrossRef]
4. Tacon, A.G. Trends in global aquaculture and aquafeed production: 2000–2017. *Rev. Fish. Sci. Aquac.* **2020**, *28*, 43–56. [CrossRef]
5. Anater, A.; Manyes, L.; Meca, G.; Ferrer, E.; Luciano, F.B.; Pimpao, C.T.; Font, G. Mycotoxins and their consequences in aquaculture: A review. *Aquaculture* **2016**, *451*, 1–10. [CrossRef]
6. Marijani, E.; Kigadye, E.; Okoth, S. Occurrence of fungi and mycotoxins in fish feeds and their impact on fish health. *Int. J. Microbiol.* **2019**, *2019*, 6743065. [CrossRef]
7. Gonçalves, R.; Schatzmayr, D.; Hofstetter, U.; Santos, G. Occurrence of mycotoxins in aquaculture: Preliminary overview of Asian and European plant ingredients and finished feeds. *World Mycotoxin J.* **2017**, *10*, 183–194. [CrossRef]
8. Gonçalves, R.A.; Naehrer, K.; Santos, G.A. Occurrence of mycotoxins in commercial aquafeeds in Asia and Europe: A real risk to aquaculture? *Rev. Aquac.* **2018**, *10*, 263–280. [CrossRef]
9. Gruber-Dorninger, C.; Jenkins, T.; Schatzmayr, G. Global mycotoxin occurrence in feed: A ten-year survey. *Toxins* **2019**, *11*, 375. [CrossRef]
10. Di Gregorio, M.C.; Neeff, D.V.; Jager, A.V.; Corassin, C.H.; Carão, Á.C.; Albuquerque, R.D.; Azevedo, A.C.; Oliveira, C.A. Mineral adsorbents for prevention of mycotoxins in animal feeds. *Toxin Rev.* **2014**, *33*, 125–135. [CrossRef]
11. EC. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* **2006**, *364*, 5–24.
12. EC. Commission recommendation (EU) 2016/1319 of July 29, 2016 amending recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin a in pet food. *Off. J. Eur. Union* **2016**, *208*, 58–60.
13. Kolawole, O.; Graham, A.; Donaldson, C.; Owens, B.; Abia, W.A.; Meneely, J.; Alcorn, M.J.; Connolly, L.; Elliott, C.T. Low doses of mycotoxin mixtures below EU regulatory limits can negatively affect the performance of broiler chickens: A longitudinal study. *Toxins* **2020**, *12*, 433. [CrossRef] [PubMed]
14. Tomašević-Čanović, M.; Daković, A.; Rottinghaus, G.; Matijašević, S.; Đuričić, M. Surfactant modified zeolites—-New efficient adsorbents for mycotoxins. *Microporous Mesoporous Mater.* **2003**, *61*, 173–180. [CrossRef]
15. Daković, A.; Tomašević-Čanović, M.; Dondur, V.; Rottinghaus, G.E.; Medaković, V.; Zarić, S. Adsorption of mycotoxins by organozeolites. *Colloids Surf. B Biointerfaces* **2005**, *46*, 20–25. [CrossRef] [PubMed]
16. Kraljević Pavelić, S.; Simović Medica, J.; Gumbarević, D.; Filošević, A.; Pržulj, N.; Pavelić, K. Critical review on zeolite clinoptilolite safety and medical applications in vivo. *Front. Pharmacol.* **2018**, *9*, 1350. [CrossRef]
17. Ariton, A.M.; Neculai Văleanu, A.S.; Sănduleanu, C.; Crivei, I.C.; Postolache, A.N.; Poroșnicu, I.; Mădescu, B.M.; Ungureanu, E.; Trincă, L.C. Applications of Clinoptilolite in Veterinary Medicine and Animal Husbandry. *Sci. Pap. J.* **2022**, *65*, 36–40.
18. Kaya, D.; Genc, E.; Palić, D.; Genc, M.A.; Todorović, N.; Sevgili, H.; Vasiljević, M.; Kanyılmaz, M.; Guroy, D. Effect of dietary modified zeolite (clinoptilolite) on growth performance of gilthead sea bream (*Sparus aurata*) in the recirculating aquaculture system. *Aquac. Res.* **2022**, *53*, 1284–1292. [CrossRef]
19. Obradović, S.; Vukašinović, M.; Šekler, M.; Rajković, M.; Kosanović, N. Adding zeolite 'minazel-plus' in feeding Oncorhynchus mykiss (Walbaum) and its influence on morphometrical characteristics. *Vet. Glas.* **2006**, *60*, 345–355. [CrossRef]
20. Zahran, E.; Risha, E.; Hamed, M.; Ibrahim, T.; Palić, D. Dietary mycotoxicosis prevention with modified zeolite (Clinoptilolite) feed additive in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2020**, *515*, 734562. [CrossRef]
21. Fornari, D.; Peixoto, S.; Ksepka, S.P.; Bullard, S.A.; Rossi, W.; Dennis, N.E.; Davis, D.A. Effects of dietary mycotoxins and mycotoxin adsorbent additives on production performance, hematological parameters, and liver histology in juvenile Nile tilapia (*Oreochromis niloticus*). *Front. Anim. Sci.* **2023**, *4*, 1281722. [CrossRef]
22. Matejova, I.; Svobodova, Z.; Vakula, J.; Mares, J.; Modra, H. Impact of mycotoxins on aquaculture fish species: A review. *J. World Aquac. Soc.* **2017**, *48*, 186–200. [CrossRef]
23. Sampantamit, T.; Ho, L.; Lachat, C.; Sutummawong, N.; Sorgeloos, P.; Goethals, P. Aquaculture production and its environmental sustainability in Thailand: Challenges and potential solutions. *Sustainability* **2020**, *12*, 2010. [CrossRef]
24. Hassaan, M.S.; Nssar, K.M.; Mohammady, E.Y.; Amin, A.; Tayel, S.I.; El-Haroun, E.R. Nano-zeolite efficiency to mitigate the aflatoxin B1 (AFB1) toxicity: Effects on growth, digestive enzymes, antioxidant, DNA damage and bioaccumulation of AFB1 residues in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2020**, *523*, 735123. [CrossRef]
25. Abd El-Hack, M.E.; El-Saadony, M.T.; Nader, M.M.; Salem, H.M.; El-Tahan, A.M.; Soliman, S.M.; Khafaga, A.F. Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). *Int. J. Biometeorol.* **2022**, *66*, 2183–2194. [CrossRef] [PubMed]
26. EU. DIRECTIVE 2010/63/EU on the protection of animals used for scientific purposes. *EU Off. J.* **2010**, *L 276*, 33–79. Available online: https://faolex.fao.org/docs/pdf/eur98296.pdf (accessed on 1 September 2024).

27. Eroglu, N.; Emekci, M.; Athanassiou, C.G. Applications of natural zeolites on agriculture and food production. *J. Sci. Food Agric.* **2017**, *97*, 3487–3499. [CrossRef]
28. Thiex, N.; Novotny, L.; Crawford, A. Determination of ash in animal feed: AOAC official method 942.05 revisited. *J. AOAC Int.* **2012**, *95*, 1392–1397. [CrossRef]
29. Ng, W.K.; Romano, N. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Rev. Aquac.* **2013**, *5*, 220–254. [CrossRef]
30. Sardar, P.; Singha, K.P.; Shamna, N.; Sahu, N. Recent advances in tilapia nutrition. In Proceedings of the Twelfth International Symposium on Tilapia in Aquaculture (ISTA 12), Chennai, India, 19–21 June 2019.
31. Royes, J.-A.B.; Chapman, F.A. Preparing Your Own Fish Feeds: Cir 97/FA097, 2/2003. *EDIS* **2003**, *2003*, 1–9.
32. Gonzalez, C.; Allan, G. *Preparing Farm-Made Fish Feed*; NSW Department of Primary Industries: Queanbeyan, Australia, 2007.
33. Witeska, M.; Kondera, E.; Ługowska, K.; Bojarski, B. Hematological methods in fish–Not only for beginners. *Aquaculture* **2022**, *547*, 737498. [CrossRef]
34. Meyers, T.R. Standard necropsy procedures for finfish. In *NWFHS Laboratory Procedures Manual*, 5th ed.; US Fish and Wildlife Service: Washington, DC, USA, 2009; pp. 64–74.
35. Salkova, E.; Gela, D.; Pecherkova, P.; Flajshans, M. Examination of white blood cell indicators for three different ploidy level sturgeon species reared in an indoor recirculation aquaculture system for one year. *Vet. Med.* **2022**, *67*, 138–149. [CrossRef]
36. Braceland, M.; Houston, K.; Ashby, A.; Matthews, C.; Haining, H.; Rodger, H.; Eckersall, P. Technical pre-analytical effects on the clinical biochemistry of Atlantic salmon (*Salmo salar* L.). *J. Fish Dis.* **2017**, *40*, 29–40. [CrossRef] [PubMed]
37. Khor, L.; Delamare-Debouteville, J.; Ali, S.; Chadag, V. *Quick Fish Sampling Guide for Disease Diagnostics-Blood Sampling Guide*; Worldfish: Penang, Malaysia, 2021.
38. Mumford, S.L. *Histology of Finfish*; USFWS, Olympia Fish Health Center: Olympia, WA, USA, 2004.
39. Hossain, M.K.; Hossain, M.D.; Rahman, M.H. Histopathology of some diseased fishes. *J. Life Earth Sci.* **2007**, *2*, 47–50. [CrossRef]
40. Streit, E.; Schatzmayr, G.; Tassis, P.; Tzika, E.; Marin, D.; Taranu, I.; Tabuc, C.; Nicolau, A.; Aprodu, I.; Puel, O. Current situation of mycotoxin contamination and co-occurrence in animal feed—Focus on Europe. *Toxins* **2012**, *4*, 788–809. [CrossRef]
41. Wielogórska, E.; MacDonald, S.; Elliott, C. A review of the efficacy of mycotoxin detoxifying agents used in feed in light of changing global environment and legislation. *World Mycotoxin J.* **2016**, *9*, 419–433. [CrossRef]
42. Zachariasova, M.; Dzuman, Z.; Veprikova, Z.; Hajkova, K.; Jiru, M.; Vaclavikova, M.; Zachariasova, A.; Pospichalova, M.; Florian, M.; Hajslova, J. Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals. *Anim. Feed. Sci. Technol.* **2014**, *193*, 124–140. [CrossRef]
43. Jakić-Dimić, D.; Jeremić, S.; Nešić, K.; Radosavljević, V. The influence of mycotoxins in food on fish health status. *Zb. Matice Srp. Za Prir. Nauk.* **2005**, *109*, 73–79. [CrossRef]
44. Jestoi, M. Emerging Fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin—A review. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 21–49. [CrossRef]
45. Wojnarowski, K.; Cholewińska, P.; Palić, D.; Bednarska, M.; Jarosz, M.; Wiśniewska, I. Estrogen receptors mediated negative effects of estrogens and xenoestrogens in teleost fishes. *Int. J. Mol. Sci.* **2022**, *23*, 2605. [CrossRef]
46. Ruiz, M.-J.; Macáková, P.; Juan-García, A.; Font, G. Cytotoxic effects of mycotoxin combinations in mammalian kidney cells. *Food Chem. Toxicol.* **2011**, *49*, 2718–2724. [CrossRef]
47. Hoseyni, S.Z.; Imani, A.; Vazirzadeh, A.; Moghanlou, K.S.; Farhadi, A.; Razi, M. Dietary aflatoxin B1 and zearalenone contamination affected growth performance, intestinal and hepatopancreas gene expression profiles and histology of the intestine and gill in goldfish, Carassius auratus. *Aquac. Rep.* **2024**, *34*, 101887. [CrossRef]
48. José Mendes dos Reis, F.; Silva de Pádua Melo, E.; Marcos Jacques Barbosa, A.; de Cássia Avellaneda Guimarães, R.; Arunachalam, K.; Juliano Oliveira, R.; Carla Pinheiro Lima, A.; Fernanda Balestieri Mariano de Souza, M.; Carla Gomes Rosa, A.; Aratuza Pereira Ancel, M.; et al. Bentonite Clays as Adsorbent Material for Mycotoxins and the Hematological Parameters Involved in Tilapia Species: A Systematic Review. *Aquac. Res.* **2024**, *2024*, 4899256. [CrossRef]
49. Pożarska, A.; Karpiesiuk, K.; Kozera, W.; Czarnik, U.; Dąbrowski, M.; Zielonka, Ł. AFB1 Toxicity in Human Food and Animal Feed Consumption: A Review of Experimental Treatments and Preventive Measures. *Int. J. Mol. Sci.* **2024**, *25*, 5305. [CrossRef] [PubMed]
50. Koletsi, P.; Schrama, J.W.; Graat, E.A.; Wiegertjes, G.F.; Lyons, P.; Pietsch, C. The occurrence of mycotoxins in raw materials and fish feeds in Europe and the potential effects of deoxynivalenol (DON) on the health and growth of farmed fish species—A Review. *Toxins* **2021**, *13*, 403. [CrossRef] [PubMed]
51. Magouz, F.; Salem, M.; Hashad, M. Effect of some mycotoxin on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*). *Iraqi J. Vet. Sci.* **2018**, *32*, 99–108. [CrossRef]
52. Accensi, F.; Pinton, P.; Callu, P.; Abella-Bourges, N.; Guelfi, J.-F.; Grosjean, F.; Oswald, I.P. Ingestion of low doses of deoxynivalenol does not affect hematological, biochemical, or immune responses of piglets. *J. Anim. Sci.* **2006**, *84*, 1935–1942. [CrossRef]
53. Bhujel, R.C.; Perera, A.D.; Todorović, N.; Raj, J.; Gonçalves, R.A.; Vasiljević, M. Evaluation of an Organically Modified Clinoptilolite (OMC) and a Multi-Component Mycotoxin Detoxifying Agent (MMDA) on Survival, Growth, Feed Utilization and Disease Resistance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings Fed with Low Aflatoxin. *Aquac. J.* **2023**, *3*, 56–69. [CrossRef]
54. Tóth, Á.; Zomborszky-Kovács, M.; Tornyos, G.; Szalai, N.; Kübler, K. Effect of low doses of the mycotoxin fumonisin B1 on the body mass gain, feed intake and feed conversion rate of pigs. *Agriculture* **2000**, *6*, 149–151.

55. Alinezhad, S.; Faridi, M.; Falahatkar, B.; Nabizadeh, R.; Davoodi, D. Effects of nanostructured zeolite and aflatoxin B1 in growth performance, immune parameters and pathological conditions of rainbow trout Oncorhynchus mykiss. *Fish Shellfish Immunol.* **2017**, *70*, 648–655. [CrossRef]
56. Marijani, E.; Charo-Karisa, H.; Gnonlonfin, G.J.B.; Kigadye, E.; Okoth, S. Effects of aflatoxin B1 on reproductive performance of farmed Nile tilapia. *Int. J. Vet. Sci. Med.* **2019**, *7*, 35–42. [CrossRef]
57. Esmaeili, N. Blood performance: A new formula for fish growth and health. *Biology* **2021**, *10*, 1236. [CrossRef]
58. Fazio, F. Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture* **2019**, *500*, 237–242. [CrossRef]
59. Witeska, M.; Kondera, E.; Bojarski, B. Hematological and hematopoietic analysis in fish toxicology—A review. *Animals* **2023**, *13*, 2625. [CrossRef] [PubMed]
60. Saei, M.; Taee, H.; Siahpoust, S.; Taheri, M. Effects of toxin binder biotox on growth performance survival, enzymatic activity, Hematologichal and biochemical parameters of fingerlings rainbow trout (*Oncorhynchus mykiss*) fed diets-contaminated with aflatoxin. *J. Aquac. Res. Dev. S* **2017**, *2*, 2. [CrossRef]
61. Raj, J.; Vasiljević, M.; Tassis, P.; Farkaš, H.; Bošnjak-Neumüller, J.; Männer, K. Effects of a modified clinoptilolite zeolite on growth performance, health status and detoxification of aflatoxin B1 and ochratoxin A in male broiler chickens. *Br. Poult. Sci.* **2021**, *62*, 601–610. [CrossRef]
62. Tola, S.; Bureau, D.P.; Hooft, J.M.; Beamish, F.W.; Sulyok, M.; Krska, R.; Encarnação, P.; Petkam, R. Effects of wheat naturally contaminated with Fusarium mycotoxins on growth performance and selected health indices of red tilapia (*Oreochromis niloticus*× *O. mossambicus*). *Toxins* **2015**, *7*, 1929–1944. [CrossRef]
63. Naiel, M.A.; Ismael, N.E.; Shehata, S.A. Ameliorative effect of diets supplemented with rosemary (*Rosmarinus officinalis*) on aflatoxin B1 toxicity in terms of the performance, liver histopathology, immunity and antioxidant activity of Nile Tilapia (*Oreochromis niloticus*). *Aquaculture* **2019**, *511*, 734264. [CrossRef]
64. Deng, S.-X.; Tian, L.-X.; Liu, F.-J.; Jin, S.-J.; Liang, G.-Y.; Yang, H.-J.; Du, Z.-Y.; Liu, Y.-J. Toxic effects and residue of aflatoxin B1 in tilapia (*Oreochromis niloticus*× *O. aureus*) during long-term dietary exposure. *Aquaculture* **2010**, *307*, 233–240. [CrossRef]
65. Selim, K.M.; El-hofy, H.; Khalil, R.H. The efficacy of three mycotoxin adsorbents to alleviate aflatoxin B 1-induced toxicity in *Oreochromis niloticus*. *Aquac. Int.* **2014**, *22*, 523–540. [CrossRef]
66. Shahafve, S.; Banaee, M.; Haghi, B.N.; Mohiseni, M. Histopathological study of common carp (*Cyprinus carpio*) fed aflatoxin-contaminated diets. *Int. J. Aquat. Biol.* **2017**, *5*, 63–70. [CrossRef]
67. Abu-Hassan, F.A.; Khalil, R.H.; Saad, T.T.; Amer, M.T.; Abdel-Latif, H.M. Histopathological outcomes designating the toxicological aspects of Fumonisin B1 on Cultured Nile Tilapia, *Oreochromis niloticus*. *Int. J. Fish. Aquat. Stud.* **2016**, *4*, 52–60.
68. Huang, Y.; Han, D.; Xiao, X.; Zhu, X.; Yang, Y.; Jin, J.; Chen, Y.; Xie, S. Effect of dietary aflatoxin B1 on growth, fecundity and tissue accumulation in gibel carp during the stage of gonad development. *Aquaculture* **2014**, *428*, 236–242. [CrossRef]
69. Tuan, N.A.; Grizzle, J.M.; Lovell, R.T.; Manning, B.B.; Rottinghaus, G.E. Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B1. *Aquaculture* **2002**, *212*, 311–319. [CrossRef]
70. Diab, A.M.; Salem, R.; Abeer, E.-K.M.; Ali, G.I.; El-Habashi, N. Experimental ochratoxicosis A in Nile tilapia and its amelioration by some feed additives. *Int. J. Vet. Sci. Med.* **2018**, *6*, 149–158. [CrossRef] [PubMed]
71. Jain, S.K. Protective role of zeolite on short-and long-term lead toxicity in the teleost fish Heteropneustes fossilis. *Chemosphere* **1999**, *39*, 247–251. [CrossRef]