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Dietary Mycotoxins Effects on Nile Tilapia (*Oreochromis niloticus*) Microbiomes Can Be Mitigated with Addition of Organically Modified Clinoptilolites

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Abstract: Mechanisms of action attributed to feed additives are of continuous research interest, increasing our knowledge about the side effects (direct or indirect) of their application. The primary role of organically modified clinoptilolite is to bind multiple polar and non-polar mycotoxins contaminating the feed and remove them during the digestion process and through feces, therefore preventing adsorption and consequences of mycotoxicosis on fish health. However, it is not fully understood if this binding action can influence bacterial communities in the fish digestive tract and possibly other organs, as well as the aquatic environment. Therefore, in this study, Nile tilapias (*Oreochromis niloticus*) (average weight: 30 ± 2 g; n = 48) were simultaneously exposed to low-level mycotoxins (AFB1 40 µg/kg, FB 600 µg/kg, ZEN 50 µg/kg, and DON 150 µg/kg) added to diet, with and without supplementation of commercially available organically modified clinoptilolite feed additive (MinazelPlus®, 2 g/kg). After 42 days of continuous exposure, gill tissue, feces, and water were collected, and DNA was extracted from the samples. Results of RT-PCR analysis have revealed significant changes in microbiomes in fish from different groups, most prominently in mycotoxin-exposed fish. No significant changes were detected in water samples between the control and MinazelPlus® groups, confirming the safety of MinazelPlus® for aquatic microbial communities. MinazelPlus® addition to the mycotoxin spiked diet, stabilized fish natural microbiota, and prevented the disbalance of microbial homeostasis observed in fish exposed to dietary mycotoxins.

Keywords: clinoptilolite; feed additives; microbiome; MinazelPlus®; mycotoxins; pathogens

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1. Introduction

Microbial communities associated with fishes are highly variable and diverse and can be influenced by the surrounding environment, diet, or age, affecting the host in multiple ways. Microbiomes influence digestion, nutrient assimilation, the immune system, metabolite production, and the overall health of the host [1]. Bacteria are the most abundant and key components of the microbial communities found on the external (skin, gills) and internal surfaces (digestive system). Microbiome composition is variable, depending on the type of tissue as well as localization. For example, the gastro-intestinal (GI) tract microbiome is dominated by anaerobes or facultative aerobes (strict aerobes have also been detected, although in much smaller quantities) [2]. The bacterial composition of the GI tract consists of about 90% organisms that belong to Proteobacteria (especially *Enterobacteriaceae* family), Firmicutes, and Bacteroidetes, and the remaining 10% belong to less numerous phyla such as Fusobacteria, Actinobacteria, Tenericutes, and Verrucomicroba [2–4]. The presence of

mucus can significantly affect the microbial composition of the gills and skin because of the presence of immunoglobulins and antimicrobial peptides (AMPs) with antibacterial or bacteriostatic effects [5,6]. Fish gills mucus cover contains bacterial populations in the range of 10^2 to 10^4 CFU cm^2 (reported for Atlantic salmon) [6]. Toxins, antimicrobial substances, and diet are contributing factors that may change digestive tract microbiota in aquatic animals and cause microbial dysbiosis, therefore impacting the immune system of fish and increasing the risk of diseases [7–10].

Mycotoxins are widespread feed-related fungal contaminant toxins in aquaculture, attributed to plant-based components of fish diets such as soybeans, wheat, grains, and corn. These secondary metabolites are produced under stress conditions by several mold species: *Aspergillus flavus* (Alphatoxin B1), *Gibberella zeae* (Zearalenone, ZEN), *Fusarium verticillioides* (Fumonisin B1) and *Penicillium chrysogenum* (Penicillin) [11–14]. Mycotoxins pose serious problems in aquaculture production worldwide [15] and present significant risks to human and animal health, causing or contributing to chronic and acute illnesses. Mycotoxins are regarded as a global health concern and included in the broad framework of the One-Health concept [16,17], following "an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems" (after: Joint Tripartite FAO, OIE, WHO commission and UNEP Statement, December 2021).

Exposure of fish to feed contaminated with mycotoxins is associated with liver and kidney pathologies, carcinogenic effects, gastro-intestinal disturbances, reproductive disorders, microbial dysbiosis, and immune system suppression [11,14,15,18,19]. Exposure to mycotoxins can, therefore, increase the risk of diseases and infections with pathogens such as *Aeromonas hydrophila*—which is one of the most common pathogens in aquaculture worldwide [20,21]. Frequent bacterial infection outbreaks may lead to overuse or misuse of antimicrobial substances, further influencing the emergence of resistance in bacteria and the increased presence of antimicrobial resistance genes (ARG) in water environments [22,23]. Therefore, mitigation measures and good manufacturing practices are necessary to reduce mycotoxin-related health problems.

Prevention of mycotoxins entering the food chain via animal feedstuff is carried out with different approaches, including monitoring and detection of residues and the addition of adsorbents. Clinoptilolite is a naturally occurring mineral, first described in the scientific literature in the early 1960s. Clinoptilolite is characterized by its exceptional ion-exchange properties, which make it a potent adsorbent. Its unique physicochemical properties include a complex structure comprised of the three-dimensional scaffoldlike framework of tetrahedrons built from Si(O4)-4 and Al_2O_3, where Al ion is in the center, and the presence of additional exchangeable cations of Na, K, and Ca that allow the clinoptilolites to exchange the ions with the chemicals present in the environment [24–27]. At a higher level, clinoptilolites are characterized by the lamellar rigid structure, further enhancing their ion-exchange properties. Such physicochemical properties are highly effective in the adsorption of various chemicals, simultaneously supporting the safety of use that has been determined in 2006 as "generally recognized as safe" by the US Food and Drug Administration, followed by the same designation in the EU in 2013 [28].

Clinoptilolite can be further modified to magnify some of its adsorptive properties and improve its selectivity, and one example of such modifications is a patented organically modified clinoptilolite-based product called MinazelPlus®. Three-step tribochemical surface modification and addition of the long-chain organic cations to the clinoptilolite surface resulted in the development of a two-fold layer of organic ligands to which non-polar mycotoxins are being adsorbed. MinazelPlus® modifications allow it to not only adsorb the mycotoxins but, at the same time, provide it with higher stability (strong binding) in the entire gastro-intestinal (GI) tract of the animals, even with large sectional differences in pH and other GI environments [24,25,29]. Modified and unmodified clinoptilolite effects on fish health and other production parameters were the subject of intensive studies in recent years. In studies performed on rainbow trout (*Oncorhynchus mykiss*) by Ergün et al. [30], supplementation of feed with a certain amount of zeolite has decreased levels of ammonia

excretion from farmed fish. In studies performed by Zahran et al. [31], MinazelPlus® supplementation to aflatoxins contaminated diet has mitigated adverse aflatoxin effects on fish health, including antioxidant and immune responses in fish [31]. Those observations correspond with studies performed on sea bream (*Sparus aurata*), where the addition of MinazelPlus® has significantly improved the production parameters of the fish [32].

Recent studies demonstrated that clinoptilolite can affect the composition of the microbiome of the gut and adsorb some of the metabolites associated with it [33,34]. Clinoptilolites are known to interact with pathogenic bacteria metabolites, such as biogenic amines and ammonia, and can influence levels of Enterobacteriaceae abundance in microbial communities [33,35]. Therefore, it is of interest to determine if MinazelPlus®, with its modifications, can potentially have an even stronger influence on the state of the gut microbiota.

The aim of the present study was to analyze the effect of commercially available organically modified clinoptilolite (MinazelPlus®) on selected bacteria in intestines (including feces) and gills of Nile tilapia (*Oreochromis niloticus*) exposed to low doses of multiple mycotoxins in food for an extended period (42 days). The selection was based on the most abundant phyla as reported in the literature, including the *Clostridiaceae* family, which contains important pathogenic bacteria [2–4,36–38].

2. Materials and Methods

2.1. Animals and Feed Preparation

Nile tilapia (*Oreochromis niloticus*) of 30 ± 2 g individual weight were distributed randomly in 12 aquaria (50 L) equipped with continuous single pass flow system sourced from conditioned water. Three aquaria were randomly assigned per each experimental group. The fish were randomly divided into the following 4 groups (n = 12/group):

C–control group (no mycotoxin, no MinazelPlus®, basal diet).

T–a mixture of mycotoxins was added to the basal diet (Aflatoxin B1/AFB1, 40 µg/kg; Fumonisin/FUM, 600 µg/kg; Zearalenone/ZEN, 50 µg/kg; and Deoxynivalenol/DON, 150 µg/kg).

M–basal diet with mycotoxin mixture as in diet T with the addition of MinazelPlus® (2 g/kg).

Z–basal diet with the addition of MinazelPlus® (2 g/kg).

Commercially available organically modified clinoptilolite (MinazelPlus®; Patent Co., Mišićevo, Serbia) was added to the basal diet according to the safety and efficacy study protocols. The determination of the mycotoxin concentrations used in the experiment was based on the analysis of mycotoxins presence in animal feeds from Southeast Asia, as reported by Gruber-Dorninger et al. [39]. The feed was analyzed by Patent Co. for mycotoxin concentration to confirm that the required amount of mycotoxin concentration was achieved (Table 1, Supplementary File Figure S1) [24,25]. Water quality was monitored daily (NO_2, temperature, pH) and weekly (NO_3, KH—Carbonate Hardness, DO—Dissolved oxygen) to ensure constant environmental conditions (Table 2). Observation of general fish behavior before and during feeding was performed twice daily as part of the general laboratory animal routine check that was recorded in the animal facility logbook. Over 42 days, no differences were observed in the case of growth performance or behavior of fish. All animals were kept in condition's accordance with the European Union Directives regarding the requirements for the use of acceptable technologies in breeding and animal welfare conditions. The research has received approval from the Ethical Committee for Animal Experiments of the Regierung von Oberbayern, Maximilianstr. 39, 80534 München (ROB–55.2-2532.Vet_02-20-142).

Table 1. Results of the feed analysis, representing diets that were utilized in the experiment. Diet 1—Control group ©, Diet 2—MinazelPlus® group (Z), Diet 3—Mycotoxins group (T), Diet 4—MinazelPlus® + Mycotoxins (M).

Sample Number	Sample Name	Results(µg/kg) or ppb Relative to Feed with Moisture Content of 12%										
		Aflatoxin				Ochratoxin A	Zearalenone	Deoxynivalenol	Fumonisin		HAT-2	T-2
		B1	B2	G1	G2				B1	B2		
S22-01-012	DIET 1 (C)	<0.4	<0.4	<0.4	<0.4	<1.6	<16	<64	<40	<40	<9.6	<9.6
S22-01-013	DIET 2 (Z)	<0.4	<0.4	<0.4	<0.4	<1.6	<16	<64	<40	<40	<9.6	<9.6
S22-01-014	DIET 3 (T)	31.67	Feb 45	<0.4	<0.4	<1.6	53.48	145	405	124	<9.6	<9.6
S22-01-015	DIET 4 (M)	35.85	Feb 41	<0.4	<0.4	<1.6	51.39	148	421	137	<9.6	<9.6

Table 2. Waters parameters from the 14th to the 57th day. C—control group; T—a mixture of mycotoxins was added to the basal diet; M—basal diet was mixed with Minazel Plus® 2 g/kg and mycotoxin mixture; Z—basal diet was mixed with Minazel Plus®.

Water Parameters	C		T		M		Z	
	Average	SD	Average	SD	Average	SD	Average	SD
DO (mg/L)	8.14	0.01	7.74	0.01	7.85	0.50	7.86	0.11
Temperature (°C)	27.16	0.50	27.16	0.50	27.16	0.51	27.17	0.50
NO_2 (mg/L)	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01
NO_3 (mg/L)	1.00	0.00	0.90	0.30	0.80	0.00	1.00	0.00
KH (°dH)	14.36	2.31	14.20	1.99	14.30	1.43	13.88	1.96

2.2. Experimental Design and Sampling

All fish were kept in individual tanks and fed a basal diet for 14 days in order to acclimatize, then fed with respective experimental and control diets for 42 days. After 42 days of exposure, fish were sampled (n = 10 per group), and intestines (including feces), gills, and aquarium water (n = 6/per group) were collected under aseptic conditions and placed in sterile containers. The samples were kept at −80 °C until analysis.

2.3. DNA Extraction

Extraction of DNA from water, intestines (including feces), and gills was performed using the Genomic Bacteria AX Mini kit (A&A Biotechnology, Gdansk, Poland). Then, the quality of the performed DNA isolations was checked using the Thermo Scientific NanoDrop 2000 Spectrophotometer device (Thermo Fisher, Waltham, MA, USA) and concentration on Qubit 4 Fluorometer (Thermo Fisher, Waltham, MA, USA). The average DNA content of the samples was 40 µg/µL (in 50 µL). The contamination at 260/230 (contamination related to, among others, reagents used for isolation): 2.0–2.2, and for 260/280 (contamination with substances such as enzymes and inhibitors): 1.8–2.0 (correct levels, according to the instruction manual of the device).

2.4. RT-PCR

Analysis of RT—PCR was performed with the use of an apparatus Agilent MX 3000 (Agilent Technologies, Inc., Santa Clara, CA, USA) with the SsoAdvanced™ Universal SYBR® Green Supermix kit (Bio-Rad Laboratories, Inc., Irvine, CA, USA) at a volume of 10 µL in 3 technical repetitions (Table 3). A no template control (NTC–without DNA sample, only primers and water with PCR mix) test was additionally performed for each amplicon. The real-time PCR analysis strategy was based on the amplification of specific amplicons for the tested cluster (Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria) and family *Clostridiaceae* against the reference amplicon for all bacteria (16S rDNA) (Table 4).

In addition, the obtained results were compared to the sample constituting the calibrator with the lowest level of the studied cluster and the lowest level of the reference amplicon in order to determine the relative level of DNA in terms of the tested amplicons [40].

Table 3. Proportion of qPCR mix.

Component	Volume Per 10 µL Reaction
SsoAdvanced™ Universal SYBR® Green Supermix	5 µL
Forward and reverse primers	1 µL (0.8 µM)
DNA template	2 µL (0.04–0.015 × 10^{-4})
Nuclease–free water	2 µL

A standard curve was performed for the genes tested to determine the efficiency of each gene. A sample dilution of 10^{-4} from the 10^{-2} to 10^{-7} series of dilutions was selected for analysis. A linear line of the fluorescent signals converted to the cycle threshold (Ct) values was plotted, and the slope of the linear equation was applied to calculate the primer efficiency according to the equation Efficiency (%) = (10 [−1/slope] −1) × 100. It was 89.4% for the Firmicutes phylum, 100.9% for Bacteroidetes, 91.6% for Actinobacteria, 94.1% for Proteobacteria, 95.4% for *Clostridiaceae* 98.4% and for the Universal Eubacterial Gene 94.4%.

The amplification was performed according to a protocol of 40 cycles: polymerase activation and DNA denaturation at 95 °C (3 min), denaturation at 95 °C (15 s), annealing at 60.5 °C (15 s), extension and plate reading at 72 °C (40 s). The analysis of the melting curves for the samples was performed at temperatures ranging from 65 °C (5 s) to 95 °C (0.5 °C increments in 2 s). The obtained Ct values were used for relative quantification of the DNA expression level according to the 2-ΔΔCt method (assigned as DNA level) [41].

Table 4. Primers used for analysis.

Name	Primer Sequence (5′ → 3′)	Forward (F)/Reverse (R)	Reference
Universal Eubacterial gene	530F (GTCCCAGCMGCNGCGG)	F	[42]
	1100R (GGGTTNCGNTCGTTG)	R	
Firmicutes	928F (TGAAACTYAAAGGAATTGACG)	F	[43]
	1040R (ACCATGCACCACCTGTC)	R	
Bacteroidetes	798cfbF (CRAACAGGATTAGATACCCT)	F	[43]
	cfb967R (GGTAAGGGTTCCTCGCGTAT)	R	
Actinobacteria	Eub338F (ACGGGCGGTGTGTACA)	F	[44]
	Act1159R (TCCGAGTTRACCCCGGC)	R	
Proteobacteria	27F (GAGTTTGATCMTGGCTCAG)	F	[45]
	1529R (CAKAAAGGAGGTGATCC)	R	
Clostridiaceae	Clos-58-f (AAAGGAAGATTAATACCGCATAA)	F	[46]
	Clos780-r (ATCTTGCGACCGTACTCCCC)	R	

2.5. Statistical Analysis

The data were analyzed using R statistics software (v. 4.2.3, freely available) with packages "dyplr", "ggpubr", "FSA", "vegan" and "devtools". The Shapiro–Wilk test was performed–the data distribution was not normal for tested bacteria. As a result, the analyses were performed using PCA and the Kruskal–Wallis test ($p < 0.05$), where the factors were diet type. Results of the Kruskal–Wallis test and the post hoc Dunn's multiple comparisons test showed the significance of differences between groups. Plots were created in R using ggplot2.

3. Results

3.1. Gills

In the case of gills, significant differences between the studied groups ($p < 0.01$) (Figure 1) were observed. Significant differences were shown in the level of bacteria (Figure 2) from the Actinobacteriota phylum between group C and Z ($p = 0.0014$), M and Z ($p = 0.00014$), and M and T ($p = 0.00144$). The highest levels of bacteria occurred in groups T and Z. Analysis of the Bacteroidota phylum showed differences between group C and Z ($p = 0.037$), where the level was lowest in group C. The Firmicutes phylum showed significant differences between groups C and M ($p = 0.024$), where the level of bacteria in group C was the lowest. In contrast, the greatest variation was observed in the Proteobacteria phylum. Highly significant differences were shown for this cluster between groups C and M ($p = 0.0079$), C and T ($p = 0.0016$), M and Z ($p = 0.0018$), and T and Z ($p = 0.000408$). The lowest level was shown in the T group and the highest in the Z group. However, for the Clostrodiaceae family analyzed, highly significant differences were shown between groups C and M ($p = 0.00421$) and M and Z ($p = 0.00392$), while significant differences were shown between groups M and T—$p = 0.025$. The highest level of Clostridiaceae bacteria occurred in group Z, while the lowest level was in group M.

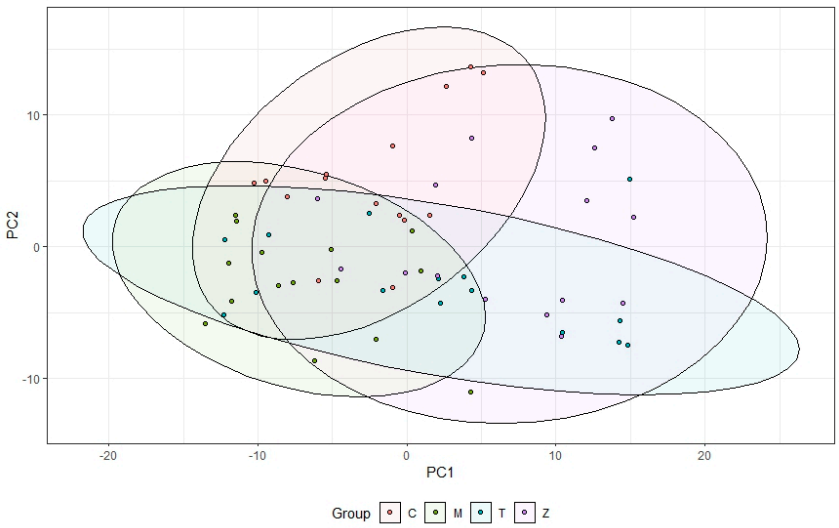

Figure 1. PCA results of analyzed gill samples: C—control group; T—a mixture of a mixture of mycotoxins was added to the basal diet; M—basal diet was mixed with Minazel Plus® 2 g/kg and mycotoxin mixture; Z—basal diet was mixed with Minazel Plus®.

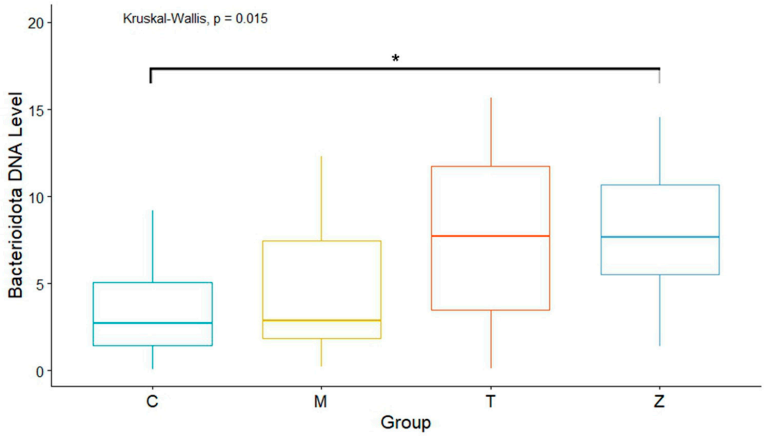

Figure 2. *Cont.*

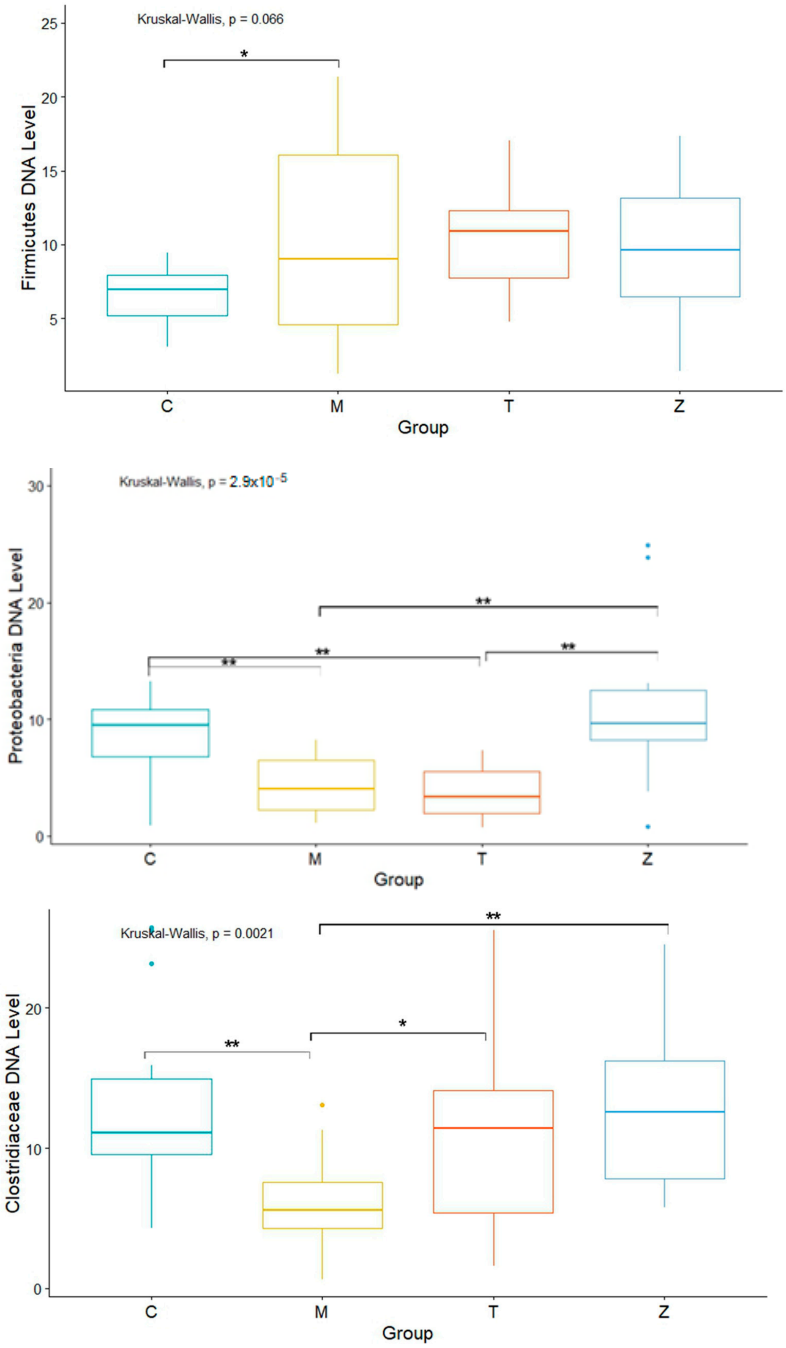

Figure 2. Differences between selected bacteria in gill samples: C—control group (basal diet); T—mycotoxins mixture was added to the basal diet; M—Minazel Plus® and mycotoxin mixture was combined with basal diet; Z—Minazel Plus® was mixed added to the basal diet. $p < 0.05$—*; $p < 0.01$—**.

3.2. Intestines

The analysis of intestines (including stool) samples showed significant differences between the analyzed groups ($p < 0.01$), which is also illustrated in Figure 3. In the case of the Firmicutes phylum (Figure 4), there were significant differences between group C and T ($p = 0.003$), where the level in group C was the highest compared to the other groups (5.84 DNA level). On the other hand, analysis of the level of the Actinobacteria phylum (Figure 4) showed significant differences between all the groups studied. Highly significant differences were shown between groups C and M ($p = 0.0016$), M and T ($p = 0.0049$), and C and Z ($p = 0.0068$). In turn, differences were found between groups T and Z at $p = 0.0222$.

The highest levels of bacteria were found in group M (22.5 DNA level), while the lowest was in group C (16.8 DNA level). Significant differences were also shown between groups M and T in the level of the *Clostridiaceae* family, with group M having the highest level.

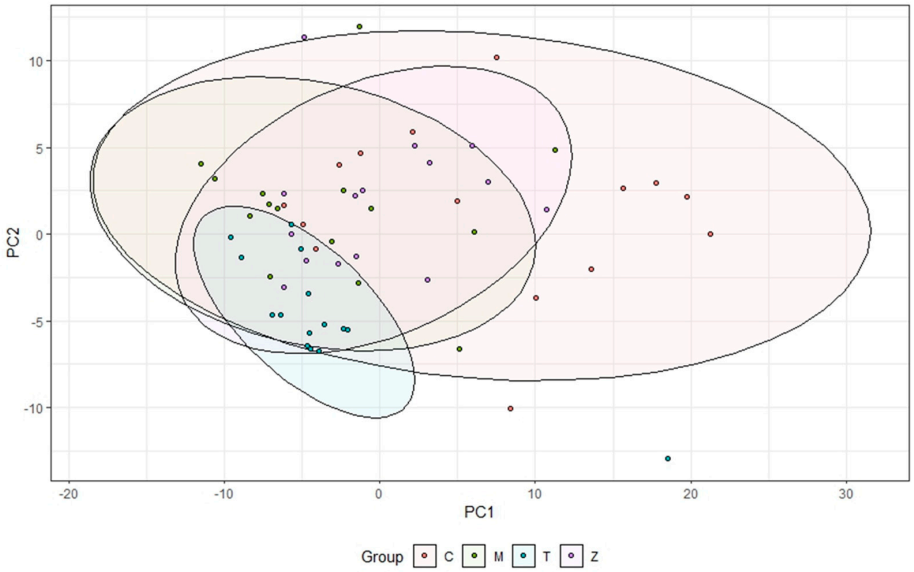

Figure 3. PCA results of analyzed intestines (including feces) samples: C—control group; T—a mixture of a mixture of mycotoxins was added to the basal diet; M—basal diet was mixed with Minazel Plus® 2 g/kg and mycotoxin mixture; Z—basal diet was mixed with Minazel Plus®.

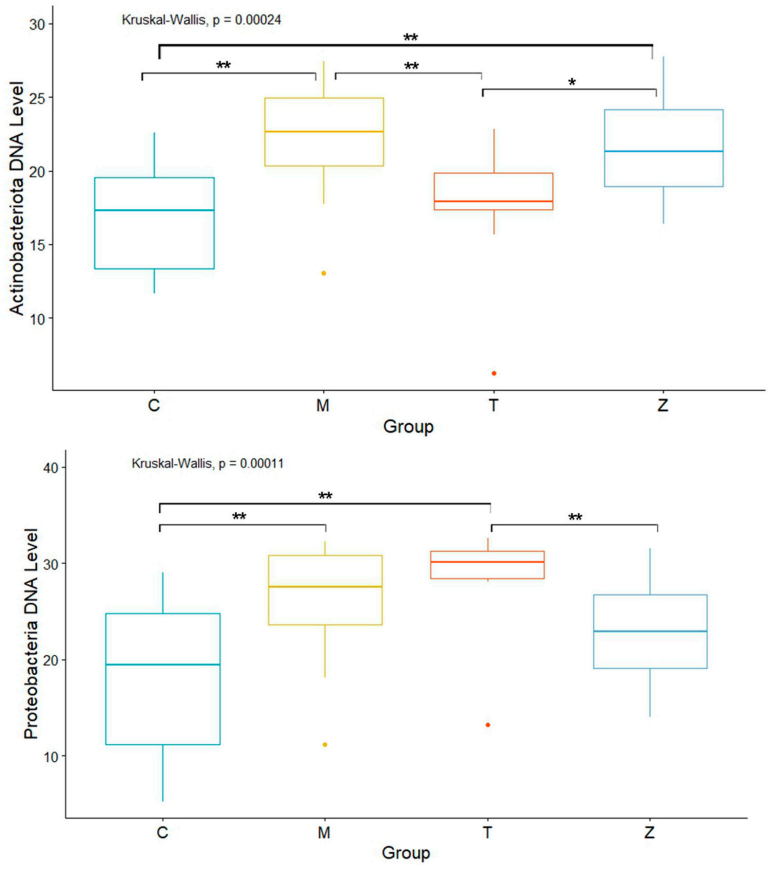

Figure 4. *Cont.*

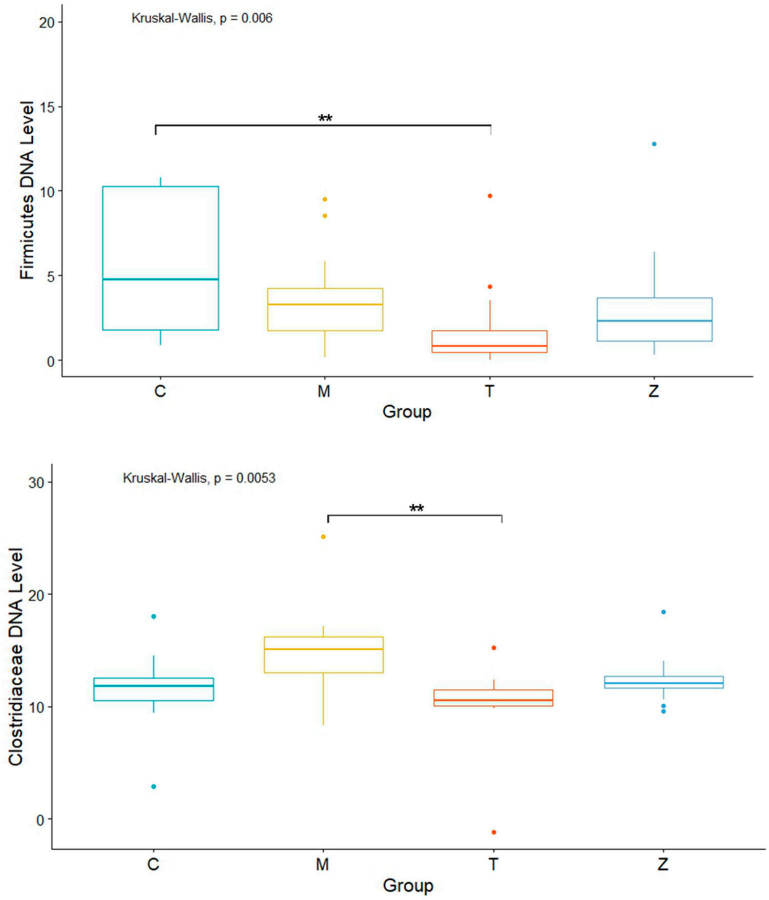

Figure 4. Differences between selected bacteria in intestines (including feces) samples: C—control group (basal diet); T—mycotoxins mixture was added to the basal diet; M—Minazel Plus® and mycotoxin mixture was combined with basal diet; Z—Minazel Plus® was mixed added to the basal diet. $p < 0.05$—*; $p < 0.01$—**.

3.3. Water

In the case of water samples, differences in the level of bacteria (Figure 5) from the Actinobacteriota phylum were shown between groups M and Z ($p = 0.0112$), where the highest level of that bacteria occurred in group Z. On the other hand, analysis of the Firmicutes phylum showed highly significant differences between groups C and M ($p = 0.0075$), where the level of this cluster was highest in group C. Analysis of the Proteobacteria phylum showed the greatest variation. Highly significant differences were shown between groups C and M ($p = 0.0018$) and C and T ($p = 0.0005$). Significant differences were shown between groups T and Z ($p = 0.0285$), and the highest level of this phylum occurred in group C and the lowest in T. Analysis of the level of the *Clostridiaceae* family showed highly significant differences between group C and M ($p = 0.00564$), where group C had the highest level of this family, while group T had the lowest.

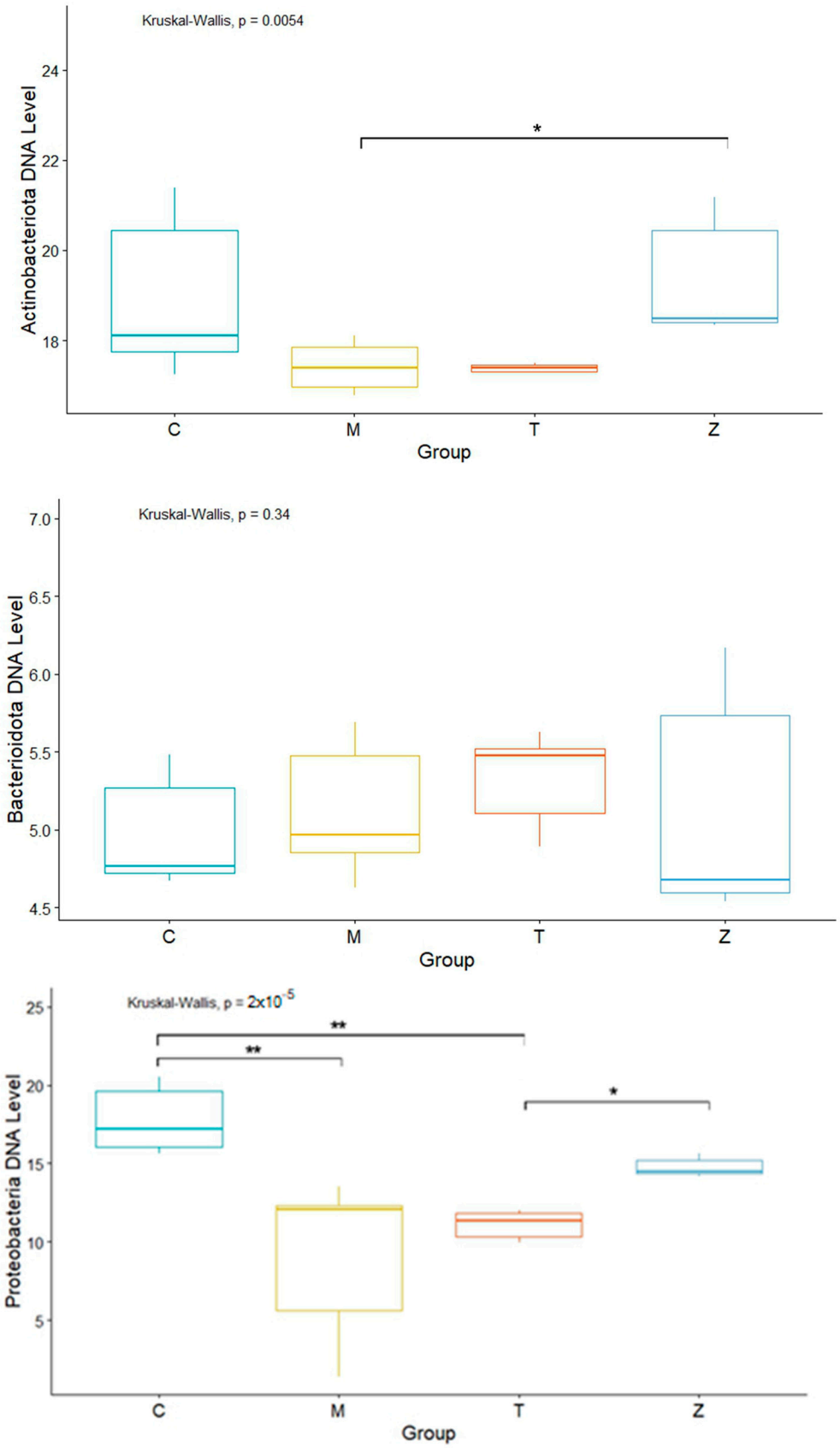

Figure 5. *Cont.*

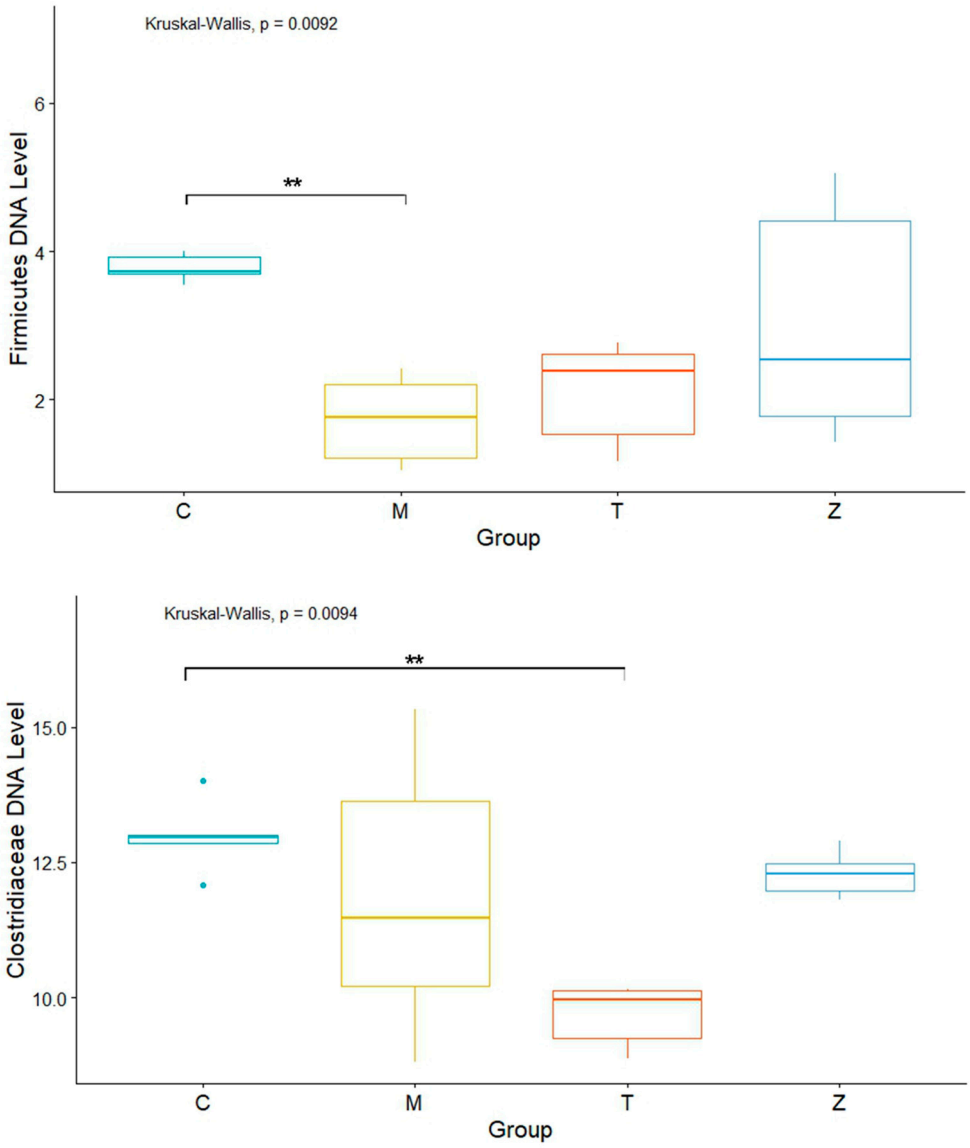

Figure 5. Differences between selected bacteria in water samples: C—control group (basal diet); T—mycotoxins mixture was added to the basal diet; M—Minazel Plus® and mycotoxin mixture was combined with basal diet; Z—Minazel Plus® was mixed added to the basal diet. $p < 0.05$—*; $p < 0.01$—**.

4. Discussion

Mycotoxins are secondary metabolites produced by filamentous fungi and prevention of their toxic effects is critical to food and feed safety. They negatively affect the health of the host by various mechanisms, including interference with proteins and peptides that have important functions in the immune system and metabolism, causing malfunction in the intestinal epithelium and leading to poor intestinal health and integrity [42]. Such changes also affect the composition of the microbial community, which can result in an increased risk of infection by pathogenic bacteria [47–49].

In this study, most of the changes in selected bacteria's DNA levels were observed in gills (Figure 2). Gills have direct contact with the water environment, which constitutes one of the most prolific habitats of microorganisms on Earth. It is the main way of their spread in nature, as well as the most microbiologically loaded animal environment [50,51]. Gill epithelium releases mucus with defensive substances, such as immunoglobulins and antimicrobial peptides (e.g., β-defensin), to reduce the colonization potential of pathogenic or

opportunistic bacteria, simultaneously supporting the abundance of neutral and beneficial bacterial microbiome composition [5,6]. Similar results to gills can also be observed in water and intestine samples. This could be due to the influence of mycotoxins on bacteria present in the water environment [52,53]. Additionally, a comparison between intestine samples, gill, and water has shown that the intestine microbial community is less vulnerable to environmental changes and toxins [50,51,54].

One of the ways to remove the mycotoxin from the feed or the environment is to use adsorption with organically modified clinoptilolite. This mineral belongs to the heulandite (HEU) group, which possesses a two-dimensional structure and manifests ion-exchange properties in water. It is used in various fields, medicine, industry, and the environment, mostly for the sequestration of toxic pollutants from industrial effluent and wastes. Additionally, it is used to absorb and remove harmful or toxic substances like mycotoxins from human and animal digestive tracts [55,56]. In animals, it may, therefore, have a stabilizing effect on the intestinal barrier due to properties that have the effect of attracting and buffering excess protons that cause acidification. It also has an inhibitory effect on the growth of pathogenic bacteria that pose a danger to both humans and farm animals, and in the case of aquatic animals, it could positively influence environmental surroundings [57,58].

In vivo studies have shown that synthetic or modified clinoptilolite can prevent pathogenic bacteria colonization (e.g., *E. coli* or *Salmonella typhimurium*), as well as selectively bind toxins (but not essential micro and macro elements, vitamins, and amino acids), further improving animal growth performance [35,57,59]. Minazel Plus® is produced by a patent-protected industrial process that embeds negatively charged organic long chains in inorganic cations from the surface of clinoptilolite (E568), allowing it to maintain a balance of positive and negative charges on the clinoptilolite surface, leading to the formation of organic-inorganic complexes on the surface of the mineral. As a result, this organic modification of clinoptilolite allows for a broader mycotoxin adsorption characteristic with improved selectivity and adsorption stability [32,60].

In our studies we have observed significant differences between group M (mycotoxin + Minazel Plus®) and T (mycotoxin). In the case of intestines, the differences were observed in Actinobacteriota phyla and the *Clostridiaceae* family, and in the case of gills, differences were observed in the level of Actinobacteriota phyla only. However, differences in water samples between M and T groups were not observed. Additionally, gill samples presented differences between the M group and C (control) samples, strongly suggesting that modified clinoptilolites have the potential to influence the composition of microbiota as also supported by other studies [32,57,59]. We further observed differences in intestine samples between groups M and C at the Actinobacteriota phylum level. Similar changes were seen in gills with Actinobacteriota and Bacteroidota phyla. There were no significant differences in phyla composition in the water environment between those groups. These findings suggest that there is a potentially selective effect of organically modified clinoptilolites on the microbial community [47,57,59]. However, further research is needed to determine the exact effect of the Minazel Plus® on the composition of microbiomes in different exposure routes and durations [61,62]. From a different perspective, and related to the use of antibiotics in aquaculture, it is important to investigate possibilities for using alternative products to support the homeostasis of the microbial community of the body without adversely affecting the aquatic environment [63–65].

5. Conclusions

In summary, the study indicates the potential for organically modified clinoptilolites to be used in the prevention of mycotoxin effects on microbiomes, as well as a tool to manipulate the fish microbiomes. The study showed that both mycotoxins and the addition of MinazelPlus® changed the composition of bacteria in intestines and gills. Observed differences in microbial communities suggest the potential of organically modified clinoptilolites to reduce mycotoxin levels and prevent mycotoxin-related microbial dysbiosis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15040149/s1, Figure S1: Results of the feed analysis, representing diets, which were utilized in the experiment. Diet 1—Control group, Diet 2—MinazelPlus® group, Diet 3—Mycotoxins group, Diet 4—MinazelPlus® + Mycotoxins.

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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 published article.

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