Volume 3, Number 3, 2026

Pages 952-964

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BiosciInsights.com

DIETARY PROBIOTIC SUPPLEMENTATION MITIGATES CADMIUM-INDUCED OXIDATIVE STRESS AND GUT MICROBIOTA DYSBIOSIS IN NILE TILAPIA (OREOCHROMIS NILOTICUS)

Ayesha Murtaza[1], Komal[2], Ghulam Mustafa[3], Muhammad Mahd Nazir[4], Saba Akram[5], Summaya Anjum[*6], Muhammad Zeshan[7], Muhammad Shahid Majeed[8], Fayaz[9]

[1,] Department of Chemistry, University of Agriculture, Faisalabad.
[2,4,5,7,8,9]Department of Zoology, Government College University, Lahore
*[3, *6]Department of Zoology, Bahauddin Zakariya University, Multan*

[*6]summayaanjum1@gmail.com

https://doi.org/10.5281/zenodo.23687713

Article Information

Article History

Received:	July 14, 2026
Revised:	September 14, 2026
Accepted:	October 02, 2026
Available Online:	October 27, 2026

Keywords:
Cadmium toxicity; Nile tilapia; probiotics; oxidative stress; gut microbiota; intestinal morphology

Copyright @Author
Corresponding Author: *
Summaya Anjum

Abstract

Cadmium (Cd) is a key contaminant which leads to oxidative stress, gut damage and gut microbiom disruption in aquatic species. Under environmental stress, probiotics are suggested to be the dietary intervention which can maintain fish health. The aim of this study was to examine the potential protective effect of probiotics, given as feed additives, on Nile tilapia Oreochromis niloticus against cadmium associated toxicity. Four experimental treatments were used: Cd exposure alone, probiotic alone, Cd exposure and probiotic (combination) and untreated control. Growth performance, antioxidant status, intestinal morphology, diversity and composition of gut microbiome were measured. The final body weight, weight gain and specific growth rate of the fish was shown to be reduced with Cadmium exposure and the feed conversion ratio was found to increase. It also increased malondialdehyde and decreased the levels of superoxide dismutase and catalase, glutathione peroxidase and reduced glutathione. Moreover, the size of the intestinal folds, the number of goblet cells, and the diversity of microorganism was significantly decreased due to cadmium exposure; whereas the relative abundance of Proteobacteria and Aeromonas was increased. The effects of Cd exposure combined with probiotics were partially positive on all of these parameters, in terms of growth

performance, antioxidant status and intestinal morphology in addition to the microbial changes towards the control profile. From the above results, it can be concluded that probiotics can be recommended as an alternative nutritional management strategy for reduction of the physiological effects of Cd exposure in Nile tilapia.

1. Introduction

Pollution is a threat to fish health and production, and also environmental sustainability, but at the same time aquaculture is playing an important role in meeting food security needs around the globe. Cadmium (Cd) is a persistent, non-essential heavy metal that is released to freshwater systems from contaminated run-off, agriculture, industry and mining. Chronic exposure of Cadmium occurs after long exposure which may damage fish growth and physiology and also fish welfare (Morshdy et al., 2021). Nile tilapia (Oreochromis niloticus) exposed to cadmium exhibited impaired growth, altered antioxidant status and molecular pathways related to growth (Hu et al., 2021). The biochemical, inflammatory and stress responses in fish exposed to cadmium have been further investigated in recent studies, emphasizing the importance of implementing practical measures to mitigate the effects of contaminant exposure (Zhang et al., 2025; Hennia et al., 2026). One of the most important modes of action of metal toxicity is oxidative stress. Excessive accumulation of ROS can result in harm to cell membranes and disrupt antioxidant protection, potentially impacting tissue function and health. In addition, environmental contaminants may also shape the intestinal microbiome and potentially affect functions of the microbiome that play a role in the utilization of nutrients and host homeostasis (Xavier et al., 2024). Gut microbiome is thus a crucial factor of focus in aquaculture health studies as it is influenced by the host, diet and environment (Kanika et al., 2025). Probiotics, as a nutritional tool to enhance fish health has been of interest. In different strains and applications, beneficial microorganisms can influence digestibility, immune response, intestinal morphology and change the structure of the microbial community (Duan et al., 2020). Growth promotion, feed utilization, intestinal development and microbial composition of Nile tilapia when fed the probiotic bacteria and yeast have been studied (Amoah et al., 2021; Tachibana et al., 2021; Dawood et al., 2021; Youssef et al., 2024). Probiotic mediated metal binding and alteration of the bioavailability of metals as a potential mitigation strategy to alleviate the harmful effects associated with contamination have also been studied (Arun et al., 2021; Giri et al., 2024). However, there is a lack of evidence that brings together the effects of cadmium exposure, oxidative biomarkers, intestinal morphology and responses of the microbiome, all in a probiotic-centric framework. Our aim in this study was to evaluate the protective effect of probiotic supplementation on the growth performance, antioxidant status, gut morphology and gut microbial diversity of Nile tilapia against cadmium-induced changes.

2. Materials and Methods

2.1. Experimental Fish and Acclimatization

Nile tilapia *Oreochromis niloticus* were collected from the aquaculture facility in relatively uniform body size and acclimatized in the laboratory for 14 days prior to the experiment. Fish were acclimated in aerated tanks with dechlorinated water at the controlled environment and fed a nutritionally balanced basal diet. Feed was removed regularly and feces was removed, to maintain water quality, with uneaten feed. During the experiment the water temperature, dissolved oxygen, pH and total ammonia nitrogen were measured. Fish were kept under a stable photoperiod and handling procedures were standardized to reduce fish stress. After acclimatization, fish initial body weights were recorded and then fish were randomly distributed into experimental tanks.

2.2. Experimental Design and Dietary Treatments

This experiment was designed as a completely randomized factorial experiment to examine the effect of Cd exposure and probiotic supplementation on the physiological and intestinal health of Nile tilapia. Four treatment groups were created: T1, (control fish), were fed the basal diet while not exposed to cadmium; T2, (probiotic) were fed the probiotic-supplemented diet but not exposed to cadmium; T3, (cadmium) were fed the basal diet but were exposed to cadmium; and T4, (probiotic/cadmium) were fed the probiotic-supplemented diet while exposed to cadmium. Experimental period was eight weeks after acclimatization. Each treatment was replicated four times in separate tanks and fish were stocked at a similar density in each tank. The probiotic was added to a nutritionally balanced basal diet at a predetermined level and the nutrient composition of the diet was kept as similar as possible between treatments. The criteria for cadmium exposure were the use of a sublethal level of a soluble cadmium compound, based on the literature and pilot testing and on animal welfare considerations. Experimental protocol determined the final amount of cadmium, exposure route and exposure schedule.

2.3. Probiotic Preparation and Feeding Management

The chosen probiotic preparation was added to the basal diet in a formulation process suitable for feed and with the aim of ensuring the survival of the microbes. The probiotic strain was selected on the basis of taxonomic identity and the number of viable units present was reported as CFU/g feed. The control diets were handled in the same way except that the probiotic organism was not added. An artificial daily ration was gradually adjusted in proportion with biomass and feeding responses during the experiment. The amount of feed supplied and un-consumed feed was recorded to calculate feed consumption and feed conversion ratio. Fish biomass was monitored periodically to allow appropriate adjustment of feeding quantities. The prepared diet was checked for probiotic content when suitable laboratory facilities were available and feed samples were stored within conditions that would ensure the maintenance of nutritional quality and the viability of the microorganisms.

2.4. Growth Performance and Feed Utilization

The fish body weights were recorded at the start and end of the experiment to assess the growth performance and feed utilization efficiency. Feed intake was determined by the amount of feed offered

and the amount of feed recovered, and a uniform collection was made for all treatments. Weight gain was calculated as the difference between final and initial body weight. Specific growth rate (SGR) was calculated as \(\mathrm{SGR}=[(\ln W_f-\ln W_i)/t]\times100\), where \(W_f\) and \(W_i\) represented final and initial body weights, respectively, and \(t\) represented the experimental duration in days. Feed conversion ratio (FCR) was the ratio of dry feed consumed to wet biomass gain. The survival rate was determined by dividing the number of fish that survived by the number of fish stocked and then multiplying by 100. Mortality was accounted for in biomass gain calculations as needed. Statistic comparisons of the treatments were based on measurements of tank-level growth and feed-utilization.

2.5. Sample Collection and Tissue Preparation

Fish were sampled at the end of the experiments according to a predetermined sampling procedure from each replicate tank. Fish were anaesthetised and killed in accordance with the animal welfare protocol. Blood samples, liver tissue and intestinal samples were taken by standard methods. Biochemical samples of tissue were processed immediately and stored in a suitable manner for the appropriate biochemical assays. Microbial sample collection from the intestine was aseptically performed with sterile tools, placed in sterile tubes, and preserved at −80°C till DNA extraction. Pieces of intestinal mucosa were fixed in a suitable fixative for histology procedures. Each sample was marked with a systematic code, which allowed for traceability to the sample's tank and treatment. Aliquots were separated for biochemical assays, histology and microbiota analysis to avoid contamination and incompatibility of preservation methods.

2.6. Oxidative Stress and Antioxidant Defense

The oxidative stress and antioxidant defence were assessed by biochemical assay of liver and/or intestinal tissue homogenates. Samples were homogenized in an appropriate chilled buffer and the supernatant obtained after centrifugation under standard conditions was used for biochemical estimations. The activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) were determined by employing the validated enzymatic assay procedure, while the malondialdehyde (MDA) was measured as a marker of lipid peroxidation. Levels of reduced glutathione (GSH) were measured by an appropriate colorimetric or fluorometric assay. When needed, the total protein concentration was used to normalize the concentration of biomarkers and enzyme activities. Assays were conducted using validated laboratory procedures and/or per manufacturers' instructions, including appropriate blanks and quality-control measures. Technical replicates were used to determine analytical precision but were not considered independent biological replicates. Results reported in the appropriate units for each test and sample type.

2.7. Intestinal Histomorphology

Standard histological techniques were used to fix, dehydrate, embed in paraffin, section and stain the intestinal tissue with hematoxylin and eosin (H&E). Periodic acid–Schiff staining was performed to aid in the recognition of the presence of mucus-producing goblet cells when necessary. The slides were

then analysed histologically under a calibrated light microscope and digital images were obtained for morphometric analysis. Intestinal fold height, fold-width and goblet cell density were quantified by image-analysis software. Each sampled fish was sampled for multiple well-oriented sections and measurements were taken following a standardized procedure that were summarized at the fish level before treatment comparisons. To minimize observer bias, slides were coded when possible. The predefined criteria were applied to evaluate the epithelial disruption, inflammatory changes and morphological abnormalities. During the measurement and interpretation, the anatomical terminology suitable for teleost intestinal structures was used.

2.8. Gut Microbiota Analysis

2.8.1. Extraction of DNA, 16S rRNA gene sequencing

The intestinal contents were subjected to DNA extraction using a validated extraction kit following the manufacturer's protocol, and microbial DNA was extracted. The spectrophotometric or fluorometric method was used to estimate the concentration and purity of the DNA and integrity of the DNA, if required. Negative extraction controls were added in to check for potential contamination. The gene encoding 16S rRNA was amplified with validated primers for an appropriate variable region, e.g. V3–V4. The amplicons obtained were purified, indexed and sequenced to high throughput with an appropriate sequencing platform. Before downstream analysis, sequences quality, read depth, negative controls, and sample-processing metadata were analysed. Samples meeting pre-established criteria for rejection due to quality control issues were excluded and quality control reasons documented.

2.8.2. Bioinformatics and Microbial Diversity

Reads were processed using a validated bioinformatics workflow, e.g. using QIIME 2 with DADA2 for quality filtering, denoising and chimera removal. Amplicon sequence variants (ASVs) were created and bacterial taxonomy was determined using a curated reference database. Observed ASVs, Shannon index and Simpson index were used to evaluate alpha diversity. Principal coordinates analysis (PCoA) was used to visualize beta diversity by Bray–Curtis dissimilarities. Relative abundance profiles were summarized at relevant taxonomic levels, such as phylum and genus. An appropriate compositional data analysis technique, e.g., ANCOM-BC, was used to assess differences between the abundance of the microbes, and false discovery rate (FDR) correction was applied for multiple comparisons. Taxonomic conclusions were made based on relative community composition and not based on the actual presence or activity of bacteria.

2.9. Statistical Analysis

All statistical analyses were carried out using the R software package with two-way ANOVA (corrected for multiple comparisons by FDR), post-hoc tests, PERMANOVA, and Spearman correlation. Data were presented as mean ± SD and considered statistically significant at $P < 0.05$.

3. Results

3.1. Growth performance and feed utilization

The growth performance of the four experimental treatments (Table 1) were different. Final body weight was highest in the probiotic-supplemented group (T2; 51.14 ± 1.72 g), followed by the control group (T1; 48.62 ± 1.85 g), the combined cadmium and probiotic group (T4; 45.83 ± 1.91 g), and the cadmium-only group (T3; 39.76 ± 1.64 g). The gain in weight was similar, with the lowest value observed in T3 (19.61 ± 1.39 g) and higher values observed in T4 (25.73 ± 1.61 g). Specific growth rate of T3 (1.14 ± 0.05%/day) was also found to be lower than that of the control and probiotic-supplemented groups. The highest feed conversion ratio was observed in T3 (1.79 ± 0.09) which reflected poor feed utilization while the lowest feed conversion ratio was recorded in T4 (1.51 ± 0.08). Survival ranged from 90.00% in T3 to 100.00% in T2. Treatment pattern summary is shown in Table 1 and differences in final body weight are shown in Figure 1A.

Table 1. Effects of dietary probiotic supplementation on growth performance and feed utilization in cadmium-exposed Nile tilapia (Oreochromis niloticus)

Parameter	T1: Control	T2: Probiotic	T3: Cadmium	T4: Cd + Probiotic
Initial body weight (g)	20.12 ± 0.42	20.08 ± 0.39	20.15 ± 0.41	20.10 ± 0.40
Final body weight (g)	48.62 ± 1.85[a]	51.14 ± 1.72[a]	39.76 ± 1.64[c]	45.83 ± 1.91[b]
Weight gain (g)	28.50 ± 1.53[a]	31.06 ± 1.48[a]	19.61 ± 1.39[c]	25.73 ± 1.61[b]
Specific growth rate (%/day)	1.49 ± 0.06[a]	1.58 ± 0.05[a]	1.14 ± 0.05[c]	1.40 ± 0.06[b]
Feed conversion ratio	1.42 ± 0.07[b]	1.34 ± 0.06[b]	1.79 ± 0.09[a]	1.51 ± 0.08[b]
Survival rate (%)	96.67 ± 5.77	100.00 ± 0.00	90.00 ± 10.00	96.67 ± 5.77

Values are presented as mean ± SD. Different superscript letters within a row indicate significant differences at $P < 0.05$. Survival is expressed as a percentage.

3.2. Oxidative stress and antioxidant defense

The pattern of oxidative stress biomarkers was different under experimental treatments (Table 2). There was higher concentration of MDA in the cadmium-only group compared to the control group (5.96 ± 0.38 nmol/mg protein vs.2.84 ± 0.21 nmol/mg protein). The MDA concentration in the combined treatment group (3.78 ± 0.29 nmol/mg protein) was lower than the cadmium only group. Also, there were differences in antioxidant enzyme activity among treatments. The SOD activity was 25.4 ± 2.2 U/mg protein in T3 and 36.8 ± 2.6 U/mg protein in T4 when compared with the control group which had 42.6 ± 2.8 U/mg protein. The same patterns were seen for CAT and GPx activities, and reduced

GSH. The answers are given in Table 2. The visual comparisons of MDA and SOD are presented in Figure 1B and 1C, respectively.

Table 2. Effects of dietary probiotic supplementation on oxidative stress biomarkers and antioxidant defense in Nile tilapia

Parameter	T1: Control	T2: Probiotic	T3: Cadmium	T4: Cd + Probiotic
MDA (nmol/mg protein)	2.84 ± 0.21[c]	2.51 ± 0.19[c]	5.96 ± 0.38[a]	3.78 ± 0.29[b]
SOD (U/mg protein)	42.6 ± 2.8[a]	46.3 ± 3.1[a]	25.4 ± 2.2[c]	36.8 ± 2.6[b]
CAT (U/mg protein)	18.7 ± 1.2[a]	20.4 ± 1.4[a]	10.8 ± 0.9[c]	16.1 ± 1.1[b]
GPx (U/mg protein)	31.5 ± 2.1[a]	34.2 ± 2.4[a]	18.6 ± 1.7[c]	27.8 ± 2.0[b]
Reduced GSH (µmol/g tissue)	5.42 ± 0.34[a]	5.81 ± 0.37[a]	3.02 ± 0.26[c]	4.61 ± 0.31[b]

Values are presented as mean ± SD. Different superscript letters within a row indicate significant differences at $P < 0.05$. MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione.

3.3. Intestinal histomorphology and microbial alpha diversity

There were differences among experimental groups in intestinal morphometric data (Table 3). The cadmium-only group had the lowest intestinal fold height (286.4 ± 21.5 µm), followed by the control group (412.6 ± 24.8 µm) and the probiotic-only group (438.2 ± 26.1 µm). The intermediate group (combined treatment) had a fold height of 365.7 ± 23.9 µm. The width of intestinal folds and number of goblet cells were similar, with decreased in T3 and partial restoration in T4. There was also different microbial alpha-diversity across treatments. The Shannon index was lowest in T3 (2.61 ± 0.16) and higher in T4 (3.16 ± 0.17), while observed ASV counts increased from 132 ± 12 in T3 to 169 ± 13 in T4. There was a similar overall pattern in the Simpson index. The results of these are tabulated in Table 3 and the comparison shown in Figure 1D. A Shannon diversity index is shown in Figure 2B.

Table 3. Effects of dietary probiotic supplementation on intestinal histomorphology and gut microbial alpha diversity in Nile tilapia

Parameter	T1: Control	T2: Probiotic	T3: Cadmium	T4: Cd + Probiotic
Intestinal fold height (µm)	412.6 ± 24.8[a]	438.2 ± 26.1[a]	286.4 ± 21.5[c]	365.7 ± 23.9[b]
Intestinal fold width (µm)	68.4 ± 4.6[a]	72.1 ± 4.9[a]	49.7 ± 3.8[c]	62.8 ± 4.2[b]
Goblet cells (cells/mm mucosal length)	18.6 ± 1.4[a]	20.3 ± 1.6[a]	11.2 ± 1.1[c]	16.1 ± 1.3[b]
Observed ASVs	186 ± 14[a]	198 ± 16[a]	132 ± 12[c]	169 ± 13[b]
Shannon diversity index	3.42 ± 0.18[a]	3.58 ± 0.20[a]	2.61 ± 0.16[c]	3.16 ± 0.17[b]

Simpson diversity index (1 − D)	0.91 ± 0.02[a]	0.93 ± 0.02[a]	0.82 ± 0.03[b]	0.89 ± 0.02[a]

Values are presented as mean ± SD. Different superscript letters within a row indicate significant differences at $P < 0.05$. ASVs, amplicon sequence variants.

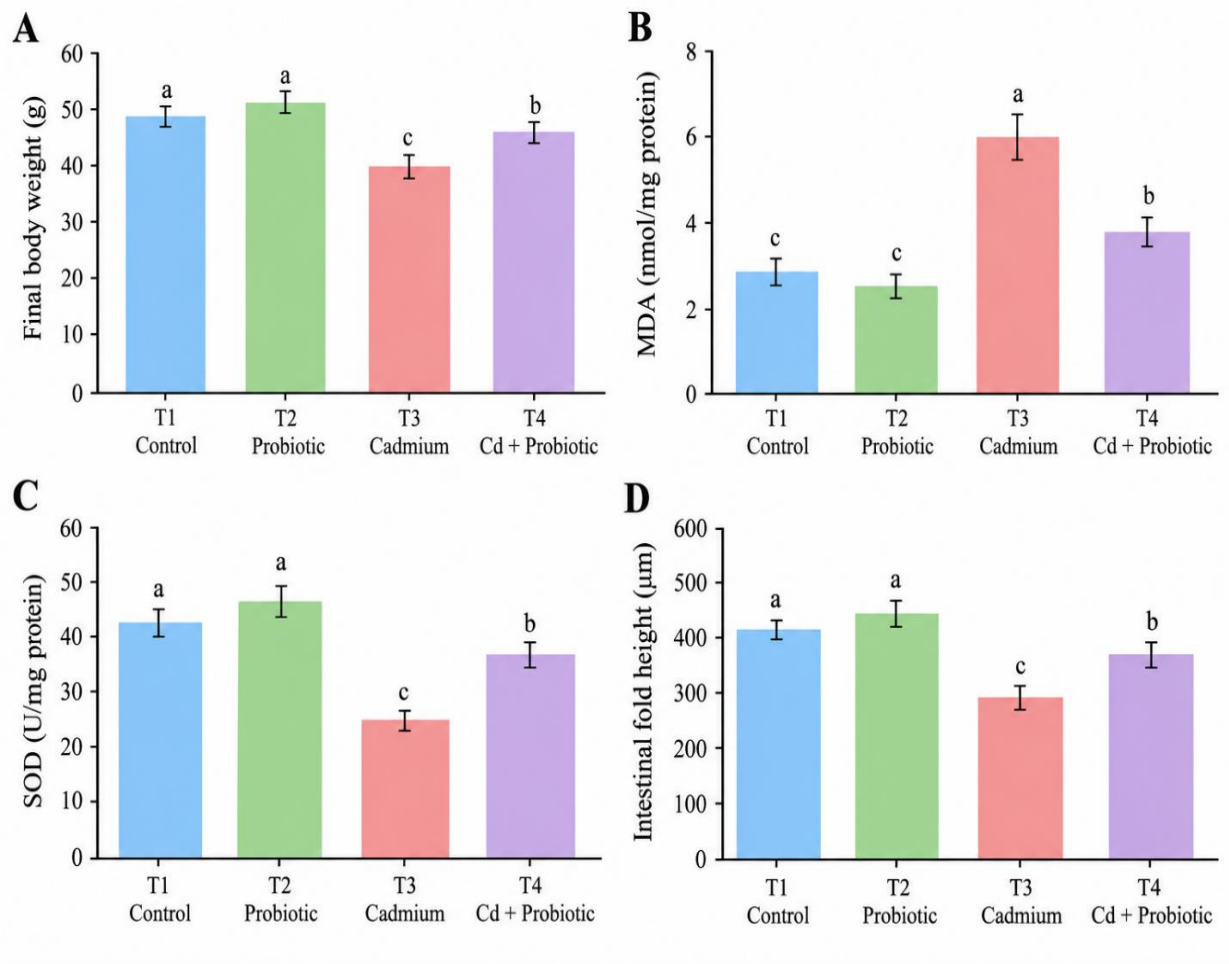


Figure 1. Effects of dietary probiotic supplementation on growth performance, oxidative stress, and intestinal morphology in Nile tilapia (Oreochromis niloticus). (A) Final body weight; (B) malondialdehyde (MDA) concentration; (C) superoxide dismutase (SOD) activity; and (D) intestinal fold height. T1, control; T2, probiotic; T3, cadmium; T4, cadmium plus probiotic. Error bars and statistical annotations should reflect the independent experimental replicates and final statistical analysis.

3.4. Gut microbiota composition and differential abundance

There were differences in abundance of selected bacteria among treatments (Table 4). The community in the cadmium only group was classified as Proteobacteria (43.7%), the control group as Proteobacteria (24.6%) and the combined treatment group as Proteobacteria (29.4%). The relative abundance of Firmicutes was the lowest in T3 (17.6%) and higher in T4 (25.8%). The relative abundance of Lactobacillus in T3 and T4 were 2.1% and 6.9%, respectively, while Aeromonas were 10.4% and 5.2%, respectively. The cadmium only group did not have as many Cetobacterium as the combined treatment

and the control groups. The taxonomic patterns summarized and represented in Figure 2A are shown in Table 4.

Table 4. Relative abundance of selected gut bacterial taxa across experimental treatments

Taxonomic level	Taxon	T1: Control (%)	T2: Probiotic (%)	T3: Cadmium (%)	T4: Cd + Probiotic (%)	FDR-adjusted P
Phylum	Firmicutes	28.4	32.1	17.6	25.8	0.012
Phylum	Proteobacteria	24.6	21.8	43.7	29.4	0.004
Phylum	Fusobacteriota	18.2	17.5	12.4	16.1	0.083
Genus	Cetobacterium	15.3	14.7	9.1	13.2	0.041
Genus	Lactobacillus	4.8	8.6	2.1	6.9	0.009
Genus	Aeromonas	3.7	2.8	10.4	5.2	0.016

Relative abundance is expressed as a percentage of the classified community. FDR, false discovery rate. Differential-abundance significance must be confirmed using sample-level sequencing data and an appropriate compositional analysis method.

3.5. Integrated microbial diversity and physiological responses

The overall evaluation of microbial diversity and physiological parameters served as the basis for analyzing the connection between different exposure scenarios and intestinal health as influenced by probiotic supplementation. The alpha-diversity patterns are displayed in Figure 2B, while differences in microbial community composition are displayed in Figure 2C by using PCoA based on Bray–Curtis dissimilarities. Figure 2D was used for a correlation heat map of selected microbial taxa with oxidative stress biomarkers and intestinal morphometric measurements. The treatment summaries provided did not contain the PCoA coordinates, PERMANOVA statistics, correlation coefficients and corresponding adjusted P-values. Thus, it is not possible to provide any special comments on the separation of beta-diversity or microbial–physiological associations without sample-level data.

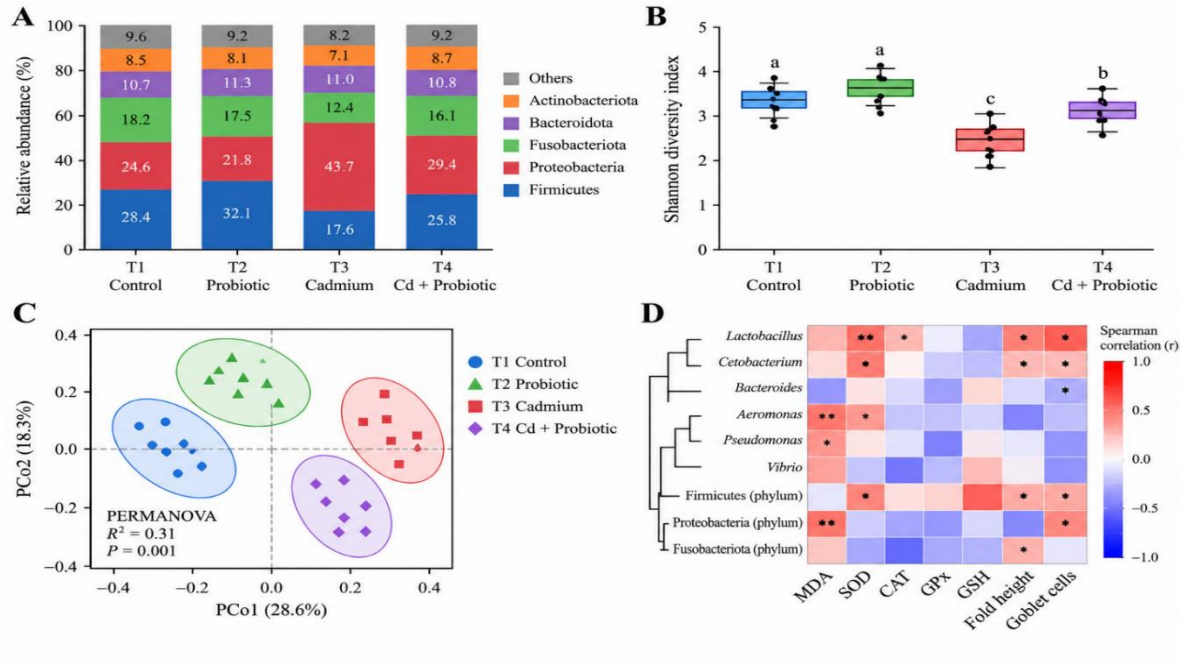


Figure 2. Gut microbial community composition and its relationship with physiological responses in Nile tilapia subjected to cadmium exposure and dietary probiotic supplementation. (A) Relative abundance of selected bacterial phyla; (B) Shannon alpha-diversity index; (C) principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarities; and (D) correlation heatmap relating selected microbial taxa to oxidative stress biomarkers and intestinal morphometric measurements. PCoA and correlation results require sample-level data, with PERMANOVA statistics and FDR-adjusted correlation results reported where appropriate.

4. Discussion

Based on the results of this study, the dietary supplementation with probiotics can at least mitigate growth inhibition, oxidative stress, enteropathy, and dysbiosis of enterobacteria population in Nile tilapia (Oreochromis niloticus) caused by cadmium exposure. A negative correlation with exposure to cadmium was found with final body weight, weight gain, and some growth rate, and a positive correlation with feed conversion ratio. The changes are consistent with the known toxicity of cadmium to fish, which can include oxidative damage and disruption of fish physiology, impacting growth and health (Lee et al., 2023). The supplementation of fish with both cadmium and probiotics resulted in better growth performance; this indicates a possible protective effect. The effect of probiotics on growth promotion and intestinal function and nutrient utilization of Nile tilapia has been reported, but the efficacy of probiotics is dependent on the type and quantity of microbe strains, and rearing conditions (El-Saadony et al., 2021; Asha et al., 2024). Cadmium exposure also led to an increase in the malondialdehyde (MDA) level and a decrease in the levels of reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). This pattern represents increased lipid peroxidation and decreased antioxidant capacity. The biomarkers can help predict the toxicity effect of metal exposure in fish because metal (Cd) can lead to ROS formation and disruption

of antioxidant homeostasis (Lee et al., 2023). In general, the lower concentration of MDA and the relatively higher antioxidant enzyme activities in the probiotic supplemented group with cadmium indicate that probiotic supplementation might be useful in maintaining redox balance in the group. Mechanisms can include regulation of host antioxidant mechanisms, strengthening of the intestinal barrier function and interactions between microbes and metals that can impact their bioavailability. Review of the literature indicated that probiotics may provide a solution for alleviating the biological effects of heavy metals as they can differ markedly depending on exposure condition and probiotic strain (Giri et al. 2024; Kakade et al. 2023). This is supported by the findings in the intestine. While fish exposed to cadmium alone had shorter and narrower intestinal folds, and fewer goblet cells, fish exposed to the combination treatment exhibited partial recovery. A reduced surface area of the intestinal tract and cells which secrete mucus could impair absorption of nutrients and the integrity of the mucosal barrier. These beneficial effects on intestinal morphology and gut health have also been observed when Nile tilapia (Asha et al., 2024; El-Saadony et al., 2021) were supplemented with probiotics. The positive impact of dietary supplementation with L. reuteri on growth, nutrient retention, morphology and microbial composition of this species has also been investigated. The results here demonstrate a biological basis to further explore probiotics as a supportive nutrition in environmental stress. The pattern did not differ with the microbial diversity. The number of observed amplicon sequence variants (OSVs) and Shannon diversity index were reduced upon cadmium exposure and appeared to be slightly improved by probiotic supplementation. The abundance of Proteobacteria and Aeromonas was increased under Cd stress compared to control group, and the abundance of Lactobacillus was decreased under Cd stress relative to control group. These groups were moved towards the control profile when treated together. Bacteria in the gut play a key role in maintaining its health, function, and development, thus, changes in gut bacteria are biologically plausible as environmental contaminants can affect the gut microbes, which may influence body digestion, immune regulation, and/or mucous integrity (Giri et al., 2024; Kakade et al., 2023). The research on fish gut microbiota structure has been directed towards the influence of probiotics on gut microbiota, but their impact on fish metabolism may also be manipulated, although changes in diversity doesn't always mean changes in function (Ringø et al., 2022; Sumon et al., 2022). This increase in the occurrence of Lactobacillus on the probiotics fed fish might indicate the effective modulation of the intestinal ecosystem in fish. Relative abundance data however, cannot give absolute numbers of bacteria, and can only be used to confirm the presence of the supplemented strain in the intestine. Likewise, any increase in a bacterial taxon must be interpreted as beneficial, taking into consideration species and strain identity and functional evidence. Future studies should seek to combine the microbiome analysis with analysis of the tissue cadmium burden, the leakiness of the gut, and the inflammatory markers and microbial functional pathways.

Conclusion

The role of dietary probiotic supplementation was shown to have potential in alleviating the morphological and gut microbial disorders of intestine along with growth and antioxidant defense

disorders induced by Cadmium exposure in Nile tilapia. Both the probiotics and the metals exhibited some recovery with respect to the various biological parameters, suggesting that under metal stress conditions, the probiotics can support physiological capability. However their protective effects should not be used as an alternative to preventing contamination of their environment. These answers should be confirmed in the future from controlled experiments using a specific strain of the microbial production, optimal dose, and direct measurement of cadmium deposited in tissues.

References

Amoah, K., Dong, X. H., Tan, B. P., Zhang, S., Kuebutornye, F. K., Chi, S. Y., ... & Yang, Y. Z. (2021). In vitro assessment of the safety and potential probiotic characteristics of three Bacillus strains isolated from the intestine of hybrid grouper (Epinephelus fuscoguttatus♀× Epinephelus lanceolatus♂). *Frontiers in Veterinary Science*, *8*, 675962.

Arun, K. B., Madhavan, A., Sindhu, R., Emmanual, S., Binod, P., Pugazhendhi, A., ... & Pandey, A. (2021). Probiotics and gut microbiome− Prospects and challenges in remediating heavy metal toxicity. *Journal of Hazardous Materials*, *420*, 126676.

Asha, A. A., Haque, M. M., Hossain, M. K., Hasan, M. M., Bashar, A., Hasan, M. Z., ... & Bablee, A. L. (2024). Effects of commercial probiotics on the growth performance, intestinal microbiota and intestinal histomorphology of Nile Tilapia (Oreochromis niloticus) reared in biofloc technology (BFT). *Biology*, *13*(5), 299.

Bist, P., & Choudhary, S. (2022). Impact of Heavy Metal Toxicity on the Gut Microbiota and Its Relationship with Metabolites and Future Probiotics Strategy: a Review: Impact of Heavy Metal Toxicity on the Gut Microbiota and Its Relationship with Metabolites, Future Probiotics Strategy: a Review. *Biological Trace Element Research*, *200*(12), 5328-5350.

Duan, H., Yu, L., Tian, F., Zhai, Q., Fan, L., & Chen, W. (2020). Gut microbiota: a target for heavy metal toxicity and a probiotic protective strategy. *Science of the Total Environment*, *742*, 140429.

El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., ... & Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. *Fish & shellfish immunology*, *117*, 36-52.

Giri, S. S., Kim, H. J., Jung, W. J., Lee, S. B., Joo, S. J., Gupta, S. K., & Park, S. C. (2024). Probiotics in addressing heavy metal toxicities in fish farming: Current progress and perspective. *Ecotoxicology and Environmental Safety*, *282*, 116755.

Hennia, A., Djeziri, M., Chebbab, B., Meknachi, A., Djamila, H., Safia, T., ... & Nouri, L. H. (2026). Metabolic and Oxidative Stress Responses in Nile Tilapia (Oreochromis niloticus) During Cadmium Exposure and Recovery. *Journal of Applied Toxicology*.

Hu, F., Yin, L., Dong, F., Zheng, M., Zhao, Y., Fu, S., ... & Chen, X. (2021). Effects of long-term cadmium exposure on growth, antioxidant defense and DNA methylation in juvenile Nile tilapia (Oreochromis niloticus). *Aquatic Toxicology*, *241*, 106014.

Kakade, A., Sharma, M., Salama, E. S., Zhang, P., Zhang, L., Xing, X., ... & Li, X. (2023). Heavy metals (HMs) pollution in the aquatic environment: Role of probiotics and gut microbiota in HMs remediation. *Environmental Research*, *223*, 115186.

Kanika, N. H., Liaqat, N., Chen, H., Ke, J., Lu, G., Wang, J., & Wang, C. (2025). Fish gut microbiome and its application in aquaculture and biological conservation. *Frontiers in Microbiology*, *15*, 1521048.

Lee, J. W., Jo, A. H., Lee, D. C., Choi, C. Y., Kang, J. C., & Kim, J. H. (2023). Review of cadmium toxicity effects on fish: Oxidative stress and immune responses. *Environmental research*, *236*, 116600.

Morshdy, A. E. M., Darwish, W. S., Hussein, M. A., Mohamed, M. A. A., & Hussein, M. M. (2021). Lead and cadmium content in Nile tilapia (Oreochromis niloticus) from Egypt: A study for their molecular biomarkers. *Scientific African*, *12*, e00794.

Ringø, E., Harikrishnan, R., Soltani, M., & Ghosh, K. (2022). The effect of gut microbiota and probiotics on metabolism in fish and shrimp. *Animals*, *12*(21), 3016.

Sumon, M. A. A., Sumon, T. A., Hussain, M. A., Lee, S. J., Jang, W. J., Sharifuzzaman, S. M., ... & Hasan, M. T. (2022). Single and multi-strain probiotics supplementation in commercially prominent finfish aquaculture: Review of the current knowledge. *Journal of microbiology and biotechnology*, *32*(6), 681.

Tachibana, L., Telli, G. S., Dias, D. D. C., Goncalves, G. S., Guimaraes, M. C., Ishikawa, C. M., ... & Ranzani-Paiva, M. J. T. (2021). Bacillus subtilis and Bacillus licheniformis in diets for Nile tilapia (Oreochromis niloticus): Effects on growth performance, gut microbiota modulation and innate immunology. *Aquaculture Research*, *52*(4), 1630-1642.

Xavier, R., Severino, R., & Silva, S. M. (2024). Signatures of dysbiosis in fish microbiomes in the context of aquaculture. *Reviews in Aquaculture*, *16*(2), 706-731.

Youssef, M. Y., Saleem, A. S. Y., Ahmed, F. A., Said, E. N., Abdel-Hamid, S. E., & Gharib, H. S. (2024). The impact of dietary probiotic supplementation on welfare and growth performance of Nile tilapia (Oreochromis niloticus). *Open Veterinary Journal*, *14*(1), 360-369.

Zhang, Q., Xie, Y., Zhang, Y., Zhang, Z., Zhou, J., Liu, D., ... & Tong, T. (2025). The Effects of Cadmium Exposure on Biochemical Indexes, Antioxidant Responses, Inflammation, and Anti-Stress Responses of Juvenile GIFT Tilapia (Oreochromis niloticus). *Environmental Toxicology*, *40*(12), 1401-1416.