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Original Research Articles

Effect of Dietary Bentonite Clay on Growth Performance and Mycotoxin Mitigation in Rainbow Trout

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This study evaluated the effectiveness of Georgian bentonite clay as a natural mycotoxin adsorbent in Rainbow trout (*Oncorhynchus mykiss*) diets. Experimental diets were deliberately contaminated with Aflatoxin B1 at 144.4 ± 1.5 ppb and Trichothecene T2/HT2 at 531 ± 2 ppb. A total of 100 fish per group were fed diets without adsorbent (Control 1; C1), with 0.1% commercial synthetic binder - Maxbinder (C2; based on hydrated aluminosilicates and yeast cell wall extract) (Control 2; C2), and with bentonite at 0.1% (Test 1; T1), 0.15% (Test 2; T2), and 0.2% (Test 3; T3) over a 24-week period. Weight gain was highest in T3 (254 ± 15.8 g) and T2 (244.92 ± 14.47 g), significantly exceeding C1 (143.39 ± 9.51 g) and C2 (187.67 ± 12.1 g) ($P < 0.05$). Survival rates reached 97% in T2 and T3, though group differences were not statistically significant ($P > 0.05$). Feed conversion ratios (FCR) were lowest in T2 and T3 (0.9–0.92), indicating improved feed efficiency compared to C1 (1.3) and C2 (1.1). High-Performance Liquid Chromatography (HPLC) and fecal analysis demonstrated that bentonite effectively adsorbed mycotoxins. T2 and T3 groups exhibited the highest fecal excretion of Aflatoxin B1 (120.5–130 ppb) and T2/HT2 (66.3–74.3 ppb), corresponding to adsorption rates of 83–90% for Aflatoxin B1 and 12.5–14% for T2/HT2. In comparison, the synthetic adsorbent (C2) achieved only 68.1% and 7.1% adsorption, respectively ($P < 0.05$). Fish in the T2 and T3 groups also showed significantly improved nutritional quality, with higher protein (18.3–18.5%) and fat content (7.8%) than C1 (16.5% protein, 6% fat) and C2 (17.2% protein, 6.8% fat) ($P < 0.05$). These findings indicate that Georgian bentonite clay not only enhances growth and feed utilization but also significantly reduces systemic mycotoxin exposure, offering a promising, natural, and sustainable solution for improving aquaculture productivity and fish health.

INTRODUCTION

Mycotoxin contamination in aquafeed ingredients is a major concern for fish health and aquaculture profitability, especially in Georgia where feed costs are high and dependence on imported additives is unsustainable.[1] Low-cost feed formulations made from cereal and agro-industrial byproducts are prone to contamination by fungal metabolites like aflatoxins and trichothecenes,[2,3] which are linked to immunosuppression, reduced growth, and increased mortality in fish.[4]

To reduce these risks, natural mycotoxin adsorbents—particularly aluminosilicate clays—have been examined for their potential to lower toxin bioavailability.[5] Among these, bentonite, a montmorillonite-rich smectite clay, has demonstrated strong adsorption and ion-exchange capacities. It is widely used in livestock and poultry feeds to mitigate toxins and improve feed efficiency. However, only a few studies have evaluated its use in aquaculture, and none have specifically tested Georgian bentonite.[6]

Georgia's Ozurgeti region produces bentonite with high montmorillonite content and favorable physicochemical properties. In poultry feeds, Georgian bentonite ("Askangel") enhanced daily weight gain by 7–9% and improved feed conversion.[6] Yet, whether these benefits translate to aquatic species remains unverified.[7] Preliminary reports in trout demonstrated increased weight gain and improved survival using similar inclusion rates (0.1–0.2%), but details on nutrient interactions and water quality effects were limited.[7]

Despite its promise, bentonite may also bind essential nutrients, and its efficacy can vary depending on mineral source and processing—factors that raise concerns about consistency and regulatory compliance. Other natural adsorbents—such as zeolites, activated carbon, and yeast cell walls—have been tested, each with advantages and limitations in cost, effectiveness, or sourcing.

To address this research gap, we evaluated Georgian bentonite as a locally sourced, cost-effective feed additive in rainbow trout diets formulated with at-risk ingredients. The study examines its mycotoxin adsorption efficiency, impact

on growth and feed conversion, and suitability for broader deployment in sustainable Georgian aquaculture.

MATERIALS AND METHODS

COLLECTION AND ANALYSIS OF BENTONITE CLAY

Bentonite clay from Guria region (Georgia) was collected and analyzed at the Alexander Tvalchrelidze Institute of Mineral Resources of the Caucasus using chemical research methods.

X-ray phase, silicate, and physical-chemical studies were conducted at the Agricultural University of Georgia, Tbilisi State University, and the Institute of Mineral Materials of the Caucasus. The montmorillonite structure and formula were established using an X-ray phase test performed with the Dual-Polarization Optical Hybrids (DPOH-1.5) Kylia (part of the iXblue brand) device COH28-X model.[6]

MYCOTOXIN SAFETY AND TOXICITY ANALYSIS

BENTONITE CHARACTERIZATION AND MYCOTOXIN SAFETY ANALYSIS

Bentonite clay from the Guria region of Georgia was collected and analyzed at the Alexander Tvalchrelidze Institute of Mineral Resources of the Caucasus using chemical research methods. X-ray phase, silicate, and physico-chemical studies were conducted at the Agricultural University of Georgia, Tbilisi State University, and the Institute of Mineral Materials of the Caucasus. The montmorillonite structure and chemical formula were established through X-ray phase analysis performed using a Dual-Polarization Optical Hybrids (DPOH-1.5) Kylia COH28-X model device.[6]

CHEMICAL COMPOSITION ANALYSIS

To determine the safety and potential toxic effects of Georgian bentonite clay in fish feed, the following analyses were performed: X-ray phase analysis, silicate testing, and physico-chemical studies. These tests confirmed that Georgian bentonite clay is primarily composed of montmorillonite, making it suitable for mycotoxin adsorption.[6]

TOXICITY STUDIES AND MYCOTOXIN LEVELS IN FEED INGREDIENTS

In this study, fish biochemical blood parameters, daily weight gain, feed conversion ratio (FCR), and survival rates were monitored to assess the health effects of bentonite-supplemented feed.[8,9] Raw materials and complete feeds were analyzed for the presence of mycotoxins—specifically Aflatoxin B1 and Trichothecenes (T2/HT2 toxins). The test was conducted at the accredited veterinary laboratory of Chirina Ltd., using express diagnostic methods with the "Aokin" fluorescence polarimeter.[10]

To simulate naturally contaminated feed, Aflatoxin and T2 toxins were deliberately introduced into the feed at controlled levels appropriate for experimental purposes.[11] Given that raw materials in the region are almost always contaminated to some degree, no group was fed a completely mycotoxin-free diet.

The analysis revealed the following levels of Aflatoxin B1: soybean meal contained 118 ppb, wheat had 16.75 ppb, and sunflower meal showed 9.9 ppb. For Trichothecenes (T2/HT2), sunflower meal contained 450 ppb, soybean meal 12.75 ppb, and wheat 68.02 ppb. Corn was included only in minimal amounts and was not tested during this phase. These results showed that while trichothecene levels in sunflower and soybean meals approached the maximum permissible limits, all mycotoxin levels remained below the regulatory safety thresholds.[12] Nonetheless, it is essential to recognize that even low-level mycotoxins can accumulate in fish tissues over time, posing potential long-term health risks.[13]

EXPERIMENTAL DESIGN

EXPERIMENTAL DIETS

The control and test group fish were fed complete diets intentionally contaminated with mycotoxins—specifically Aflatoxin B1 and T2 toxin—to simulate realistic exposure levels. Contamination was carefully calibrated to ensure consistency across all experimental groups. A 4 mm floating pellet feed was used for this trial to suit the size and feeding behavior of the fish.

Three different concentrations of Georgian bentonite clay—0.1%, 0.15%, and 0.2%—were incorporated into the experimental diets. These inclusion rates were selected based on previous research demonstrating the efficacy of similar aluminosilicate mycotoxin binders in both poultry and aquaculture feeds.[11] These levels have been shown to reduce mycotoxin toxicity effectively without negatively impacting feed quality or animal health, while also promoting improved growth performance.[12]

Additionally, studies by Pestka et al.[13] indicated that bentonite clay at comparable dosages could enhance feed conversion efficiency and mitigate toxic effects in fish. In poultry nutrition, bentonite has been proven to bind aflatoxins effectively and reduce their deleterious effects,[14] with similar benefits observed in aquatic species as well.[15]

The percentage composition of experimental diets supplemented with different levels of mycotoxin adsorbents is shown in Table 1.

Nutritional Composition of Experimental Diets is presented in Table 2.

FISH AND EXPERIMENTAL SETUP

Rainbow trout (Salmonidae subspecies "rainbow trout") fry (n=2500) were obtained, with initial weights ranging from 16-21g. Test ponds were allocated at an aquaculture farm in Shida Kartli. Five experimental groups were established. The study included five replicate sections per group, with 100 fish per section, for a total of 500 fish per group (Table 3).

Water quality parameters, trout health status, and productivity were monitored throughout the study using standard zootechnical methods, as described by Costas et al.[16]

Table 1. Percentage and proximate composition of the experimental diets supplemented with different levels of adsorbents (% in 100 kilograms of extruded feed)

Ingredient composition in feed (%)	Experimental groups				
	Control 1	Control 2	Test 1	Test 2	Test 3
Wheat	20	19.9	19.9	19.85	19.8
Meat Meal	20	20	20	20	20
Fish Meal	15	15	15	15	15
Soybean meal	12	12	12	12	12
Sunflower meal	13	13	13	13	13
Poultry Blood Meal	7	7	7	7	7
Fish Oil	5	5	5	5	5
Fish premix (vitamins, minerals, amino acids)	8	8	8	8	8
Georgian bentonite clay	-	-	0.1	0.15	0.2
Synthetic mycotoxin adsorbent	-	0.1	-	-	-
Total (%)	100	100	100	100	100

Table 2. Percentage nutritional composition of experimental diets supplemented with different levels of adsorbents (% in 100 kilograms of extruded feed)

Parameters	%
Dry mater (%)	93.8
Crude protein (% DM.)	42
Crude fat (% DM.)	20
Crude fiber (% DM.)	1.3
Ashes (%)	8.5
Starch (%)	14.5
Lysine	3.2
Methionine + Cystine	1.3
Threonine	1.5
Methionine	0.78
Gross energy (kJ/g) 22.5	

DM = dry mater / kJ/g = kilojoules per gram (a unit of energy)
The daily average weight gain is calculated by dividing the monthly weight by the number of days in each month; and feed consumption per kg of fish weight gain (FCR) is calculated by dividing the amount of feed consumed by the amount of weight gained by the animals. Steps to calculate FCR: Determine total feed consumed: Measure the total amount of feed provided to the fish during the study period.

EXPERIMENTAL CONDITIONS AND FISH MANAGEMENT

The trial lasted 24 weeks (168 days). For the initial 70 days, trout were hand-fed to satiation three times per day, after which feeding frequency was reduced to twice daily for the remainder of the study. Monthly weight measurements were taken following a 24-hour fasting period. Daily records were maintained for mortality and clinical observations. Water quality was monitored weekly to ensure optimal conditions for fish health, with key parameters—including temperature, dissolved oxygen (mg/L), pH, and ammonia (NH_3) concentration (mg/L)—maintained within recommended limits.[17] All experimental procedures were reviewed and approved by the Animal Care and Use Committee of the Agricultural University of Georgia (Institutional Animal Care and Use Committee [IACUC]).

GROWTH AND FEED EFFICIENCY

Fish were individually weighed at 3, 4, 5, and 6 months to monitor growth performance. Feed Conversion Ratio (FCR) was calculated as the total amount of feed consumed divided by the total weight gained. Survival rate was determined using the formula: (Number of surviving fish / Initial number of fish) × 100.[18]

FECES COLLECTION AND MYCOTOXIN ANALYSIS

Feces samples were collected monthly from each raceway using fine mesh nets, with one sample obtained per group simultaneously and preserved for analysis. At the conclusion of the trial, these samples were assessed for mycotoxin content—specifically Aflatoxin B1 and Trichothecene T2/HT2—using High-Performance Liquid Chromatography (HPLC) with fluorescence detection.[19]

BLOOD SAMPLE ANALYSIS

At the end of the experiment, blood samples were collected from 15 fish in each group for hematological and biochemical analyses. Hemoglobin concentration was measured using a HemoCue Hb-201+ hemoglobinometer. Erythrocytes and leukocytes were counted using a Neubauer chamber, while erythrocyte, leukocyte, and thrombocyte counts were further validated using automated hematology analyzers such as Sysmex and Coulter Counter models.[20,21] Biochemical parameters—including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), bilirubin, total protein, and creatinine—were measured using the Humalyzer Primus biochemical analyzer.

Table 3. Experimental Design with Replicates, and Mycotoxin Adsorbent Treatments

Group Number	Group Description	Mycotoxin Treatment	Number of Fish per Section	Number of Replicates (Sections)	Total Number of Fish per Group
1	Control 1 (C1)	No Adsorbent	100	5	500
2	Control 2(C2)	Synthetic Adsorbent (0.1%)	100	5	500
3	Test 1 (T1)	Georgian bentonite clay 0.1%	100	5	500
4	Test 2 (T2)	Georgian bentonite clay 0.15%	100	5	500
5	Test 3 (T3)	Georgian bentonite clay 0.2%	100	5	500

Groups names: C1= Control 1 / C2= Control 2/ T1= Test 1 / T2= Test 2 / T3= Test 3
The preparation and condition of the aquaculture facility were evaluated prior to the experiment to ensure compliance with standard biosecurity protocols. The functionality of portable equipment—including a digital scale, oximeter, pH meter, and mineralization (TDS) meter—was verified.

FISH MEAT QUALITY ANALYSIS

Proximate Composition. Moisture content was determined through oven drying, protein by the Kjeldahl method, fat via Soxhlet extraction, and ash by incineration at 550°C.[22, 23]

Organoleptic Properties. Aroma, taste, and tenderness were assessed using cooked samples—three fish from each group were boiled, and three were fried. A trained panel of sensory experts, experienced in food quality evaluation, conducted the tasting. The procedure was carried out in a controlled environment to ensure unbiased evaluations. A double-blind method was employed, with panelists unaware of whether the samples came from control or experimental groups. Samples were presented in random order with coded labels to prevent preconceived notions from influencing the evaluation. Each sample of trout meat (boiled and fried) was rated on a 1-point or 5-point hedonic scale for aroma, taste, tenderness, and overall impression.[24] All panelists were selected based on their expertise and prior experience in sensory analysis and food quality assessments. Before the sensory evaluation, the panelists underwent training sessions to ensure consistency and reliability.

Mycotoxin Analysis. To ensure food safety, meat samples from each group were analyzed for potential mycotoxin residues. The levels of aflatoxin B1 and trichothecene (T2/HT2) were determined using enzyme-linked immunosorbent assay (ELISA) kits specifically designed for detecting these mycotoxins in animal products.[25,26] In parallel with the organoleptic tasting, additional analyses were conducted to ensure that meat from groups exposed to high mycotoxin content did not pose a health risk to consumers.[27]

STATISTICAL ANALYSIS

Data was analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's HSD post hoc test to determine significant differences between treatment groups. A significant level of $p < 0.05$ was used for all comparisons. Statistical analysis was conducted using SPSS version.

RESULTS

WATER QUALITY PARAMETERS

During the experimental period, water quality parameters remained within normal ranges for all groups, with no significant differences observed in key metrics ($P > 0.05$). The following values were recorded: Raceways setup: raceway system, stocking density: 10 kg live weight/ $1m^2$, photoperiod: 13-hour light:11-hour dark cycle, total dissolved solids (TDS): <500 mg/l, water Hardness (as $CaCO_3$): 100 mg/l, water velocity: 0.5 to 0.6 meters per second (m/s), dissolved oxygen: 6.2 ± 1 mg/l to 6.46 ± 0.71 mg/l, pH: 6.6 ± 0.99 to 7 ± 0.68, temperature: 13 ± 0.79°C to 16 ± 0.71°C, ammonia-nitrogen: 0.09 ± 0.03 mg/l to 0.01 ± 0.18 mg/l, water exchange rate was performed daily.

Water quality parameters were monitored daily. These findings suggest that water quality was adequately maintained throughout the experiment, ensuring that observed biological and growth effects were not confounded by environmental factors.

According to Table 4, the initial live weight of fish across all five groups was comparable, ranging from 16.5 to 19.2 g. This indicates a high degree of uniformity among the fish fry selected for the study. Over the 24-week experimental period (180 days), all groups exhibited significant growth, with the T3 group achieving the highest final weight (272.59 ± 17.49 g) ($P < 0.01$) and the C1 group the lowest (162.36 ± 14.15 g). Fish in the treatment groups (T1, T2, T3) exhibited significantly improved growth rates compared to the control groups (C1 and C2) ($P < 0.01$).

At week 24, the T3 group also showed the highest absolute weight gain (254 ± 15.8 g), which was 77% higher than that of the C1 group (Table 4). The highest daily weight gain was observed in the T3 group (1.41 ± 0.09 g) ($P \geq 0.05$), followed by T2 and T1, indicating enhanced growth performance due to dietary inclusion of Georgian bentonite clay.

The coefficient of variation (CV) values ranged between 4.4% and 10%, suggesting moderate variability in growth responses among groups.[28] Statistical comparisons re-

Table 4. Growth Performance of Fish Under Different Experimental Conditions (grams), Including Effects of Aflatoxin B1 (AFB1), Trichothecene T2/HT2, and Toxin Binders.

Indicators (Body mass)	Groups				
	C1	C2	T1	T2	T3
1st Month	18.97±1.01^{a}	16.5±0.45^{b}	19.12±0.88^{a}	19.23±0.85^{a}	18.55±0.98^{ab}
3th Month	78.96±6.27^{a}	97.35±5.32^{ab}	113.14 ±6.31^{bc}	116.59±7.08^{bc}	122.82 ±7.40^{c}
4th Month	95.22±10.05^{a}	121.61±8.91^{b}	142.56±5.85^{b}	151.91±10.30^{b}	155.72±12.14^{b}
5th Month	125 ±10.06^{a}	158.34±13.99^{b}	200.95±15.1^{c}	206.9±15.14^{c}	212.34±15.93^{c}
6th Month	162.36±14.15^{a}	204.17±16.48^{ab}	256.15±18.14^{bc}	264.15±18.35^{bc}	272.59±17.49^{c}
Absolute Weight Gain (0-6 month)	143.39±9.51^{a}	187.67±12.1^{b}	237 ±14.7^{c}	244.92±14.47^{c}	254±15.8^{c}
Daily Weight Gain (0-6 month)	0.8±0.06^{a}	1.04±0.07^{a}	1.32±0.08^{b}	1.36±0.08^{b}	1.41±0.09^{b}

Mean values marked with different superscripts (a, b, c) are significantly different at $P < 0.05$

Table 5. Effects of aflatoxin B1 (AFB1), Trichothecene T2/HT2 and toxin binders on survival and feed conversion rate (FCR) Efficiency Indicators

Indicators	Groups				
	C 1	C2	T1	T2	T3
Feed conversion rate (FCR)	1.3±0.23^{b}	1.1±0.20^{ab}	1±0.17^{ab}	0.92±0.15^{ab}	0.9±0.18^{a}
Survival %	87±1.23^{a}	92±1.20^{b}	95±1.19^{bc}	97±1.16^{c}	97±1.18^{c}

Mean values marked with different superscripts (a, b, c) are significantly different at $P < 0.05$
FCR = Feed conversion rate

vealed significant performance enhancements in the T2 and T3 groups, likely attributed to the inclusion of Georgian bentonite clay at 0.15% and 0.2%, respectively. These additives may have counteracted the negative effects of aflatoxins and trichothecene toxins, thereby promoting superior growth (see Table 4).

The test groups, particularly T2 and T3, exhibited superior growth performance, with T3 showing the highest final weight and absolute weight gain among all groups. These improvements were statistically significant and are likely attributable to the inclusion of Georgian bentonite clay at 0.15% and 0.2%, which may have mitigated the negative impacts of dietary toxins such as aflatoxins and trichothecenes (Table 4).

SURVIVAL AND FEED CONVERSION EFFICIENCY

The feed conversion ratio (FCR) and survival rate were evaluated across all experimental groups (Table 5). The control group C1 exhibited the highest FCR (1.3 ± 0.23), indicating the least efficient feed utilization. Group C2 showed modest improvement (1.1 ± 0.20). Among the test groups, T3 achieved the lowest FCR (0.9 ± 0.18) ($P < 0.01$), followed by T2 (0.92 ± 0.15) and T1 (1.0 ± 0.17), reflecting enhanced feed efficiency likely due to dietary supplementation with bentonite clay.

In terms of survival rates, the highest percentages were observed in T2 and T3 (both at 97%), followed by T1 (95%). The control groups reported lower survival, with C2 at 92% and C1 at 87%.

While both C2 and the test groups (T1–T3) were administered toxin binders, T2 and T3 significantly outperformed C2, indicating a key difference in the efficacy of the binders used. Specifically, the synthetic binder in C2 offered moderate protection—as reflected in improved growth over the untreated control C1—but it was notably less effective than the Georgian bentonite clay used in T2 and T3. The superior efficacy of Georgian bentonite clay over the synthetic binder used in C2 may be attributed to several key factors: (i) Higher Adsorption Capacity: Natural bentonite has a layered aluminosilicate structure with a high surface area and strong cation exchange properties, which enhance its ability to adsorb a wide spectrum of mycotoxins, including aflatoxins and trichothecenes. In contrast, many synthetic binders are formulated primarily for aflatoxins and may be less effective against multiple toxin types simultaneously. Broader Binding Spectrum: Georgian bentonite likely provides broader-spectrum detoxification, potentially neutralizing both polar and non-polar toxins more effectively than some synthetic binders, which may have narrower specificity. (ii) Enhanced Bioavailability and Safety: Unlike some synthetic compounds, natural bentonite clay has been shown to be safe and non-toxic at the tested inclusion levels. It may also support better gut health and nutrient absorption, indirectly contributing to better growth and feed utilization. (iii) Stability Under Aquatic Conditions: Bentonite clay maintains its binding efficacy under a range of pH and temperature conditions, making it more reliable in dynamic aquatic environments.

These findings suggest that Georgian bentonite is not only a viable alternative but may be a *superior* solution

Table 6. The amount of Aflatoxin B1 and Trichothecene T2/HT2 adsorbed levels (binding) Detected in in fish feces (ppb)

Groups	Aflatoxin B1 (ppb)	Trichothecenes T2/HT2 (ppb)
C1 (without adsorbent)	0	0
C2	98.5±1.58^{a}	37.8±0.88^{a}
T1 (0.1% Bentonite)	110.6±0.93^{b}	53.3±1.99^{b}
T2 (0.15% Bentonite)	120.5±1.22^{c}	66.3±2.00^{c}
T 3 (0.2% Bentonite)	130±1.75^{d}	74.3±1.88^{d}

Mean values marked with different superscripts (a, b, c, d) are significantly different at $P < 0.05$

to synthetic binders in mitigating mycotoxin impacts in aquaculture. Its use can lead to improved productivity, resilience, and economic efficiency, particularly in systems where feed contamination is a recurrent challenge.

Mycotoxin Adsorption Efficiency, Fish Health Status, Organoleptic and Physical Indicators, and rainbow trout (Oncorhynchus mykiss) Production Efficiency

An analysis of fecal samples from both control and treatment groups was conducted to evaluate mycotoxin adsorption efficiency, specifically targeting aflatoxin B1 and trichothecene T2/HT2 residues. The presence of higher mycotoxin concentrations in feces indicates effective binding and excretion of these toxins, reducing their bioavailability and systemic impact on fish.

Results showed significantly higher excretion of mycotoxins in the T2 and T3 groups, indicating more effective adsorption due to dietary inclusion of Georgian bentonite clay. The highest mycotoxin levels in feces were recorded in the T3 group (B1 = 130 ppb; T2/HT2 = 74.3 ppb), followed by T2 (B1 = 120.5 ppb; T2/HT2 = 66.3 ppb) ($P < 0.05$), demonstrating enhanced mycotoxin-binding capacity. The T1 group also showed improved binding (B1 = 110.6 ppb; T2/HT2 = 53.3 ppb) compared to control C2 (B1 = 98.5 ppb; T2/HT2 = 37.8 ppb) (Table 6).

These findings suggest a dose-dependent effect of Georgian bentonite clay on mycotoxin adsorption. The improved excretion patterns in treatment groups correlated with better growth performance, feed efficiency, and health indicators, including higher survival rates and improved organoleptic properties of the meat.

Overall, the inclusion of Georgian bentonite clay at 0.15% and 0.2% in rainbow trout (Oncorhynchus mykiss) diets not only improved growth and feed utilization but also significantly reduced systemic mycotoxin exposure, thereby enhancing production efficiency and product quality in rainbow trout farming.

MYCOTOXIN ADSORPTION EFFICIENCY

In the test groups, the adsorption efficiency of mycotoxins was notably higher in the groups supplemented with Georgian bentonite clay. Specifically, in the T2 (0.15%) and T3 (0.2%) groups, the adsorption rate for aflatoxin B1 was 83-90%, while the adsorption efficiency for T2/HT2 mycotoxins was significantly lower, ranging from 12.5% to 14%. In contrast, the T1 group (with a lower bentonite concentration) showed a B1 adsorption rate of 76.4%, while T2/HT2 adsorption remained low at only 10%. The control group C2 demonstrated significantly lower adsorption rates for both aflatoxin B1 (68.1%) and T2/HT2 (7.1%) ($P < 0.05$) (Table 7).

GENERAL AND BIOCHEMICAL BLOOD ANALYSIS

Biochemical analyses of blood revealed significant improvements in hemoglobin and erythrocyte levels in all test groups compared to the control group ($P < 0.05$). Hemoglobin levels increased by 10-26%, while erythrocyte counts rose by 6-12%. Notably, the T3 group (with a 0.2% bentonite clay additive) showed the most pronounced increases in these parameters (Table 8).

Additionally, the total protein content in blood serum was lower than the physiological norm in the control group, measuring at 34 g/l. In contrast, the experimental groups demonstrated a 38-47% increase in protein content, with levels ranging from 47 to 50 g/l ($P < 0.05$) (Table 9). These results indicate that the inclusion of Georgian bentonite clay as a mycotoxin adsorbent positively influenced both the general health and biochemical status of the fish.

CHEMICAL COMPOSITION OF FISH MEAT

Analysis of the chemical composition of rainbow trout meat revealed that the protein content was highest in the T2 and T3 groups ($P < 0.05$), at 18.3-18.5%. These values were 1.1-1.3% higher ($P < 0.05$) than those of the C2 group. The fat content was highest in T2 ($P < 0.05$), while the C2 group exhibited lower protein content (16.5%) and fat content (6%). The C1 group showed the lowest fat content (Table 10).

These differences in protein and fat content were statistically significant ($P < 0.05$), indicating that the inclusion of Georgian bentonite clay significantly improved both protein and fat levels in rainbow trout (Oncorhynchus mykiss) meat, likely contributing to higher nutritional quality (Table 10).

TOXICOLOGICAL SAFETY AND IMPACT OF BENTONITE ON FISH HEALTH

No direct indications of toxicological reactions or harmful effects were observed due to the addition of Georgian bentonite clay in the rainbow trout diet. On the contrary, the results suggest that bentonite had beneficial effects on the

Table 7. Adsorption Rate (%) Efficiency of Aflatoxin B1 and Trichothecene T2/HT2 in Feces

Groups	Aflatoxin B1 Adsorption Rate (%)	Trichothecene T2/HT2 Adsorption Rate (%)
C1 (without adsorbent)	No adsorption	No adsorption
C 2	68.1 ± 1.22^{a}	7.1 ± 2.18^{a}
T1 (0.1% Bentonite)	76.4 ± 2.10^{ab}	10 ± 2.00^{ab}
T2 (0.15% Bentonite)	83 ± 1.08^{bc}	12.5 ± 0.99^{ab}
T3 (0.2% Bentonite)	90 ± 2.03^{c}	14 ± 1.11^{b}

Mean values marked with different superscripts are significantly different at $P < 0.05$

Table 8. Effects of aflatoxin B1, (AFB1), Trichothecenes T2/HT2 and toxin binders on hematological values (%) in Fish.

Groups	Hemoglobin g/l	Erythrocytes 1012 l	Hematocrit (%)	Platelets 1000 mcg/L	Lymphocytes (%)	Eosinophils (%)
C 1	60 ± 5.86^{a}	1 ± 0.1^{a}	18 ± 2.29^{a}	147 ± 9.70^{a}	62 ± 0.01^{a}	1 ± 0.03^{a}
C2	77 ± 6.67^{b}	1.6 ± 0.18^{b}	26 ± 3.02^{ab}	170 ± 10.30^{ab}	78 ± 0.05^{b}	5 ± 0.05^{d}
T1 (0.1% Bentonite)	80 ± 5.50^{bc}	1.7 ± 0.30^{b}	28 ± 2.03^{b}	180 ± 15.09^{b}	77 ± 0.05^{b}	2 ± 0.11^{b}
T2 (0.15% Bentonite)	85 ± 6.01^{bc}	1.7 ± 0.17^{b}	27 ± 1.99^{b}	190 ± 17.08^{b}	76 ± 0.15^{b}	5 ± 0.13^{d}
T3 (0.2% Bentonite)	97 ± 7.10^{c}	2 ± 0.49^{c}	30 ± 3.32^{b}	200 ± 16.99^{b}	75 ± 0.20^{b}	4 ± 0.10^{c}

Mean values marked with different superscripts are significantly different at $P < 0.05$

Table 9. Effects of aflatoxin B1, (AFB1), Trichothecenes T2/HT2 and toxin binders on blood metabolites of fish. U/l =Units per Liter; g/l = grams per liter; mg/dl = milligrams per deciliter; mmol/l = millimoles per liter

Groups	Alanine Aminotransferase U/l	Aspartate Aminotransferase U/l	Total protein g/l	Albumin g/l	Creatinine mg/dl	Glucose mmol/l
C1	20 ± 3.35^{a}	155 ± 11.2^{a}	23 ± 0.25^{a}	7 ± 0.03^{a}	0.32 ± 0.01^{a}	2.2 ± 0.11^{a}
C2	29 ± 3.90^{a}	180 ± 14.00^{ab}	34 ± 0.13^{b}	12 ± 0.04^{b}	0.50 ± 0.02^{b}	3.0 ± 0.17^{b}
T1 (0.1% Bentonite)	32 ± 4.10^{ab}	189 ± 12.14^{ab}	40 ± 0.10^{c}	13 ± 0.07^{b}	0.52 ± 0.01^{b}	3.2 ± 0.20^{b}
T2 (0.15% Bentonite)	34 ± 5.12^{ab}	193 ± 15.09^{b}	47 ± 0.16^{d}	14 ± 0.04^{b}	0.53 ± 0.04^{b}	3.3 ± 0.12^{b}
T3 (0.2% Bentonite)	34 ± 4.33^{b}	198 ± 17.90^{b}	50 ± 0.19^{e}	16 ± 0.06^{c}	0.57 ± 0.03^{b}	3.5 ± 0.18^{b}

Mean values marked with different superscripts (a, b, c, d, e) are significantly different at $P < 0.05$

Table 10. Proximate carcass composition of experimental fish

Groups	Protein Content (%)	Fat Content (%)	Moisture (%)	Ash (%)
C1	16 ± 0.32^{a}	6 ± 0.18	72 ± 0.16	1 ± 0.8
C 2	17.2 ± 018^{b}	6.8 ± 0.07	71.1 ± 0.12	1.2 ± 0.18^{b}
T1 (0.1% Bentonite)	17.8 ± 0.06^{b}	7.1 ± 0.13^{b}	71.6 ± 0.03	1.1 ± 0.12
T2 (0.15% Bentonite)	18.3 ± 0.18^{bc}	7.8 ± 0.06	71 ± 0.05^{a}	1.3 ± 0.10
T3 (0.2% Bentonite)	18.5 ± 0.19^{c}	7.5 ± 0.20	71.5 ± 0.17	1.5 ± 0.22

Mean values marked with different superscripts (a, b, c) are significantly different at $P < 0.05$

growth and health of the rainbow trout. No signs of toxicity or adverse effects were reported in the blood composition or meat quality of the fish. Biochemical analyses showed that protein content in the blood of the test groups was higher than in the control group (Table 9), further supporting the positive impact of bentonite.

Table 11. Results of Organoleptic, physical indicators and Taste Properties (boiled and fried conditions) Rating with 1 being the least favorable and 5 being the most favorable.

Sample Group	Appearance	Flavor	Texture	Taste	Evaluation average score
C1	3.4	3.3	3.5	3.4	3.4
C 2	3.7	3.6	3.8	3.9	3.8
T1 (0.1% Bentonite)	4	3.8	3.9	4.1	4
T2 (0.15% Bentonite)	4.7	4.5	4.8	4.7	4.7
T3 (0.2% Bentonite)	5	4.9	5	5	5

5 point hedonic scale, Equivalent score: excellent - 5, good - 4, fair - 3, poor - 2, very poor - 1.

In terms of meat quality, the texture, taste, and fat content were significantly improved in the test groups, indicating a beneficial effect of bentonite on the nutritional value and sensory characteristics of the rainbow trout. These findings suggest that the addition of bentonite to the diet of rainbow trout is both safe and effective, with no observed toxicological reactions, and it provides a positive impact on fish health and growth.

ORGANOLEPTIC AND PHYSICAL INDICATORS

Sensory evaluation, including taste testing for aroma, taste, tenderness, and overall impression, was conducted with rainbow trout from the T2 and T3 test groups. The results showed superior performance in all tasting parameters for rainbow trout from these groups, both in boiled and fried conditions (Table 11).

DISCUSSION

This study evaluated the efficacy of Georgian bentonite clay as a natural mycotoxin adsorbent in rainbow trout diets formulated with lower-quality feed ingredients. The findings demonstrate that bentonite inclusion at 0.15% to 0.2% significantly improves growth performance and feed conversion efficiency. These results align with previous research in poultry nutrition, where bentonite enhanced feed utilization and mitigated mycotoxin toxicity,[6,29] suggesting potential cross-species benefits of this clay mineral.

Improved growth parameters in the bentonite-fed groups likely stem from multiple mechanisms. Bentonite's capacity to bind harmful mycotoxins such as aflatoxins and trichothecenes reduces their bioavailability and subsequent negative impacts on fish intestinal health and systemic physiology. Mycotoxins are known to disrupt gut barrier integrity and induce oxidative stress, impairing nutrient absorption and immune responses.[12] By preserving intestinal tight junctions and adsorbing these toxins, bentonite may protect gut morphology and function, resulting in better nutrient uptake and improved overall health. This protective effect is supported by the observed reduction in feed conversion ratio, indicating more efficient feed use likely facilitated by toxin detoxification.

The binding efficiency data reveal that Georgian bentonite is particularly effective against aflatoxin B1, achieving adsorption rates of 83–90%, consistent with findings from other studies on bentonite clays.[7,30] This is significant given aflatoxin's high toxicity and prevalence in aquafeeds. However, bentonite's lower adsorption of T2/HT2 toxins (12.5–14%) highlights its limitations and suggests that a single adsorbent may not suffice to combat the full spectrum of mycotoxins encountered in aquaculture feeds. Future research should explore combining bentonite with other adsorbents to enhance efficacy against diverse toxin profiles.

Interestingly, the synthetic adsorbent tested (C2) underperformed compared to bentonite in terms of growth and feed efficiency. This unexpected result may reflect differences in toxin binding affinities, adsorbent composition, or physiological interactions within the fish gut. The finding underscores the importance of critically evaluating synthetic additives, as their presumed consistency does not always translate to superior functional outcomes. More detailed characterization and comparative studies are warranted to clarify these differences.

Regarding meat quality and sensory attributes, while preliminary observations suggest positive effects of bentonite supplementation, these claims remain speculative without rigorous sensory evaluation or compositional analyses. Future studies should incorporate structured sensory panels and objective measures of chemical composition to verify and quantify these potential benefits.

It is also important to acknowledge potential risks associated with bentonite use. Non-specific binding of essential nutrients could occur at higher inclusion levels, potentially impairing mineral and vitamin availability. Additionally, the physical properties of clay might alter gut motility or interfere with drug absorption during treatments. These concerns highlight the necessity for optimizing dosage and conducting long-term safety assessments of the fish species. From an applied perspective, Georgian bentonite offers a promising, locally available, and cost-effective strategy to mitigate mycotoxin risks in aquaculture, supporting sustainable production and reducing reliance on expensive imported additives. However, successful commercialization will require further research to address several key areas, including mechanistic studies on bentonite's interaction with fish digestive physiology and nutrient metabolism, optimization of inclusion rates to balance toxin adsorption with nutrient bioavailability, and exploration of multi-adsorbent blends targeting a broad spectrum of mycotoxins. Additionally, comprehensive evaluation of meat quality and consumer sensory acceptance, detailed comparisons between natural and synthetic adsorbents in terms of

efficacy, safety, and cost-effectiveness, as well as economic analyses and supply chain assessments, are essential to facilitate large-scale adoption in Georgian aquaculture.

In conclusion, this study fills a critical research gap by demonstrating the benefits of Georgian bentonite in rainbow trout diets and identifying areas for further investigation to maximize its potential as a natural mycotoxin adsorbent in aquaculture feed formulations.

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AUTHORS' CONTRIBUTION

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COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

All experimental procedures applied in this research were approved by the Research Ethics Committee of the animal feeding and husbandry faculty, with number 077/ EG-FKH/ int./2024. The sentences are included here.The Research Ethics Committee, Faculty of animal feeding and husbandry, Agricultural University of Georgia having reviewed the research proposal, confirms its approval of the title. According to the review, this research proposal satisfies ethical standards and is hereby approved. The Agricultural university of Georgia Faculty of animal feeding and husbandry Research Ethics Committee reserves the right to conduct ongoing monitoring.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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