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Application of New Dietary Bentonite Clay as an Aquaculture Feed Additive

Yeni Diyet Bentonit Kilinin Su Ürünleri Yetiştiriciliği Yem Katkı Maddesi Olarak Uygulanması

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ABSTRACT

This study evaluated the effectiveness of Georgian bentonite clay as a natural mycotoxin adsorbent in rainbow trout (*Oncorhynchus mykiss*) diets. The experiment involved 100 fish per group, fed diets without adsorbent (Control 1; C1), with 0.1% synthetic adsorbent (Control 2; C2), and with bentonite at 0.1% (Test 1; T1), 0.15% (Test 2; T2), and 0.2% (Test 3; T3) for 24 weeks. 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) ($p<.05$). Survival rates were 97% in T2 and T3, but differences among groups were not statistically significant ($p>.05$). Feed conversion ratios (FCR) were lowest in T2 and T3 (0.9–0.92), indicating superior feed efficiency compared to C1 (1.3) and C2 (1.1). High-performance liquid chromatography (HPLC) analysis revealed that bentonite effectively adsorbed mycotoxins, with T2 and T3 removing 83-90% of aflatoxin B1 and 12.5-14% of T2/HT2. Chemical composition analysis showed significantly higher protein (18.3-18.5%) and fat content (7.8%) in T2 and T3 compared to C1 (16.5% protein, 6% fat) and C2 (17.2% protein, 6.8% fat) ($p<.05$). These findings suggest that Georgian bentonite clay enhances fish growth, feed efficiency, and nutritional quality while effectively mitigating mycotoxin contamination. This natural adsorbent offers a promising alternative for improving aquaculture sustainability and fish health.

Keywords: Adsorbent, Fish, Mycotoxin binders, Nutrition

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Introduction

The global consumption of fish and seafood is increasing yearly, as they are rich sources of protein. Statistically, 65-70% of the total protein consumed by humans comes from farm animals and poultry (including milk, eggs, and meat), while the remaining 30-35% is derived from fish and other seafood (Guillen et al., 2019). Over the past 50 years, global fish production has more than tripled. Fifty years ago, total fish production was around 60 million tons, with almost all of it coming from capture fisheries. Today, capture fisheries contribute 91 million tons, while aquaculture has grown to 94 million tons. In total, global fish production now reaches 186 million tons, rising from 9 kilograms per capita to 21 kilograms, and is projected to reach 30 kilograms per capita in the next decade (FAO, 2020). However, approximately 60-65% of fish farming costs stem from feeding, with Georgian fish farmers relying on expensive imported fish feed (FAO, 2024).

Today, fish feed enterprises face the challenge of producing complete, high-quality feed at reduced prices by utilizing cheaper raw materials (such as cereals or byproducts from various industries). The quality of these low-cost raw materials is a concern, as about 50-55% are often contaminated with mycotoxins—metabolic products of microscopic fungi (Saini et al., 2021).

Mycotoxins pose significant risks to farm animals, poultry, and fish due to their pronounced toxicity (Gruber-Dorninger et al., 2020). Even in small quantities, they can diffuse into feed raw materials and aquaculture feed. To date, around 300 types of mycotoxins have been isolated from 450 different fungal species, with up to 20 considered particularly dangerous due to their cancer-promoting properties in humans (Mahato et al., 2022).

Consequently, fish feed companies must find affordable methods to combat mycotoxins, ensuring the safety and quality of their products while remaining competitive with imported alternatives. Recent research has focused on the effectiveness of mycotoxin adsorbents, with numerous options available on the global market. In Georgia, many of these are synthetic, imported, and costly. One natural solution is aluminosilicate clays—specifically bentonites—which are widely used globally in the diets of agricultural animals and poultry (Kihal et al., 2022). These clays can adsorb mycotoxins, preventing their adsorption into the bloodstream and facilitating their excretion (Oliveira et al., 2023). Georgia's geological history, marked by numerous volcanic eruptions, has endowed the country with various clay deposits, including the essential bentonite clay deposit known as Georgian clay, in the Ozurgeti District (Western Georgia). Although the use of this bentonite in poultry feed for mycotoxin adsorption has been studied (Chkuaseli et al., 2016), the results indicated that incorporating Georgian bentonite clay into poultry diets led to several beneficial outcomes: improved poultry physiological condition and livability with 2-4% enhanced feed adsorption and FCR with 0.08%-0.12% reduced negative effects of mycotoxins toxicity, and increased daily weight gain with 7-9% improved meat safety. These findings suggest that Georgian bentonite clay serves as an effective mycotoxin adsorbent, positively influencing the growth, health, and safety of poultry meat. However, its application in aquaculture remains unexplored. This recent research has focused on evaluating the efficacy of locally produced bentonite clay for detoxifying mycotoxins at a comparatively low price.

The purpose of this study is to evaluate alternative feed additives using low-quality raw materials to reduce dependency on expensive traditional ingredients, while maintaining the growth performance and health of farmed trout. The significance of this research lies in its potential to reduce feed costs in trout farming, thus improving the economic efficiency of fish production and enhancing the safety of the final product (trout meat). Increased access to affordable aquaculture products can elevate community well-being. We believe the results will contribute to the sustainable development of the aquaculture industry, optimize resource (low-quality raw materials in feed) use, promote environmental awareness, and address One Health issues.

Method

Collection and Analysis of Bentonite Clay

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

An 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, model COH28-X (Chkuaseli et al., 2016).

Mycotoxin Safety and Toxicity Analysis

Chemical composition analysis

To determine the safety and potential toxic effects of Georgian bentonite clay in fish feed, the following analyses were conducted: chemical composition analysis: X-ray phase analysis, silicate tests, and physical-chemical studies confirmed by Chkuaseli et al., 2016, that Georgian bentonite clay is primarily composed of montmorillonite, making it suitable for mycotoxin binding.

Toxicity studies and mycotoxin levels in feed ingredients

In this context, fish biochemical blood tests and fish growth (daily weight gain), feed conversion, and daily survival data were monitored (Miller, 2020; Pereira, 2021). Raw materials and complete feed were tested for mycotoxins (aflatoxin B1 and trichothecenes T2/HT2 toxins). The analysis was conducted in the accredited veterinary laboratory of Chirina Ltd., using express methods on the Aokin fluorescence polarimeter (Sullivan, 2021). The mycotoxin content in feed ingredients was measured, ensuring that contamination levels did not exceed maximum allowable limits. The control and test group fish were provided with complete feed contaminated with mycotoxins (aflatoxin and T2 toxins). The contamination in this experiment was induced by the intentional addition of these mycotoxins (aflatoxin and T2 toxins) (Bøhn, 2020). Due to the fact that the raw materials of the feed are practically always contaminated with mycotoxins (sometimes more, sometimes less) in our region, we did not consider it necessary to provide any control group with feed free of mycotoxins. The concentration of these mycotoxins in the feed would have been carefully controlled and calibrated to ensure that the levels were appropriate for testing the effects on the fish. The content of mycotoxins (aflatoxin B1 and trichothecenes T2/HT2) in the fish feed ingredients from the research groups was assessed.

The results indicated the following levels of aflatoxin B1: soybean meal contained 118 ppb (micrograms), wheat had 16.75 ppb, and sunflower meal showed 9.9 ppb. For trichothecenes T2/HT2: sunflower meal had 450 ppb, soybean meal had 12.75 ppb, and wheat contained 68.02 ppb. Corn was used in minimal amounts in the diet; therefore, there was no need to test it at this stage. The results showed that the levels of T2/HT2 trichothecenes in sunflower and soybean meals were close to the maximum limit, whereas the content of aflatoxin and trichothecenes in the raw materials did not exceed the maximum allowable limits (Gruber-Dorninger et al., 2020).

However, it is important to note that these mycotoxins can accumulate in the fish's body over time (Pestka et al., 2019).

Experimental Design

Experimental diets

The control and test group fish were provided with complete feed contaminated with mycotoxins (aflatoxin and T2 toxins). The contamination in this experiment was induced by the intentional addition of these mycotoxins (aflatoxin and T2 toxins). For this trial, we used 4 mm size floating feed.

adversely affecting feed quality or fish health, leading to improved growth performance (Gruber-Dorninger et al., 2020). Furthermore, research by Pestka (2019) and Jian (2020) suggests that bentonite clay at similar concentrations not only mitigates mycotoxin toxicity but also enhances feed conversion efficiency in fish. In poultry, studies have confirmed that bentonite clay effectively binds aflatoxins and reduces their harmful effects (Van Der Merwe et al., 2018), with similar outcomes observed in aquatic species (Mohd Zain et al., 2021).

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

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 Matter (%)	93.8
Crude Protein (% D.M.)	42
Crude Fat (% DM.)	20
Crude Fiber (% D.M.)	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

The selection of additive levels (0.1%, 0.15%, and 0.2%) for bentonite clay in the experimental diets was based on prior studies and literature where similar mycotoxin adsorbents have been tested in poultry and fish feed (Bøhn et al., 2020). These concentrations were chosen because research has shown that they are effective in reducing mycotoxin concentrations without

Nutritional composition of experimental diets

The daily average weight gain is calculated by dividing the monthly weight by the number of days in each month; 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.

Fish and experimental setup

Rainbow trout (*Salmonidae* subspecies *Oncorhynchus mykiss*) fry (n=2,500) 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).

The preparation and condition of the fish farm for conducting the experiment were assessed, including biosecurity norms and the operation of portable equipment (scale, oximeter, pH meter, and mineralization meter). Water parameters, trout health, and productivity were evaluated using zootechnical methods (Costas, 2021; Pulcini, 2020).

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

Experimental conditions and fish management

The trial duration was 24 weeks (180 days). During the first 70 days, trout were hand-fed to satiation three times daily, with the feeding frequency reduced to twice daily for the remaining period. Monthly weight monitoring was conducted following a 24-hour fasting period. Mortality and clinical responses were recorded daily. Water quality was assessed weekly to ensure optimal fish health, with the following parameters maintained: temperature, dissolved oxygen (mg/L), pH, and ammonia (NH_3) mg/L (Smith et al., 2019).

All 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]).

Data Collection and Analysis

Growth and feed efficiency

The research involved individual weighing of fish at regular intervals (3, 4, 5, and 6 months) and calculating the Feed Conversion Ratio (FCR) as the ratio of total feed consumed to total weight gain. The survival rate was calculated using the formula: (Surviving fish / Initial fish) × 100 (Seppälä et al., 2022).

Feces collection and mycotoxin analysis

Feces were collected monthly from tanks using fine mesh nets, one from each group at the same time, and stored. At the end of the experiment, samples were analyzed for mycotoxin content (aflatoxin B1 and trichothecene T2/HT2) using high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) (Sulyok et al., 2020).

Blood sample analysis

Blood samples were collected from 15 fish per group at the end of the experiment. Hematological parameters, including hemoglobin, erythrocytes, leukocytes, and thrombocytes, were determined using the following methods and equipment: HemoCue Hb-201+ hemoglobinometer (for hemoglobin concentration), Neubauer Chamber (for erythrocyte and leukocyte counts), and automated hematology analyzers (e.g., Sysmex, Coulter Counter) for erythrocyte, leukocyte, and thrombocyte counts (Hossain et al., 2020; Nash, 2021).

Biochemical parameters such as AST, ALT, GGT, bilirubin, total protein, and creatinine levels were determined using the Humalyzer Primus biochemical analyzer (Zhou, 2020).

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. (Central Inland Fisheries Research Institute [CIFRI] Guidelines, 2022; FAO's Laboratory Manual, 2021).

Organoleptic Properties: Aroma, flavor, 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 tasting procedure was carried out in a controlled environment to ensure unbiased evaluations. A double-blind method was employed to eliminate bias, 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, flavor, tenderness, and overall impression (Civille et al., 2024).

All panelists were selected based on their expertise and previous experience in sensory analysis and food quality assessments. Prior to conducting the sensory evaluation, the panelists underwent training sessions to ensure consistency and reliability in their evaluations.

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 (Abcam, 2023; ProGnosis Biotech, 2023). In parallel with the organoleptic tasting, additional analyses were performed to ensure that meat from groups fed high mycotoxin content did not pose a health risk to consumers (Mago et al., 2020).

Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA). Significant differences between mean values were determined by Duncan's multiple range tests ($p<.05$) using a statistical package for social sciences (SPSS). Values were expressed as means (± S.E).

Results

Field Study Findings, Results of Production Indicators

According to Table 4, the live weight of fish at the beginning of the experiment is the same in all five groups and ranges from 16.5 to 19.2 g. This figure is an indicator of the high uniformity of the fish fry placed in the experiment. The fish groups demonstrated significant growth over the 24-week period (180 days), with the highest weight observed in the T3 group (272.59±17.49) ($p<.01$) and the lowest in C1 (162.36±14.15). Fish in the test groups (T1, T2, T3) showed better growth rates compared to the control groups (C1 and C2) ($p<.01$). At 24 weeks, T3 showed the highest absolute weight gain (254±15.8), 77% higher than C1 (Table 4). The highest daily weight gain was also recorded in T3 (1.41g ± 0.09) ($p\geq 0.05$), followed by T2 and T1 (Table 4), indicating improved growth efficiency due to the addition of Georgian bentonite clay.

The coefficient of variation (CV) values ranged between 4.4% and 10%, indicating moderate variability in growth trends (Sadek et al., 2004). Statistical comparisons suggest significant improvements in the T2 and T3 groups, likely due to the inclusion of Georgian bentonite clay (0.15% and 0.2% respectively), which may have mitigated the negative effects of aflatoxins and trichothecene toxins on fish growth (Table 4).

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

Indicators (Body mass)	Unit	Groups				
		Control 1	Control 2	Test 1	Test 2	Test 3
1st Month	g	18.97±1.01	16.5±0.45	19.12±0.88 *	19.23±0.85*	18.55±0.98
3rd Month	g	78.96±6.27	97.35±5.32	113.14 ±6.31	116.59±7.08	122.82 ±7.40*
4th Month	g	95.22±10.05	121.61±8.91	142.56±5.85	151.91±10.30	155.72±12.14
5th Month	g	125 ±10.06*	158.34±13.99	200.95±15.1	206.9±15.14	212.34±15.93
6th Month	g	162.36±14.15	204.17±16.48	256.15±18.14	264.15±18.35	272.59±17.49
Absolute Weight Gain (0-6 months)	g	143.39±9.51	187.67±12.1	237 ±14.7*	244.92±14.47	254±15.8
Daily Weight Gain (0-6 months)	g	0.8±0.06	1.04±0.07	1.32±0.08	1.36±0.08	1.41±0.09

*Mean values without superscript are significantly different ($p<.05$).

The test groups (T2 and T3) showed superior growth, with T3 having the highest growth and absolute weight gain. Significant improvements were noted in T2 and T3, likely due to the inclusion of Georgian bentonite clay)0.15% and 0.2%), which mitigated the negative effects of toxins (aflatoxins and Trichothecene) (Table 4).

Survival and Feed Conversion Rate Efficiency Findings

The feed conversion rate (FCR) and survival percentage were analyzed across all experimental groups: C1 had the highest FCR (1.3 ± 12.88), indicating lower efficiency in feed utilization. C2 showed improvement (1.1 ± 13.34). Among the test groups, T3 (0.9 ± 14.25) had the lowest FCR ($p<.01$), followed by T2 (0.92 ± 13.31) and T1 (1 ± 14.7), suggesting improved feed efficiency (Table 5). Survival rates were highest in T2 and T3 (both 97%), followed by T1 (95%). Control groups had lower survival rates, with C1 at 87% and C2 at 92% (Table 5).

Table 5.
Effects of Aflatoxin B1 (AFB1), Trichothecene T2/HT2, and Toxin Binders on Survival and Feed Conversion Rate (FCR) Efficiency Indicators

Indicators	Groups				
	Control 1	Control 2	Test 1	Test 2	Test 3
Feed Conversion Rate (FCR)	1.3±0.23	1.1±0.20	1±0.17*	0.92±0.15	0.9±0.18 *
Survival %	87±1.23	92±1.20	95±1.19*	97±1.16	97±1.18

*Mean values without superscript are significantly different ($p<.05$).

These findings indicate that the test groups with Georgian bentonite clay (0.15 and 0.2%) demonstrated better feed utilization efficiency and higher survival rates compared to the control groups, reinforcing the positive impact of toxin binders in aquaculture.

Mycotoxin adsorption efficiency, fish health status, organoleptic and physical indicators, and comparison of the results and efficiency of trout production.

An analysis of the fish feces from both the test and control groups was conducted. The fish feces from the research groups were analyzed for the content of mycotoxins (trichothecene T2/HT2 and aflatoxin B1). The study revealed significant binding of these mycotoxins in the T2 and T3 test groups, as confirmed by the amounts of aflatoxin B1 and trichothecene T2/HT2 detected in the feces. The highest values were observed in the group T2 (B1 = 120.5 ppb / T2/HT2 = 66.3 ppb) (Table 6) and the group T3 (B1 = 130 ppb / T2/HT2 = 74.3 ppb) ($p<.05$), followed by the group T1 (B1 = 110.6 ppb / T2/HT2 = 53.3 ppb), and the control group C2 (B1 = 98.5 ppb /T2/HT2 = 37.8 ppb) (Table 6).

Table 6.
The Amount of Aflatoxin B1 and Trichothecene T2/HT2 Adsorbed Levels (binding) Detected in Fish Feces (ppb)

Groups	Aflatoxin B1 (ppb)	Trichothecenes T2/HT2 (ppb)
Control 1 (Without Adsorbent)	0	0
Control 2	98.5±1.58*	37.8±0.88
Test 1 (0.1% Bentonite)	110.6±0.93	53.3±1.99
Test 2 (0.15% Bentonite)	120.5±1.22	66.3±2.00
Test 3 (0.2% Bentonite)	130±1.75	74.3±1.88

*Mean values without superscript are significant different ($p<.05$).

In the test groups T2 with 0.15% and T3 group 0.2% Georgian bentonite clay, the adsorption rate of aflatoxin B1 reached 83-90%, while the adsorption efficiency for T2/HT2 mycotoxins was lower, at only 12.5-14%. In the T1 group, the adsorption rate for B1 was 76.4%, with T2/HT2 adsorption remaining low at 10%. The group C2 exhibited significantly lower adsorption rates ($p<.05$), with B1 at 68.1% and T2/HT2 at 7.1% (Table 7).

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

Groups	Aflatoxin B1 Adsorption Rate (%)	Trichothecene T2/HT2 Adsorption Rate (%)
Control 1 (Without Adsorbent)	No adsorption	No adsorption
Control 2	68.1±1.22	7.1±2.18*
Test 1 (0.1% Bentonite)	76.4±2.10	10±2.00
Test 2 (0.15% Bentonite)	83±1.08	12.5±0.99
Test 3(0.2% Bentonite)	90±2.03	14±1.11

*Mean values without superscript are significantly different ($p<.05$).

General and biochemical analyses of blood showed that the levels of hemoglobin and erythrocytes in all test groups were higher compared to the control group ($p<.05$), with hemoglobin levels increasing by 10-26% and erythrocyte counts by 6-12%. In the T3 test group (with a 0.2% additive), the increases were particularly notable (Table 8). The total protein content in the blood serum was below the physiological norm in the control group at 34 g/l. In contrast, the experimental groups showed a 38-47% higher protein content ($p<.05$), ranging from 47 to 50 g/l (Table 9). Thus, the use of Georgian bentonite clay as a mycotoxin adsorbent in trout feed positively affected both general and biochemical blood indicators.

Table 8.
Effects of Aflatoxin B1 (AFB1), Trichothecenes T2/HT2, and Toxin Binders on Hematological Values (%) in Fish.

Groups	Hemoglobin g/l	Erythrocytes (10^{12})	Hematocrit (%)	Platelets 1000 mcg/L	Lymphocytes (%)	Eosinophils (%)
Control 1	60±5.86	1.0±0.1	18±2.29	147±9.70	62±0.01	1.0±0.03
Control 2	77±6.67	1.6±0.18*	26±3.02	170±10.30	78±0.05	5.0±0.05
Test 1 (0.1% Bentonite)	80±5.50	1.7±0.30	28±2.03	180±15.09	77±0.05	2.0±0.11
Test 2 (0.15% Bentonite)	85±6.01	1.7±0.17	27±1.99	190±17.08	76±0.15*	5.0±0.13
Test 3 (0.2% Bentonite)	97±7.10	2.0±0.49	30±3.32	200±16.99	75±0.20	4.0±0.10

*Mean values without superscript are significantly different ($p<.05$).

Table 9.
Effects of Aflatoxin B1 (AFB1), Trichothecenes T2/HT2, and Toxin Binders on Blood Metabolites of Fish.

Groups	Alanine Aminotransferase U/l	Aspartate Aminotransferase U/l	Total protein g/l	Albumin g/l	Creatinine mg/dl	Glucose mmol/l
Control 1	20±3.35	155±11.2	23±0.25	7±0.03	0.32±0.01	2,2±0.11
Control 2	29±3.90	180±14.00	34±0.13	12±0.04	0.50±0.02	3±0.17
Test 1 (0.1% Bentonite)	32±4.10*	189±12.14	40±0.10	13±0.07	0.52±0.01	3.2±0.20
Test 2 (0.15% Bentonite)	34±5.12	193±15.09	47±0.16	14±0.04 *	0.53±0.04	3.3±0.12
Test 3 (0.2% Bentonite)	34±4.33	198±17.90	50±0.19	16±0.06	0.57±0.03	3.5±0.18

*Mean values without superscript are significantly different (p<.05).

As for the results of water quality parameters obtained during the experimental period, no significant difference (p>.05) was observed in dissolved oxygen, temperature, pH, and ammonia-nitrogen. Dissolved oxygen ranged from 6.2±1 mg/l –6.46±0.71 mg/l, pH ranged from 6.6±0.99 –7±0.68, temperature ranged from 13±0.79°C –16±0.71°C and ammonia-nitrogen ranged 0.09±0.03mg/l –0.01±0.18 mg/l.

Regarding the chemical composition of the fish meat, protein content was highest in the T2 and T3 groups (p<.05) at 18.3-18.5%, which was 1.1-1.3% higher (p<.05) than the C2 group. The fat content was highest in T2 (p<.05) compared to the lower in the C2 (protein (16.5%) and fat content (6%) was lowest in C1 (Table 10). These differences were statistically significant (p<.05), indicating that the inclusion of Georgian bentonite clay improved both protein and fat content in trout meat (Table 10).

There is no direct indication in the study of toxicological reactions or harmful effects caused by the addition of bentonite. In fact, the results suggest that bentonite has beneficial effects on the growth and health of the trout. No signs of toxicity or adverse reactions were reported in terms of fish blood composition or meat quality. On the contrary, the biochemical analyses revealed improved protein content in the fish blood, which was higher in the test groups than in the control group (Table 9). Furthermore, meat quality, including texture, flavor, and fat content, was also enhanced in the test groups. Thus, based on the results, the addition of bentonite in the diet of trout appears to be safe and effective, with no observed toxicological reactions, and it provides a positive impact on fish health and growth. Organoleptic and physical indicators of the meat were assessed through tasting. All tasting parameters (aroma, flavor, tenderness, and overall impression) were notably superior in trout from the T2 and T3 test groups, both in boiled and fried conditions (Table 11).

Table 10.
Proximate Carcass Composition of Experimental Fish

Groups	Protein Content (%)	Fat Content (%)	Moisture (%)	Ash (%)
Control 1	16±0.32	6±0.18	72.0±0.16	1.0±0.80
Control 2	17.2±018*	6.8±0.07	71.1±0.12	1.2±0.18
Test 1 (0.1% Bentonite)	17.8±0.06	7.1±0.13*	71.6±0.03	1.1±0.12
Test 2(0.15% Bentonite)	18.3±0.18	7.8±0.06	71.0±0.05*	1.3±0.10
Test 3 (0.2% Bentonite)	18.5±0.19	7.5±0.20	71.5±0.17	1.5±0.22

*Mean values without superscript are significantly different (p<.05).

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
Control 1	3.4	3.3	3.5	3.4	3.4
Control 2	3.7	3.6	3.8	3.9	3.8
Test 1 (0.1% Bentonite)	4	3.8	3.9	4.1	4
Test 2 (0.15% Bentonite)	4.7	4.5	4.8	4.7	4.7
Test 3 (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.

Discussion

The aim of this research was to evaluate the effectiveness of Georgian bentonite clay as a natural mycotoxin adsorbent in aquaculture. Previous studies have demonstrated the positive impact of Georgian bentonite clay on mycotoxin adsorption, primarily in poultry feed (Biomin, 2020; Chkuaseli & Khutsishvili-Maisuradze, 2016). The study demonstrates that the inclusion of Georgian bentonite clay at 0.15% to 0.2% in trout feed significantly improves fish growth parameters: The average weight gain and feed conversion ratio (FCR) show a positive correlation with the bentonite clay concentration, indicating that it helps optimize feed utilization. Enhancements in the growth rates of the fish were also observed Abdellaoui et al. (2019) and Enyidi et al. (2020).

The increased growth rates observed in the test groups can be attributed to better nutrient adsorption and improved feed efficiency facilitated by the mycotoxin-binding properties of the bentonite clay (Gruber-Dorninger et al., 2020). The reduced FCR in the test groups further supports the conclusion that the fish are utilizing the feed more effectively, likely due to the reduced toxic impact of mycotoxins on metabolism and digestion. Mycotoxins are known to cause immune system suppression, reduced disease resistance, and poor growth in aquaculture species (Gruber-Dorninger et al., 2020).

Therefore, the ability of Georgian bentonite clay to mitigate the harmful effects of these toxins may explain the improved health outcomes and survival rates seen in the test groups. In addition, the clay's ability to detoxify mycotoxins could result in lower mortality rates and fewer incidences of disease, further enhancing fish welfare. A key highlight of the study is the mycotoxin-binding efficiency of Georgian bentonite clay.

The results show that it was especially effective at adsorbing aflatoxin B1, with a binding rate ranging from 83% to 90% in the test groups. This result is in agreement with that from Oliveira et al. (2023) and Kihal et al. (2022). Aflatoxins are some of the most harmful mycotoxins, and their presence in fish feed can lead to liver damage, immunosuppression, and growth impairment (Oliveira et al., 2023). By reducing the exposure to aflatoxins, bentonite clay can have a significant protective effect on fish health and feed safety. However, the adsorption efficiency for T2/HT2 mycotoxins was lower, which could suggest that different mycotoxin structures or properties influence the efficiency of bentonite clay in binding them. Despite this, the clay still offered a modest improvement in mycotoxin adsorption, with 12.5%–14% binding efficiency. Bentonite's positive impact on African catfish feed was similarly observed (Enyidi et al., 2020).

The study also evaluated the impact of Georgian bentonite clay on the meat quality and sensory properties of the fish. The test groups fed with bentonite showed significant improvements in chemical composition and taste properties, which were particularly beneficial for the consumer experience and market value of the fish. Meat quality factors like protein content, fat composition, and overall palatability were positively influenced by the addition of bentonite, potentially due to better overall fish health and improved metabolic processes. The enhancement of taste properties suggests that reducing the negative effects of mycotoxins on fish could lead to better quality products for the aquaculture market, where flavor, texture, and nutrient content are noted key factors in consumer choice (Oliveira et al., 2020).

This research points to several important implications for the aquaculture industry. Georgian bentonite clay could be a cost-effective solution to the growing problem of mycotoxin contamination in fish feed, which can affect both fish health and product quality. By incorporating bentonite as an additive in aquafeeds, farmers could:

1. Enhance fish growth and feed conversion while reducing the environmental and economic impacts of poor feed efficiency.

2. Improve fish health by reducing the toxic effects of mycotoxins, leading to higher survival rates and better resilience to diseases. (It has been shown that bentonite possesses detoxifying properties and can help improve immune responses and reduce disease susceptibility in aquaculture, as described by Kolawole et al. (2019).

3. Produce higher-quality fish products with improved taste and chemical composition, making them more appealing to consumers.

In terms of cost-benefit analysis, the use of Georgian bentonite clay could potentially offer long-term savings by reducing the need for expensive mycotoxin remediation technologies, improving feed utilization and lowering disease treatment costs, well-documented by Mendes dos Reis et al. (2024). The potential for cost-effective mycotoxin adsorbents to lower operational costs has also been discussed by Kolawole et al. (2019).

This finding suggests that while Georgian bentonite clay is highly effective against certain types of mycotoxins (like aflatoxins), further optimization may be required to enhance its binding capacity for other mycotoxins like T2/HT2. Additionally, it may be worthwhile to explore combinations of bentonite with other adsorbents to improve the overall mycotoxin-binding spectrum. Kihal et al. (2022) and Kolawole et al. (2019), mentioned similar challenges with the efficiency of bentonite in adsorbing certain types of mycotoxins, particularly T2/HT2.

Further studies could also evaluate the economic feasibility of using bentonite in large-scale aquaculture operations, considering factors such as costs, availability, and supply chains.

Conclusion and Recommendations

The preliminary and primary experiments on trout indicate that the application of Georgian bentonite clay, a locally produced aluminosilicate bentonite clay, is highly effective for mycotoxin detoxification in trout feed. The addition of Georgian bentonite clay at 0.15-0.2% achieved an aflatoxin B1 adsorption rate of 83-90% and a T2/HT2 mycotoxin adsorption rate of 12.5-14%.

The results of our experiments confirm our hypothesis regarding the efficacy of Georgian bentonite clay for detoxifying certain mycotoxins in fish feed. This suggests that Georgian bentonite clay can be successfully utilized for mycotoxin detoxification in aquaculture.

Based on the results of our research involving Georgian bentonite clay in trout feed, we can conclude that:

1. Locally produced bentonite clay of aluminosilicate origin can effectively detoxify mycotoxins in trout extruded feed when used at proportions of 0.15-0.2%.

2. There will be an increase in the positive dynamics of trout body mass, including both absolute and daily growth +30-35%.

3. Trout feed utilization and conversion efficiency will improve +16-18%.

4. Survival rate will increase +4-5%.

5. There is a possibility of producing a safer final product (fish meat).

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