

Using new adsorbent Georgian bentonite clay “Askangel” in trout feed

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World Journal of Advanced Research and Reviews, 2024, 24(02), 2710–2716

Publication history: Received on 21 September 2024; revised on 22 November 2024; accepted on 25 November 2024

Article DOI: <https://doi.org/10.30574/wjarr.2024.24.2.3315>

Abstract

The purpose of this research was to study the effectiveness of bentonite clay of aluminosilicate origin (Askangel) from Georgia as a natural adsorbent of mycotoxins in aquaculture, specifically in trout feeding. The study was conducted on rainbow trout divided into three test groups, each receiving different percentages of Askangel (0.1%, 0.15%, 0.2%) in their feed. A synthetic adsorbent was added at 0.1% to the control group's feed.

At the end of the experiment, the weight gain for fish was as follows: 205±0,62 g in the IV test group and 203±0,72 g in the III test group, 189±0,85 g in the II test group, and 175 ±0,83 g in the control group. Fish survival rates were 97% in the III and IV test groups, 95% in the II test group, and 92% in the control group. The feed conversion ratio (FCR) was 0.9-0.92 in the III and IV test groups, 1 in the II test group, and 1.1 in the control group.

The adsorption rate of aflatoxin B1 reached 83-90% in the III and IV test groups, while T2/HT2 mycotoxin adsorption was 12.5-14%. In the II test group, the adsorption rate for B1 was 76.4%, and for T2/HT2 it was 10%. In the control group, the adsorption rates were 68.1% for B1 and 7.1% for T2/HT2. The results indicate that Askangel serves as an effective mycotoxin adsorbent, positively influencing the growth, health, and chemical composition of trout meat

Keywords: Adsorbent; Bentonite; Mycotoxin binders; Mycotoxins; Nutrition

1. 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 [1,2]. Over the past 50 years, fish production has doubled worldwide, rising from 9 kilograms per capita to 21 kilograms, and is projected to reach 30 kilograms per capita in the next decade [3,4]. However, approximately 60-65% of fish farming costs stem from feeding, with Georgian fish farmers relying on expensive imported fish feed [5].

Today, fish feed enterprises face the challenge of producing high-quality, complete 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 [6,16,19,20].

Mycotoxins pose significant risks to farm animals, poultry, and fish due to their pronounced toxicity [7,17,22,23]. 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 [8, 21, 15, 13]. Consequently, fish feed companies must find affordable

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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 in the diets of agricultural animals and poultry globally [9,14,25,26]. These clays can adsorb mycotoxins, preventing their absorption into the bloodstream and facilitating their excretion [11,12,21,24]. 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 Askangel in the village of Askana, Ozurgeti district (west Georgia).

Although the use of Askangel in poultry feed for mycotoxin adsorption has been studied [10,26] its application in aquaculture remains unexplored. This recent research has focused evaluate the efficacy of locally produced bentonite clay (Askangel) for detoxifying mycotoxins at a comparatively low price.

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 taste properties 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.

2. Materials and methods

Bentonite clay from Askana was collected for analysis at the Alexander Tvalchrelidze Institute of Mineral Resources of the Caucasus, employing chemical research methods. X-ray phase, silicate, and physical-chemical studies were conducted on the Askangel bentonite clay at the laboratories of the Agricultural University of Georgia, Tbilisi State University, and the Institute of Mineral Materials of the Caucasus. Based on the test results, the montmorillonite structure and formula were established. An X-ray phase test was performed using the DPOH-1.5 device.

The raw materials and complete feed were tested for contamination with mycotoxins (Aflatoxin B1 and Trichothecenes T2/HT2 toxins). In the zootechnical laboratory of "Chirina," a complete feed producer equipped with modern equipment, the determination of mycotoxins in feed and fish manure was conducted in the accredited veterinary laboratory of "Chirina" Ltd using express methods on the "Aokin" fluorescence polarimeter.

For the test and control groups, diets were formulated using the "Bestmix" program in collaboration with consultants from Dutch and Turkish experts. Rainbow trout (*Salmonidae* subspecies "rainbow trout") were purchased (400 fish), and test ponds were allocated at an aquaculture farm in Shida Kartli, operated by an individual entrepreneur from "Gori."

The test groups of fish were provided with complete feed contaminated with mycotoxins (Aflatoxin and T2 toxin). The control group received feed with 0.1% synthetic mycotoxin adsorbent. The II experimental group contained feed with 0.1% Askangel bentonite clay of local origin as the adsorbent. Experimental groups III and IV were provided with 0.15% and 0.2% Askangel bentonite clay, respectively, as mycotoxin adsorbents. One goal of the physiological experiment was to collect rainbow trout manure at 6-7 months of age (marketable weight: 250-300 grams) to detect mycotoxin uptake by Askangel bentonite clay. The technological parameters of fish growth during the trial were identical for all groups and met the standardized requirements for breeding. The trout were fed in phases, adhering to nutritional norms. All four groups of fish were housed in separate sections of the pond, with 100 individuals in each.

During the trial period, the storage technological parameters were consistent across all groups and complied with the requirements for rainbow trout rearing. The trout were fed with 4 mm complete combined feed. We studied the following main indicators during the trial: live mass dynamics of trout at 3, 4, 5, and 6 months through individual fish weighing; absolute weight gain during the growing period; daily weight gain; maintenance during the growing period (%); and feed consumption per kg of fish weight gain.

Table 1 The physiological test was performed according to the following scheme

Nº	Group	Fish (Trout)	Quantity	Quantity "Askangel" (%)	of	Quantity of the Added Adsorbent (%)	Notes
I	Control	100		-		0,1	Synthetic adsorbent
II	Test	100		0,1		-	Aluminosilicate
III	Test	100		0,15		-	Aluminosilicate
IV	Test	100		0,2		-	Aluminosilicate

I-Control: 99,9 % major combined feed with 0,1% imported aluminosilicate adsorbent; II-Testing: 99,9 % major combined feed with 0,1% local bentonite adsorbent (Askangel); III-Testing: 99,85% major combined feed with 0,15% local bentonite adsorbent (Askangel); IV-Testing: 99,8 % major combined feed with 0,2% local bentonite adsorbent (Askangel).

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, acidity meter, and mineralization meter). Water parameters, trout health, and productivity were evaluated using zootechnical methods. Product (meat) quality was analyzed in the bio-organic laboratory of the Agrarian University (biochemical analyses of meat and manure). Blood counts of the fish were studied in the laboratory of the "New Veterinary Clinic." Laboratory equipment used for the research included enzyme-linked immunosorbent assay (ELISA) kits for mycotoxins (Aflatoxin and T2), a chemical analyzer, near-infrared spectroscopy, and a drying cabinet for determining protein, fat, carbohydrates, and ash in finished products, along with other technical equipment.

Chemical parameters of fish meat (moisture, protein, fat, ash, etc.) were studied in the "Etelon" laboratory in Tbilisi, and meat tasting was conducted to evaluate texture and taste. Experimental material obtained during all trials was published in specialized journals and statistically processed using the ANOVA method. Special tables were used to present the quantitative and qualitative data collected during the trial period. The advantage of these research methods lies in their established application in both international science and research, as well as the author's prior experience in the field of animal husbandry and poultry.

The physiological research encompasses two periods: a 1-month preparation phase and a 6-month observation phase (the preparatory period will conclude at the end of the first year of the project, and the observation will take place in the first part of the second year). During the physiological research, fish in all four groups will be weighed, livability rates will be recorded, and disease signs will be monitored. The results obtained in the experimental groups will be compared with the data from the control group.

3. Results

3.1. Control Findings Before the Field Study

Based on the obtained results, it was determined that Askangel belongs to the group of highly colloidal, high-performance bentonite clays. Its adsorption and exchange capacities indicate that it is a high-quality adsorbent. The study revealed that montmorillonite in Askangel is semicrystalline, with quartz and mica present in trace amounts. It is classified as a calcium-sodium mixed montmorillonite. Askangel bentonite clay exhibits high adsorption capacity and exchange rates, making it a valuable mycotoxin adsorbent.

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, and 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 are close to the maximum limit, whereas the content of aflatoxin and trichothecenes in the raw materials did not exceed the maximum allowable limits. However, it is important to note that these mycotoxins can accumulate in the fish's body over time. The typical farming cycle for raising trout can range from 7 months (for marketable fish) to 1.5 years (for breeding stock), which increases the risk of mycotoxin accumulation.

3.2. Field Study Findings

Fish productivity (weight) was assessed, and the live weight of the control group (I) and experimental groups (II, III, and IV) of trout at the beginning of the experiment was similar, ranging from 17 to 20 g. This indicated a high homogeneity of fish within the groups. After 6 months of growth, the results showed that trout in the III (0.15% additive) and IV (0.2% additive) test groups exhibited the best growth intensity. The trout in the II group (0.1% additive) demonstrated higher productivity at all growth stages compared to the control group, but it was still lower than that of the III and IV groups.

At 3 months of age, trout in the III and IV test groups had the highest live weight, ranging from 110 to 115 g, which is 12.5-14% more than that of the control group. The live weight of the trout in the II test group (0.1% additive) was 4-9 g lower than that of the III and IV groups but 7-8% higher than that of the control group. Similar trends were observed at 4 and 5 months of age.

In the trout farm where the test was conducted, trout are typically marketed at 6-7 months of age (live weight: 220-250 g). Therefore, we recorded the productivity indicators of the test fish at the end of the 6-month growing period (December-May). During this period, trout in the III and IV test groups showed an absolute weight increase, with weights of 220-225 g, while the control group had the lowest weight at 193 g. The average weight in the control group was 208 g.

Regarding average daily gain, trout in the III and IV test groups had the highest values at 1.13-1.14 g, while the control group had the lowest at 0.97 g. The II test group recorded an average daily gain of 1.05 g. These results indicate that the inclusion of 0.15% and 0.2% Askangel had a comparable positive effect on growth intensity, yielding relatively higher results than the inclusion of 0.1%.

3.2.1. Livability and Feed Conversion Rate Efficiency Findings

The assessment of livability showed that the retention rate of trout during the entire rearing period was 97% in the experimental groups III and IV. The lowest retention was observed in the control group at 92%, while experimental group II had a retention rate of 95%.

The study of feed consumption efficiency (conversion) indicated that the lowest feed conversion rate was recorded in the III and IV test groups, at 0.9–0.92 kg of feed per kg of weight gain, representing the best indicator. In contrast, the control group had the highest feed conversion rate of 1.1 kg, while the II test group recorded a rate of 1 kg of feed for 1 kg of weight gain.

An analysis of the fish feces from both the study and control groups was conducted.

3.3. Mycotoxin Adsorption Efficiency

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 III and IV test groups, as confirmed by the amounts of aflatoxin B1 and trichothecene T2/HT2 detected in the feces. The highest values were observed in group III (B1 = 120.5 ppb / T2/HT2 = 66.3 ppb) and group IV (B1 = 130 ppb / T2/HT2 = 74.3 ppb), followed by group II (B1 = 110.6 ppb / T2/HT2 = 53.3 ppb), and the control group (B1 = 98.5 ppb / T2/HT2 = 37.8 ppb).

In the test groups with 0.15% and 0.2% bentonite clay (Askangel), 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 II test group, the adsorption rate for B1 was 76.4%, with T2/HT2 adsorption remaining low at 10%. The control group exhibited significantly lower adsorption rates, with B1 at 68.1% and T2/HT2 at 7.1%.

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, with hemoglobin levels increasing by 10-26% and erythrocyte counts by 6-12%. In the IV test group (with a 0.2% additive), the increases were particularly notable. The total protein content in the blood serum, which is crucial for carbohydrate and fat metabolism, 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, ranging from 47 to 50 g/l. Thus, the use of Askangel as a mycotoxin adsorbent in trout feed positively affected both general and biochemical blood indicators.

We also evaluated the chemical composition and taste properties of the trout meat. The protein content was highest in the III and IV test groups, ranging from 18.3% to 18.5%, which is 0.15-0.2% higher than in the control group. The fat content, both in natural and air-dried meat, was highest at 7.8% in group III and lowest at 6.8% in the control group.

Organoleptic and physical indicators of the meat were assessed through tasting. All tasting parameters (aroma, taste, tenderness) were notably superior in trout from the III and IV test groups, both in boiled and fried conditions.

4. Discussion

The challenge of producing animal protein at lower costs involves the use of inexpensive raw materials that are often contaminated with mycotoxins and metabolic byproducts from molds. To address this issue, we explored the potential of aluminosilicate Georgian bentonite clay as a natural remedy to mitigate the negative effects of mycotoxins.

Fish feed companies in Georgia are currently facing the challenge of finding new, relatively inexpensive means to combat mycotoxins. This is essential to ensure that the final product (fish feed) is safe, of high quality, and competitively priced against imported fish feeds.

Previous studies have demonstrated the positive impact of Georgian bentonite clay (Askangel) on mycotoxin adsorption, primarily in poultry feed. The aim of this research was to evaluate the effectiveness of Askangel as a natural mycotoxin adsorbent in aquaculture, specifically in trout feeding, and the results have been promising.

Table 2 Analysis of Trial Results Production Indicators

Indicators	unit	Groups №			
		I (control)	II (test)	III (test)	IV (test)
Body Mass -1st Month	g	18±0,30	19±0,63	17±0,43	20±0,44
Body Mass – 3th Month	g	97±0,40	106±0,51	110±0,52	115±0,39
Body Mass – 4th Month	g	121±0,65	132±0,69	138±0,41	142±0,65
Body Mass - 5th Month	g	158±0,80	171±0,84	181±0,68	187±0,71
Body Mass – 6th Month	g	193±0,79	208±0,89	220±0,73	225±0,80
Absolute Weight Gain (0-6 month)	g	175 ±0,83	189±0,85	203±0,72	205±0,62
Daily Weight Gain (0-6 month)	g	0,97	1,05	1,13	1,14
Livability (0-6 month)	%	92	95	97	97
Feed conversion rate (FCR)		1,1	1	0,92	0,9

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

- Locally produced bentonite clay of aluminosilicate origin (Askangel) can effectively detoxify mycotoxins in trout extruded feed when used at proportions of 0.15-0.2%.
- The detrimental effects of contaminated fish feed will be reduced.
- There will be an increase in the positive dynamics of trout body mass, including both absolute and daily growth.
- Trout feed utilization and conversion efficiency will improve.
- This approach will minimize the outflow of currency spent on imported adsorbents.

5. Conclusion

The preliminary and primary experiments on trout indicate that the application of Askangel, a locally produced aluminosilicate bentonite clay, is highly effective for mycotoxin detoxification in trout feed. The addition of Askangel 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%.

Overall, the use of Askangel enhances trout production results and physiological condition, improves feed absorption in the gastrointestinal tract, reduces toxicity, and lowers feed costs. Furthermore, it minimizes the risks associated with contaminated fish feed, increases meat production, and importantly, enhances the safety of trout meat for consumers.

The results of our experiments confirm our hypothesis regarding the efficacy of Askangel for detoxifying certain mycotoxins in fish feed. This suggests that Askangel can be successfully utilized for mycotoxin detoxification in aquaculture. Future research could explore its effectiveness in other fish species, such as common carp, sturgeon, catfish, sea bass, and sea bream. We believe that these findings will contribute to the sustainable development of the aquaculture industry.

Compliance with ethical standards

Acknowledgments

We express our deep gratitude to the Agricultural University of Georgia and the Shota Rustaveli National Science Foundation for their financial support in implementing project YS-22-2184.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Availability of Data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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