

Short Communication

# Effect of Dietary Sodium Bentonite Supplement on Microbial Contamination of Mink Feed

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## Abstract

Our research material involved *Mustela neovison* mink feed collected at a farm during two feeding seasons: in preparation for animal reproduction and winter fur coat development. The feed was divided into 3 treatment groups – the control with no bentonite supplement and 2 others with dietary sodium bentonite additive at doses of 1% and 1.5% of weighed feed portion. The material was studied for total count of aerobic mesophilic bacteria, total fungal numbers, hemolyzing *streptococci*, and *Salmonella* rods. The analyzed material did not show the presence of hemolyzing *streptococci* or *Salmonella* rods that evidences good sanitary condition of feed. The highest percentage of microbial reduction was determined in the feed samples obtained from the period of animal preparation for reproduction.

**Keywords:** mink, feed, bentonite, microbial contamination

## Introduction

Poland recently has seen steady development of mink farms where animals are fed animal-origin by-products that are unfit for use in other sectors of agricultural production. Most commonly, these are animal waste – post-slaughter by-products that spoil easily at storage or feed preparation period. Then products are exposed to an infection with the abundant saprophytic bacteria and opportunistic pathogens, i.e. haemolytic *streptococci*, coli group bacteria, faecal *streptococci*, moulds, and anascogenic yeasts. Importantly, the occurrence of pathogenic flora favors the putrifaction of feedstuff ingredients. Consequently, an inadequate hygiene control system at feedstuff handling practices directly affects animal reproduction efficiency [1-3]. Therefore, to eliminate harmful factors and protect animal feedstuff, antioxidants are included in the diet. However, the measures introduced often prove ineffective and do not solve the problems related to the microbiological hazard that undoubtedly exists in such objects.

The studies conducted in the carnivorous fur animal farms have confirmed the presence of high microbial load in this environment [3]. However, the situation reported in the objects may be improved through administration of aluminosilicates – sodium bentonite in the feed. Bentonite is widely known for its unique sorptive properties. It binds toxins through minerals by ion electrostatic adsorption occurring on the surface as well as inside the material structure. Binding impurities, bentonite inhibits their intestinal absorption but passes through the digestive tract to be voided in the feces. Bentonite feed additive is primarily valued for its excellent sorptive properties combined with high cation exchange rate [4-6]. There is no work in available literature on the use of aluminosilicates in carnivorous fur-bearing animals feeding. Therefore, the present research objective was to determine the impact of dietary sodium bentonite supplement for minks on a feed microbial contamination level. Statistical analysis was performed using Tukey's test ( $p < 0.05$ ). Mean with the same letter do not differ significantly.

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Table 1. Mean values of mink feed analysis in the winter fur coat development period.

Treatment group	Total count of aerobic mesophilic bacteria in 1 g mink feed	Total fungal count in 1 g mink feed	Hemolyzing <i>streptococci</i> in 0.1 g mink feed	<i>Salmonella</i> in 20 g mink feed
Group A	9.1×10 <sup>5</sup> CFU/g A	4.7×10 <sup>4</sup> CFU/g A	does not appear	does not appear
Group B	11×10 <sup>5</sup> CFU/g A	3.1×10 <sup>4</sup> CFU/g A	does not appear	does not appear
Group C	10.9×10 <sup>5</sup> CFU/g A	5.7×10 <sup>3</sup> CFU/g A	does not appear	does not appear

A – control group without the addition of bentonite

B – experimental group with 1.0% addition of bentonite to daily dose of feed

C – experimental group with the addition of bentonite to 1.5% daily dose of feed

Mean values marked with the same letters (A, A) did not differ significantly

## Experimental Procedures

The study material included *Mustela neovison* mink feed collected at a farm into sterile containers during two feeding seasons, i.e. at the reproduction preparation time with a low-calorie and high-energy diet, when EM (metabolic energy) is available mainly from protein, and at the time of winter fur coat development with a high protein level and EM (metabolic energy) from carbohydrates. Sodium pyrosulfate at 0.2-0.3% ready feed mass and Rendox antioxidant at 300g/t ready feed were used as preservatives. The samples were immediately cooled to 4°C temperature using a refrigerator car and transported to the laboratory. Then the feed was divided into 3 study groups: A – control group with no bentonite additive, B and C made treatment groups. The B group diet was supplemented with sodium bentonite at 1% feed weighed portion, whereas that of C group had 1.5% bentonite additive. The test weight of mink feed was 100 grams. The prepared weighed portions were put aside for an hour, then homogenized, diluted, and inoculated in compliance to the norm PN-R-64791:1994 [7]. The studied material was examined for total aerobic mesophilic bacteria count, total fungal numbers, hemolyzing *streptococci*, and *Salmonella* rods. Incubations were conducted in the following conditions: mesophilic aerobic bacteria – 48 hours at 37 degrees, sticks of *Salmonella* – 16 to 20 hours at 37 degrees, hemolytic *streptococcus* – 18 to 24 hours at 37 degrees, and mushrooms for 5 to 7 days at 25°C. After incubation, grown colonies were counted, then the results substituted in the corresponding models in accordance with PN-6-64791 to determine the level of microbial contamination of the tested mink feed.

## Results

The analysis results are summarized in Tables 1-3. The first analyses included the feed collected from the period of winter fur coat formation. In group A control, the mean number of aerobic mesophilic bacteria maintained at 9.1×10<sup>5</sup> CFU/g level, while total fungal count reached 4.7×10<sup>4</sup> CFU/g, (Table 1). Compared to control, both treatment groups showed a higher content of aerobic mesophilic bacteria. In the group with 1% bentonite supplement (group B), the level was 11×10<sup>5</sup> CFU/g, whereas in that with 1.5%

bentonite additive (group C) it was 10.9×10<sup>5</sup> CFU/g feed. The feeding of the fourth period (making a winter coat), there were no statistically significant differences between groups. However, we found statistically significant differences compared to the overall number of fungi between test group C in relation to research group B and control group A.

We identified fungi from *Rhizopus oryzae*, *Penicillium* sp., *Candida famata*, and *Candida glabrata* genus in the feed. Mold fungi were identified microscopically using a key for the determination of fungi [8] and yeast-like fungi using commercial biochemical tests API 20°C AUX, bioMerieux Poland Ltd., and ApiWeb software. In group C, with the highest bentonite concentration, the microbial contamination level appeared to be the lowest and averaged 5.7×10<sup>3</sup> CFU/g. Similarly, group B, with 1% dietary bentonite additive, showed markedly lower quantity of fungi as compared to the control. The analyzed research material did not display the presence of hemolyzing *streptococci* or *Salmonella* rods that gives evidence of appropriate sanitary condition of feed (Table 1).

Further studies focused on the feed samples obtained from the preparation period for mink reproduction. Then there were exhibited only minimal reducing properties of sodium bentonite toward aerobic mesophilic bacteria (Table 2). The quantitative differences recorded between the investigated groups proved small. Group B, with 2.3×10<sup>4</sup> CFU/g, had the lowest aerobic mesophilic bacteria level compared to groups A and C (Table 2). Thus, the consecutive trials reconfirmed the reducing properties of sodium bentonite with relation to total fungal count. The best results were obtained for a feed with 1.5% bentonite level that averaged 1.1×10<sup>3</sup> CFU/g (Table 2). Whereas group B, with 1% bentonite additive, also showed lower fungal concentration compared to the control samples (9.8×10<sup>3</sup> CFU/g). During this period, statistically significant differences were found for total mesophilic bacteria, where Group B differed significantly from research group C and control group A.

Also in this research period, no presence of hemolyzing *streptococci* or *Salmonella* rods was recorded in the analyzed material, which indicates good sanitary state of the feed (Table 2) [7].

The highest percentage of microbial elimination was noted in the feed samples collected at the period of animal preparation for reproduction. In both research periods, the

Table 2. Mean values of mink feed analysis at mink preparation for reproduction period.

Treatment group	Total count of aerobic mesophilic bacteria in 1 g mink feed	Total fungal count in 1 g mink feed	Hemolyzing <i>streptococci</i> in 0.1 g mink feed	<i>Salmonella</i> in 20 g mink feed
Group A	2.2×10 <sup>4</sup> CFU/g A	9.8×10 <sup>3</sup> CFU/g A	does not appear	does not appear
Group B	2.3×10 <sup>4</sup> CFU/g B	5.0×10 <sup>3</sup> CFU/g B	does not appear	does not appear
Group C	1.0×10 <sup>5</sup> CFU/g A	1.1×10 <sup>3</sup> CFU/g A	does not appear	does not appear

A – control group without the addition of bentonite

B – experimental group with 1.0% addition of bentonite in daily dose of feed

C – experimental group with 1.5% addition of bentonite in daily dose of feed

Mean values marked with the same letters (A, A) did not differ significantly

Table 3. Percentage of microbial reduction in mink feed with bentonite in both research periods.

Treatment group	Winter fur coat development period		Preparation for reproduction period	
	Total count of aerobic mesophilic bacteria	Total fungal count	Total count of aerobic mesophilic bacteria	Total fungal count
Group B	No reduction	34.04%	89.54%	48.98%
Group C	No reduction	87.87%	54.54%	88.78%

B – experimental group with 1.0% addition of bentonite in daily dose of feed

C – experimental group with the addition of 1.5% bentonite in daily dose of feed

highest reduction level of total fungal numbers was obtained for the feed supplemented with 1.5% bentonite (Table 3).

## Discussion

Feed supplied to animals may be a source and transmission factor of microorganisms in those that are pathogenic for both humans and animals. The presence of pathogens and toxins poses the risk of their spread in the environment and, infrequently, inclusion into the food chain [1, 9]. Hence, in the search for factors preventing feed contamination at its storage or deposition time on racks or in feeders, extensive studies have been conducted [3]. Sodium bentonite seems to meet requirements. Absorbing impurities in its structure, it carries them away safely through the digestive tract. The main constituent of bentonite is a clay mineral – sodium montmorillonite. It is a member of the smectite family, layer silicates designated as a 2:1 layer mineral. The layers have strong ion-atomic bonding, with no interlayer hydrogen bond formation. Due to bentonite surface structure, it is characterized with high sorptive area, i.e. 700-800 m<sup>2</sup>/g. Importantly, the major compensation cations, Na, K, Mg, and Ca ions, have the ability to attract, among others, feed mycotoxins [6].

Besides, bentonite with urea phosphate was shown as an effective disinfectant of poultry manure by Grata et al. [9] and the strong bactericidal efficiency was reported as early as after 2 weeks of study. The studies on animals prove that bentonite is the preferred choice to aid in diarrhea treatment and can take effect right away [5, 6]. As for fatteners diet, halosit supplement was used for decontamination of bacteria, fungi, and mycotoxins [10, 11].

Mycotoxins remain a challenging problem for minks, as cereal grains are an important component of their diet. Morgan et al. [12] administered moniliformin to minks in acute and subacute trials and observed the following ultra-structural changes in the heart tissue using an electron microscope. They indicated that male minks fed a diet containing ochratoxin [4] displayed markedly decreased feed intake, whereas in the case of moniliformin, characteristic changes in the heart were detected. The female minks exposed to dietary zearalenone had increased uterine weight. Glucomannan added to the diet did not alleviate toxin effects.

Inclusion of sodium bentonite to broiler diet contaminated with aflatoxin proved effective for reducing its deleterious effects. Bentonite ameliorates adverse results of the toxin through its reduced bioavailability in the gastrointestinal tract [13]. Dobrzański et al. [14] reported considerable fungal reduction in the broilers exposed to bentonite as early as after 2 weeks, and a decline of aerobic mesophilic bacteria concentration even up to 70%. In the present research, a level of aerobic mesophilic bacteria reduction at the preparation period for animal reproduction averaged 54.54% and 89.54%, and appeared higher at 1% bentonite supplementation. A reverse relationship, yet higher than in Dobrzański et al. [14] studies, was noted in fungal reduction in both research periods.

During the winter fur coat development time, a factor reducing bacteria elimination was likely to be too rich feed. High-fat feed probably limits water availability and, in turn, bentonite hydration, that directly translates into its sorptive capacity.

Bentonite's unique sorption potential and low cost is likely to make it an effective feed additive that improves its

microbial quality and at the same time limits the penetration of microbial contaminants into the environment.

### Conclusions

1. Sodium bentonite supplementing feed promotes the reduction of total fungal count.
2. Feed fat content diminishes sodium bentonite reducing properties toward the total number of aerobic mesophilic bacteria.
3. Sodium bentonite added to mink feed reduces fungal contamination that can positively influence animal health.

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