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Original Scientific Article

THE EFFECT OF SODIUM BENTONITE SUPPLEMENTATION IN THE DIET OF MINK (*Neovison vison*) ON THE MICROBIOLOGICAL QUALITY OF FEED AND ANIMAL HEALTH PARAMETERS

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Summary: The objective of the study was to assess the effect of mink feed supplementation at weaning on the hygiene and sanitary conditions of feed and selected parameters of animal health . The experiment was carried out in two stages. In the first stage of the study, as 0.5% addition of bentonite and a 1% supplement are incorporated into the daily feed intake mink experimental groups D1 and D2, respectively. In the second step, the increase in the proportion of bentonite in the two groups is up to 1% and 1.5%. K group (control group) was not supplemented. The microbiological examinations of the chosen indices of mink feed were performed twice in each feeding period. The mink health state was monitored and evaluated via hematological analyses and lysozyme activity. The research results enable a conclusion that the application of sodium bentonite in the analyzed amounts does not negatively influence the analyzed parameters of mink blood. In fact, it may be recommended to reduce fungus numbers in feed.

Key words: mink; bentonite; feed quality; haematological parameters; lysozyme

Introduction

Montmorillonite is commonly included into an animal diet due to its adsorptive properties. Bentonite is part of this group. Some authors tend to highlight bentonite therapeutic efficacy which is similar to antibiotics (1). Breeders feed fur carnivores with animal-origin by-products from other sectors of animal production. Under such conditions, saprophytic bacteria and opportunistic pathogens can quickly develop; and their occurrence favors the putrification of feed stuff ingredients. According to the study by Kopczewski et al. (2), poor hygiene feed control directly affects animal reproductive performance, which leads to infertility, reduced litter size and even miscarriages. Feeds administered to fur animals are relatively seldom microbiologically evaluated and, importantly, animal health is unequivocally impacted by feed hygiene and sanitary practices (3,4). Hematological examinations are used to indicate animal welfare, i.e. to determine the optimal physical and psychical standard against the environmental conditions where a feeding strategy and management systems are most important (5).

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Lysozyme is one of the key components of nonspecific defensive mechanisms in azurophilic granules, specific granules, neutrophil gel granules, as well as in grains of monocytes and macrophages (6). Its activity in blood is enhanced at lower airway disorders, renal diseases, and some diseases of the cardiovascular system (7,8,9). The objective of the study was to assess the effect of mink feed supplementation at weaning on the hygiene and sanitary conditions of feed and selected parameters of animal health.

Material and methods

The study concerned pastel-type minks at weaning, caged in the farm pavilion system. All of the animals had prophylactic treatments that are appropriate for the species. The animals received the same diet in terms of composition, nutritive value and as specified by feeding standards (10). The experiment proceeded in two stages. In the first research stage, a 0.5% bentonite additive and a 1% one were added as feed additive supplement into the daily feed intake into in the D1 and D2 experimental groups, respectively. In the second stage, the feed additive level was increased in both of the groups: D1 received 1% of bentonite in a daily feed intake, whereas 1.5% of bentonite was administered in D2. Feed for Group C (control group) was not supplemented. Each group consisted of 30 animals. Sodium bentonite was analyzed in the Polish Geodesic Institute in Warsaw (11) before it was used in the study. The microbiological examinations of the chosen indices of mink feed were performed twice in each feeding period. The sample analyses were made in two replications to determine total bacterial and fungal counts in feed according to feeding standards (12,13). The mink health state was monitored and evaluated cia hematological analyses of erythrocytes, hematocrit, hemoglobin, leucocyte level and lysozyme activity. The animal blood was collected from a clipped claw after previous local anesthetic administration (ointment with lidocaine) and from the heart at mink slaughter after previous stunning. The material was collected in test tubes with EDTA K2 PROFILAB and analyzed with an automatic blood analyzer MS4 VET. Lysozyme concentration was determined using a plate with the method modified by Hankiewicz (14). The experiment was conducted with the consent of the local ethical committee (No. 40/2009). The results were analyzed statistically with statistical program Statistica10.0 (StatSoft, Poland).

Results

The results of the microbiological evaluation of mink feed (*Neovison vison*) with the sodium bentonite supplement are summarized in Table 1. Total bacterial numbers in the first research stage reached the highest level in the experimental animal feed. The average concentration of mesophilic aerobic bacteria was $2.x10^6$ cfu/g/ feed in group D1 with a dietary 0.5% sodium bentonite additive, whereas in group D2 with a 1% bentonite supplement – $2.3x10^6$ cfu/g/feed. The lowest load of mesophilic aerobic bacteria, i.e. 1.5x10 cfu/g/feed was determined in the control group (C). The statistical analysis showed no significant differences in the numbers of studied microbes ($p>0.05$) between the groups in the first research stage. Different values, however, were noted for total fungal numbers. The highest level of fungal contamination of feed was recorded in the control group (C) as its average concentration was $2.1x10^4$ cfu/g/feed (Table 1). The experimental groups D1 and D2 had a similar but lower total fungal number. The fungal number obtained in the investigated mink feed did not exceed the statistical significance threshold between the groups ($p>0.05$), (Table 1). The studies on the microbiological quality of mink feed in the second research stage are illustrated in Table 1. The examination of total mesophilic aerobic bacteria counts showed their highest number in the feed of the experimental groups, i.e. D1 and D2. The mean bacteria concentration in this period reached 2.8x106 cfu/g/ feed and $2.7x10^6$ cfu/g/feed in group D2 and group D1, respectively, whereas $1.7x10^6$ cfu/g/feed in the control group (C). The statistical analysis in the second research stage did not exhibit significant differences between the groups in total mesophilic aerobic bacteria counts either. ($p>0.05$), (Table 1).

In the second study stage, just like in the first stage, the control group feed had the highest total fungal numbers which averaged $7.4x10^4$ cfu/g, while the lowest numbers were found in group D2: on average $3.1x10^3$ cfu/g/feed (Table 1).

Table 1: Total count of bacteria and fungi in animal feed in I and II research stage (cfu/g)

Group	I research stage				II research stage			
	Total bacteria count		Total fungal count		Total bacterial count		Total fungal count	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
K	$1.5x10^6$	$4.8x10^5$	$2.1x10^4$	$1.5x10^4$	$1.7x10^6$	$9,6x10^4$	$7,4x10^3$	$6,1x10^3$
D1	$2.4x10^6$	$4.8x10^5$	$1.1x10^4$	$2.8x10^3$	$2.8x10^6$	$1,7x10^5$	$4,7x10^3$	$2,3x10^3$
D2	$2.3x10^6$	$9.3x10^5$	$1.1x10^4$	$7.0x10^3$	$2.7x10^6$	$1,6x10^6$	$3,1x10^3$	$3,1x10^3$
Analysis	H=3.43; p=0.18		H=0.86; p=0.65		H=1.14; p=0.56		H=0.96; p=0.62	

Explanation: - the arithmetic mean, SD - standard deviation, Z-Mann Whitney U test, H-Kruscala-Wallis test

Table 2: Lysozyme activity in mink blood in I and II research stage

Group	I research stage				II research stage			
	I collection		II collection		I collection		II collection	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
K	3.27	0.51	3.29	0.43	2,86	0,35	3,75	0,65
D1	4.30	0.32	3.90	0.35	2,36	0,78	4,10	0,26
D2	4.06	0.59	4.23	1.53	1,36	0,73	4,41	2,68
Analysis	H=8.19; p=0.02*		H=2.86; p=0.24		H=0.57; p=0.75		H=8.58; p=0.01*	

Designation as in Table 1

Table 3: Level of chosen morphological parameters in research stage I

Parameter	Collection	Group						Statistical analysis, p
		K		D1		D2		
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Erythrocyts [m/mm^3]	I	9.59	1.32	10.37	0.57	9.31	2.78	H=3.17 p=0.20
	II	9.65	0.71	10.42	0.48	10.01	0.56	H=6.53p=0.04*
Hematocrit [%]	I	55.07	8.17	56.94	3.23	52.08	15.08	H=1.09; p=0.58
	II	58.07	2.68	61.52	2.08	60.27	2.72	H=7.35;p=0.03*
Hemoglobin [g/dl]	I	17.64	2.63	19.86	1.53	16.82	5.17	H=6.47;p=0.04*
	II	17.53	0.85	18.56	0.77	17.84	0.85	H=7.06;p=0.03*
Leucocytes [m/m^3]	I	12.89	4.55	7.88	3.39	8.11	5.00	H=7.02 p=0.03*
	II	10.17	2.26	8.90	2.39	10.68	2.37	H=3.14 p=0.21

Designation as in Table 1

Table 4: Level of chosen morphological parameters in research stage II

Parameter	Collection	Group						Statistical analysis, p
		K		D1		D2		
			SD		SD		SD	
Erythrocyts [m/mm^3]	I	10.05	0.53	10.35	0.87	10.69	0.87	H=2.54;p=0.28
	II	9.82	0.60	9.69	1.55	10.12	0.28	H=1.54;p=0.46
Hematocrit [%]	I	57.53	2.23	58.29	4.12	59.22	5.33	H=1.07; p=0.58
	II	56.00	3.35	55.49	7.01	59.32	3.13	H=4.57; p=0.10
Hemoglobin [g/dl]	I	19.00	1.57	18.66	1.15	19.14	1.98	H=0.47; p=0.79
	II	18.80	2.21	18.94	2.20	19.14	1.62	H=0.47; p=0.79
Leucocytes [m/m^3]	I	7.60	2.97	7.68	3.45	8.71	4.10	H=0.40; p=0.81
	II	9.25	3.13	7.69	3.58	7.56	5.66	H=1.69; p=0.43

Designation as in Table 1

The examination indicated higher lysozyme activity in the blood plasma of experimental animals in both the first and second study stages. The analysis performed in the first research stage showed the increased lysozyme activity in the animals from the experimental groups D1 and D2 compared to control group C (p=0.02). The lowest lysozyme content was in control group C (3.27μg/ml), whereas it was the highest in the experimental group D2 (4.30 μg/ ml). Significant differences between the groups in the second study stage (p=0.01) were determined. The highest lysozyme activity was again observed in the animals from the D2 group (4.1μg/ml) and the lowest in control group C (2.86μg/ml). In the first collection in the second stage, the differences in this parameter level were statistically negligible (Table 2).

The hematological evaluation in the first blood collection showed significant differences in the evaluation of leucocytes (p=0.03) and hemoglobin (p=0.04), while the differences in other parameters were statistically insignificant (p>0.05). The leucocyte content in the first collection was the highest in the control group (C) (12.89 m/m3), whereas it was lower though still similar in the experimental groups (Table 3). Similarly, an increase in average WBC count in the control group (C), though statistically insignificant, was observed in the second stage. The obtained white blood cell count in all of the analyzed groups was within the reference range presented by Hunter (16) and in the upper limits given by Berestov et al. (17). One of the basic blood parameters conditioning its transportation function is hemoglobin (Hb). This parameter level is endorsed as a tool for diagnosing anemia in an organism. An Hb level in the first research stage was statistically significant in both of the analyzed blood samplings. The highest Hb level in the first stage was observed in the minks from the D1 group, while in control groups C and D2 its concentration had similar values (Table 3). The obtained hemoglobin content in the mink blood in all of the groups under study was found to slightly surpass the mean values reported by Hunter (16) and within the interval depicted by Berestov et al. (17). In the second blood collection, statistically significant differences were determined in the evaluation of erythrocytes (p=0.04), hematocrit (p=0.04), hemoglobin (p=0.03). In the first research stage, the erythrocyte level was highest in experimental group D1 (10.37 m/mm^3 in the first sampling and 10.42 m/mm^3 in the second one (Table 3). The values obtained in the second sampling were statistically significant (p=0.04). This parameter in all of the groups under investigation exceeded the values suggested by Berestov et al. (17) but fell within the upper limits presented by Hunter (1996). The hematocrit value in the second blood collection was higher in both of the experimental groups, i.e. D1, D2, compared to the control one. The statistical analysis showed that the differences were significant (p=0.04) (Table 3). In both of the analyzed samplings, the parameter was found in the upper limits given by Hunter (16).

Studying the results of the hematological assessment of mink blood, no statistically significant differences were determined in the evaluation of erythrocytes, hematocrit, and hemoglobin ($p>0.05$) in the second research stage in the first and second blood collection (Table 4). Similarly, the analysis of the white blood cells of mink blood in the second stage did not exhibit statistically significant differences between the groups ($p>0.05$).

Discussion

The research results available thus far have indicated that dietary bentonite included into a diet of animals that show varied degrees of diarrhea can cause the symptoms to regress. In the case of calf diarrhea, bentonite improves therapeutic efficacy better than antibiotics and chemotherapeuticals do. A bentonite-supplemented diet for pigs enhances growers' body conditions and increases weight gains. Dobrzański et al. (18) administered bentonite to chicken broilers' feed for 2 weeks and then observed a substantial reduction in fungal numbers and a decrease in mesophilic bacteria count, up to 70%. Grata et al. (19) studied the use of urea phosphate for disinfecting poultry liquid manure, and its strong bactericidal properties were observed as early as after 2-week studies. The examples of research on animals indicated that bentonite could improve the efficiency of treatment and relieve the symptoms of various cases of diarrhea (20, 21). In the studies by Kulok et al. (22) and Kołacz et al. (23), halloysite (aluminosilicate clay mineral) was used in fatteners' diet to decontaminate bacteria, fungi and mycotoxins in feed mixtures as well as reduce ammonia emission; the authors highlighted its high efficiency. The studies by Pasha et al. (24) also confirmed the strong adsorptive properties of this aluminosilicate towards aflatoxins. These authors indicate a beneficial effect of 0.5% of sodium bentonite used per 100 (mcg/kg) aflatoxins in feed regarding boosting the bird immune system measured by the antibody titers and phagocytosis process rate. Sodium bentonite has proven to be efficient in binding adverse aflatoxins in feed as it can prevent the "depression" of immune response by the elevation of antibody titers against hemagglutinin (HA) as well as improving feed conversion by 23.8% in birds whose diet included a sodium bentonite additive.

The obtained results of total mesophilic aerobic bacteria count in mink feed are lower than those given by Urlings et al. (25). The authors studying the possibility of using fermented poultry by-products in a mink diet determined a level of mesophilic aerobic bacteria in the range from 7 to 7.4×10^6 cfu/g. Similarly, the values reported by Powell et al. (26) for the total bacteria count exceed those obtained in this study. The authors assessed the impact of formalin as a preservation means for mink feed and established mesophilic aerobic bacteria count at the level of 7.7×10^6 cfu/g feed.

Lysozyme is known to have a bacteriolytic function and can degrade the mucin cell wall. The studies on ferrets by Wells et al. (27) showed the abundance of lysozyme in tracheal submucosal glands, which secrete proteins of antibacterial properties that in turn favor airway protection against bacterial infections. The significant increase of lysozyme activity proves the activation of innate immunity cellular mechanisms (28). In contrast, the maintenance of significantly higher lysozyme activity as compared to the initial values and the control group indicates the efficiency of phagocytic system cells as the main source of its production. Lysozyme activity grows in the presence of immunoglobulins. Interpreting the obtained research results can be challenging because there are no reference values for the lysozyme level in mink blood plasma. The vast majority of reports addressing mink blood parameters concerns studies on small populations and employs only a few parameters. The white blood cell count in each organism is frequently considered as an organism response to diverse environmental stressors (physical, chemical, biological). In carnivorous animals, leucocytosis is often recognized as soon as feed contaminated by bacteria is administered. In this research, the WBC count in the experimental groups of D1 and D2 was lower or similar, which may be attributed to the detoxificative operation of a sodium bentonite dietary additive. Grosicki and Kowalski (29), who analysed the health state of rats fed with preparations with a 2% bentonite supplement, showed no statistically significant differences in a level of erythrocytes, leucocytes, hemoglobin or hematocrit. Similar values of the hematological parameters of mink blood were reported by Douglas et al. (30), who aimed to establish a reference range. In this study, slightly

higher values for red blood cell components (RBC, Ht, Hb) were recorded. The results obtained in this research were consistent with these given by Fletch and Karstad (31). Analyzing the hematological parameters of mink blood, the authors focused on the influence of color on each blood parameter.

The divergences observed are likely to arise from a method of blood collection for analyses. An increase in erythrocyte numbers in many animal species results from spleen contraction due to animal's stress and excitement at blood sampling. In minks, however, this effect has not been studied in detail.

The research results enable a conclusion that the application of sodium bentonite in the analyzed amounts does not influence negatively the analyzed parameters of mink blood. Reducing fungus numbers may be recommended in feed as it lowers mycotoxin concentration and improves the feed's sanitary state.

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VPLIV DODATKA NATRIJEVEGA BENTONITA V HRANI KANADSKIH KUN ZLATIC (*Neovision vision*) NA MIKROBIOLOŠKO KAKOVOST KRME IN ZDRAVSTVENE PARAMETRE HRANJENIH ŽIVALI

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Povzetek: Cilj raziskave je bil oceniti učinek dodatka natrijevega bentonita v krmi kun na higieno in sanitarne lastnosti krme ter izbrane parametre zdravja živali ob njihovi odstavitvi. Poskus je bil opravljen v dveh fazah. V prvem delu je bilo v dnevno količino krme vključenih 0,5 odstotka oziroma 1 odstotek bentonita pri poskusnih skupinah kun D1 in D2. V drugem delu poskusa je bil delež bentonita v obeh skupinah povečan na 1 oziroma 1,5 odstotka. Skupina K (kontrolna skupina) v krmi ni imela dodatka. Mikrobiološke preiskave krme kun so bile opravljene po dvakrat v vsakem obdobju hranjenja. Zdravstveno stanje kun smo spremljali in ocenjevali preko hematoloških analiz in meritve aktivnosti encima lizocima. Rezultati raziskave omogočajo sklep, da uporaba natrijevega bentonita v analiziranih količinah ne vpliva negativno na analizirane parametre krvi kun. V resnici je dodatek priporočljiv za zmanjšanje števila glivic v krmi.

Ključne besede: kanadska kuna zlatica; bentonit; kakovost krme; hematološki parametri; lizocim