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TRENDS
IN LIVESTOCK
PRODUCTION

PROCEEDINGS

11th - 13th October 2017 - Belgrade, Serbia

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Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
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BENTONITE IN NUTRITION OF DAIRY CATTLE

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Original scientific paper

Abstract: There are numerous methods for decontamination of animal feed, and a relatively inexpensive method is the application of inorganic adsorbents. Adsorbents from the group of alumosilicates are particularly important, especially bentonite. Inorganic adsorbents are able to detoxify foods contaminated with mycotoxins by their adsorption power, while minimizing the negative effects on the organism. The aim of this study was to examine and determine whether the use of natural bentonite has an effect on adsorption of aflatoxins from food. The influence of daily intake of bentonite at different doses (30 and 50 g / head) on the content of aflatoxin M_1 (AFM_1) in milk of examined cows was examined. By mycotoxicological analysis of the feed it was determined that maize grains were infested with aflatoxin B_1 (1.24 μg/kg) and that this was the cause of the AFM_1 metabolite in raw milk of cows. Based on the results obtained, it was concluded that the content of AFM_1 in milk of cows consuming food with a daily intake of 30 and 50 g bentonite was significantly reduced.

Key words: dairy cows, aflatoxins, bentonite

Introduction

A significant problem in the field of intensive livestock production is the occurrence of mycotoxycoses that are directly reflected as a problem of contamination of raw milk, reduced growth, condition and preservation of health of all categories of cattle.

In some years, natural conditions (high temperatures during the summer, heavy rainfall during autumn, etc.) can be very suitable for the development and activity of the muolds. In addition to the detection of mycotoxins in food, it is also important to specify their amount (*Žust et al., 1989).* About 30-40% of all moulds create mycotoxins dangerous for humans and animals. The most common are moulds from the genera *Aspergillus, Penicillium, Fusarium, Stachybotrys* and others. Apart from the general toxicity to the cells of an organism, some mycotoxins especially have pronounced effect on the liver (hepatotoxicant), the kidneys (nephrotoxins), nerves (neurotoxins), hematopoiesis (hematotoxins), heart (cardiotoxins), or digestive organs (gastrointestinal toxins). Some have a specific cytostatic, carcinogenic, mutagenic, teratogenic, emetic, immunosuppressive, estrogenetic or photosensitive effect (*Djordjevic and Dinić 2011*). The main producers of aflatoxin are *Aspergillus* species, in particular *A. flavus, A. parasiticus, A. orizae* and others. In addition, they are synthesized by some of the *Penicillium* and *Rhizopus* species. There are several aflatoxins in animal feed: B_1, B_2, G_1, G_2, and colostrum and milk secrete their metabolites M_1 and M_2. Aflatoxins inhibit the synthesis of DNA and RNA (*Diekman and Green, 1992*). It is believed that Aflatoxin B_1 is one of the strongest known hepatic carcinogens, which causes liver cancer in animals and humans, and also has mutagenic, teratogenic, and immunosuppressive action (*Jurić and Pupavac, 1994, Jurić et al., 2003*). Biochemical blood tests show a change in the activity of individual enzymes, which is a sign of damage to the liver (*Đorđević and Dinić, 2011*). *Škrinjar et al. (2011)* state that 91% of feeds for cows are contaminated with moulds of 20 genera and 72 types of moulds. Also in the tropical region with extremely hot conditions for contamination of the fodder with aflatoxin B_1, aflatoxin M_1 occurs in milk of cows after 10 hours subsequent to the consumption of food (*Sumantri et al., 2012*). Aflatoxin M_1 as a natural metabolite of aflatoxin B_1, which occurs in the liver of animals, is excreted in the milk, feces and urine (*Polovinski-Horvatović et al., 2009-a*). EU legislation is one of the most restrictive in regard to this mycotoxin and allows the concentration of 0.05 mg/litre, while in other countries of Europe, Asia, Africa and America ten times the amount of 0.5 g/l (*Polovinski-Horvatović et al., 2009-b*) is permitted.

Bentonite is an aluminosilicate clay made of colloidal and plastic materials, predominantly of montmorillonite minerals. The toxic effect of clay has not been recorded so far, but the elimination ability of toxins is observed (*Stojiljković, 2010*). Sodium bentonite as a binding agent improves the quality of the pellets, in the pelleting of animal feed, it has a positive effect on the utilization of ammonia nitrogen in the rumen of ruminants, positive effect on the ratio of the concentration of acetates and propionates in the rumen, exerts a buffering effect in the rumen and absorbs efficiently mycotoxins present in feeds. Sodium bentonite is included in the mixture for the appropriate categories of cattle when using diets with a high proportion of the concentrate, and diets with a high protein content particularly degradable in the rumen (*Stojanović et al., 2008*). The effects of using bentonite concentrates for calves, indicate realized higher average daily gain and higher average feed intake, better conversions and a higher pH of the rumen at the age of 80 and 120 days (*Stojanović et al., 2009, Adamović et al., 2011- Adamović et al., 2011-b, Adamović, 2005*). Bentonites, as well as zeolites, significantly influence the degree of acidity of the rumen and blood parameters (*Nešić et al., 2010)*. The positive effects of the use of bentonite are also shown for the detoxification of the organism and the prevention of diarrhea of animals of all species (*Trackova et al., 2004*). Bentonite reduces the level of radioactivity in animal feed and their products (*EFSA Journal 2010*), the milk has a higher content of calcium, phosphorus and glucose, while the value of urea in the blood is lower (*Radivojević et al., 2010*). Aluminosilicate clays (Min-a-Zel) in the sugar beet pulp silage, 60 days after ensiling, increase the lactic acid production of silage, reduce the content of acetic acid and pH (*Koljajić et al., 2003*), very significantly influence all the parameters of the maize silage quality, its chemical composition and biochemical changes (*Đorđević et al., 2006*).

HSCAS (hydrated sodium calcium aluminosilicate) in diet for dairy cows reduces the concentration of aflatoxin M_1 in milk, similarly also in the nutrition of lambs (*Harvey et al., 1991*), i.e. it immobilizes mycotoxins in the gastrointestinal tract of animals (*Phillips et al., 1990*). Bentonite is a good transporter of immunomodulators and vitamin complexes (vitamins A, C, E), and a reliable mould antagonist (*Neustroy and Tarabukin, 1995*).

Bentonite can adsorb the harmful gases (ammonia and carbon dioxide) and thus improve the microclimate of livestock facilities (*Avakumović et al., 1990*). Bentonite is material often used in environmental protection and water treatment (*Ranđelović et al.2011*).

Due to the possible risk of the occurrence of increased concentrations of aflatoxins M_1 in cow's milk, the aim of this study was to determine the effect of the application of bentonite in animal feed on the production performance, the health condition and the quality of the milk of the examined cows.

Material and methods

The trial studies were carried out at the farm of Holstein-Friesian cows in Bečej, where the animals were reared in the free housing system. Within 40 days, starting from mid-January 2014, the quality of milk obtained from cows at different stages of lactation was examined. According to the AT_4 method of milk control, average milk fat content and crude protein content were examined. The average milk fat content was determined by the Gerber method (*Rulebook on methods for sampling and methods of chemical and physical analyses of milk and milk products*

- *"Official Gazette of SFRY", No. 32/83*), and the content of raw proteins was determined by the method of total combustion.

The fodder base for feeding of dairy cows throughout the year on the farm is: whole maize plant silage and triticale silage, alfalfa hay, sugar beet noodles, sugar beet pulp, maize and wheat grains, soya cake, sunflower grain, bran, retread palm fats, sodium-carbonate ($NaCO_3$) and premixture for dairy cows. Meal is prepared in the form of TMR (*total mixed ration*), twice a day, with balanced cows' needs for daily production of 35-38 kg of milk.

The random samples of four kinds of feeds (maize silage, sunflower meal, sugar beet pulp and the maize kernel) intended for the feeding of the test group of dairy cows were analysed to determine the content of aflatoxin B_1 (AFB_1), at the beginning of the trial, prior to supplementation of the diet with bentonite. Aflatoxin M_1 content (AFM_1) was determined in the milk of test dairy cows from the samples of milk from animals that consumed feed without the addition of the bentonite and milk samples from animals that have received food containing the bentonite. AFB_1 and AFM_1 analyzes were performed according to the ELISA method.

Biochemical analysis of blood, which comprised the determination of the concentration of glucose, total protein, total β-hydroxy butyric acid (BHBA), the concentration of total bilirubin, total albumin, the concentration of the urea, and the concentration of calcium and phosphorus minerals was carried out on the eight randomly selected animals from free housing system. All the parameters were analysed from blood, except for glucose derived from blood serum. The comparison of the obtained values was performed according to "The Merck Veterinary manual" (www.merckmanuals.com) based on reference values. Blood samples for analysis of the metabolic profile were taken at the beginning and at the end of the experiment, by the method of puncture the tail vein (*lat. vena coccigea*) with manual compression in vacutainer tubes. Statistical analysis of the obtained data of the analysis of blood was carried out with the program package "Statistica Statsoft V. 6, 2003" (www.statsoft.com).

Results and discussion

Table 1 shows the results of the analysis of aflatoxin B_1 (AFB_1) in feed for the examined dairy cows sampled prior to the addition of bentonite. The average level of AFB_1 in all tested feed samples did not exceed the maximum allowed limit (5 μg/kg) according to the Rulebook on the quality of animal feedingstuffs of the Republic of Serbia (*Official Gazette of the Republic of Serbia, 4/2010, and 27/2014 113/2012*). Among the examined samples of dairy cow feeds, the highest content of AFB_1 was found in maize kernel samples (1.24 μg/kg) (Table 1).

Table 1. Average content (Mean level) of AFB_1 in examined samples of feed for dairy cows

Feed for dairy cows	AFB_1 (µg/kg)
Maize silage	< 0.03
Sunflower meal	< 0.03
Sugar beet pulp	< 0.03
Mize, kernel	1.24

In the milk samples tested, the average level of aflatoxin M_1 (AFM_1) was not above the maximum allowed limit (0.05 µg/kg) according to the Rulebook on the amendment of the Rulebook on maximum permitted quantities of residues of plant protection products in food and animal feed and on food and animal feed for which maximum residue limits for plant protection products are determined (*Official Gazette, 2014*) (Table 2).

Table 2. Average content (Mean level) of AFM_1 in examined samples of milk from trial dairy cows

Date of sampling	AFM_1 (µg/kg)	The amount of daily bentonite intake (g)
06.12.2013.	0.303	0
10.01.2014.	0.390	0
07.02.2014.	0.150	30
20.02.2014.	0.126	50

Bentonite has shown the ability and efficacy of aflatoxin adsorption, but not completely, for several reasons; bentonite, although it has a 70-90% share of montmorillonite, can not bind to all mycotoxins in its crystal lattice, it is not selective for aflatoxin but also for other mycotoxins, as well as all other free radicals from the animal organism. This is similar to the results of *Harvey et al. (1991)* and *Phillips et al., (1990).* Other authors have confirmed that bentonite adsorbs many vitamins, minerals and organic molecules with a free group (*Neustroy and Tarabukin, 1995, Tomašević - Čanović et al., 2000*).

During regular monthly AT_4 milk controls, milk production, average milk fat content and average content of raw milk proteins were monitored.

Table 3. Milk performance parameters during the trial

Periods within the trial	Number of milking cows	Average milk yield (kg)	Average milk fat content (kg)	Average protein content (kg)	Daily bentonite intake (g)
I - 10 days	81	40.80	4.55	3.61	0
II - 10 days	70	41.44	4.54	3.62	0
III - 10 days	82	48.16	4.39	3.48	30
VI - 10 days	91	47.12	4.26	3.59	50

The content of bentonite slightly affected the higher milk yield and protein content, and did not have a significant effect on fat content, which decreased with the stage of cow lactation.

Table 4. Metabolic profile of cows, initial and final

Trial phase	Number of animals	Glucose	BHBA	Bilirubin	Proteins	Albumins	Urea	Calcium	Phosphorus
	Ref. values	2,2-4,2 mmol/l	0,7-1,0	0,7-8,5 µmol/l	58-81 g/l	28-40 g/l	2,0-7,5 mmol/l	2,0-3,0 mmol/l	1,4-2,7 mmol/l
Beginning	**Average**	**1,443**	**0,683**	**3,900**	**77,493**	**36,843**	**5,750**	**3,335**	**1,799***
	St. dev.	0,397	0,251	2,300	4,341	3,607	1,349	0,255	0,235
	St. error	0,141	0,089	0,813	1,535	1,275	0,477	0,090	0,083
End	**Average**	**1,339**	**0,836**	**5,486**	**77,660**	**37,205**	**4,509**	**3,255**	**2,125***
	St. dev.	0,667	0,248	1,159	4,146	2,668	1,031	0,088	0,335
	St. error	0,236	0,088	0,410	1,466	0,943	0,365	0,031	0,118

* Differences are statistically significant (P=0.04)

The values of the metabolic profile parameters were within the limits of the reference values, similarly to *Nešić et al., (2010).* Glucose was at a somewhat lower level because all selected dairy cows were in the lactation. Total β-hydroxybutyric acid (BHBA) was within normal limits. Cows at the beginning of lactation have an indicative level of β-hydroxybutyric acid, sometimes even higher than 1.20 mmol/l in blood, which is characteristic of subclinical ketosis (*Đoković et al., 2013*), and for this reason this parameter has been monitored. If the concentrations of albumin, glucose, β-hydroxybutyric acid (BHBA) and calcium are significantly below normal values, the metabolic profile can be predicted and then confirmed by incidence of fatty livers in the cows after calving (*Šamanc et al., 2011*). The concentration of bilirubin in the blood of all sampled cows was within the limits of the reference values. Proteins, albumin and urea were also within optimal limits. Values for calcium were over 3.0 mmol/l in all examined cows, the reason being that the basic roughage feed was alfalfa hay. Thus, the needs of cows in calcium were overrated. Phosphorus values were in the range of average values.

After 40 day trial and consumed amounts of bentonite, the blood of the same cows was taken again for the analysis of the metabolic blood profile (Table 4).

The glucose values remained below the average values for almost all animals, and in case of cow 8 the value was within the limits of the average values. The total β-hydroxybutyric acid (BHBA) was slightly increased in animals 1 and 5, and decreased in animal 8. The values shifted slightly towards the upper limit during the duration of the experiment, but no cows entered the state of the metabolic imbalance or ketosis. This is also confirmed by the parameters of bilirubin and urea, which kept their values in line with the reference. For all proteins and albumin, the values were optimal, except for slight transitions of animals 4 and 8 towards the upper limit. The calcium intake was too high, and phosphorus changed its values. Analysis of the variance of the obtained data (Table 4) showed that there were no statistically significant differences between the observed parameters, except for the phosphorus share in the blood. At the end of the experiment, there was significantly more phosphorus in the blood of 2.125 mmol/l ($P = 0.04$) than at the beginning 1.799 mmol/l, although the values in both cases were within physiologically normal limits.

Conclusion

- The examined food for the dairy cows in which the bentonite was added was balanced according to the cow requirements and contained all the necessary ingredients for satisfying the nutritional needs of the cows.
- The mycotoxicological analysis of feeds used in the diet for dairy cows, showed that only maize kernels were infected with AFB_1 (1.24 μg / kg) and that this was the cause of AFM_1 in raw milk of the animals from the examined dairy farm.
- It has been confirmed that daily intake of bentonite in the amount of 30 and 50 g/animal significantly reduces the content of AFM_1 in milk. Both doses of bentonite administered during the experiment had a similar AFM_1 adsorption effect.
- The effect of bentonite added to the dairy cow's diet did not show significant influence on milk parameters and on the biochemical parameters of the blood, condition and health condition of the examined dairy cows. The obtained values of all examined blood constituents were within normal physiological limits.
- Based on the conducted tests, it can be concluded that the use of bentonite, as an inorganic mycotoxin adsorbent, is one of the important and justifiable preventive measures in reducing the content of aflatoxins in the food chain.

Bentonit u ishrani muznih krava

Nenad Mićić, Goran Grubić, Milan M.Petrović, Vlada Pantelić, Bogdan Cekić, Miloš Marinković, Marina Lazarević

Rezime

Postoje brojne metode kojima se može izvršiti dekontaminacija stočne hrane, i relativno jeftin metod jeste primena neorganskih adsorbenata. Poseban značaj pripada adsorbentima iz grupe alumosilikata, u okviru njih i bentonitu. Neorganski adsorbenti su u stanju da svojom adsorpcionom moći izvrše detoksikaciju hrane kontaminirane mikotoksinima, a da pri tom negativni efekti na organizam budu što manji.
Cilj ovoga rada je bio da se ispita i utvrdi da li upotreba prirodnog bentonita ima efekat na adsorpciju aflatoksina iz hrane. Ispitivan je uticaj dnevnog unosa bentonita u različitim dozama (30 i 50 g/grlu) na sadržaj aflatoksina M_1 (AFM_1) u mleku ispitivanih krava. Mikotoksikološkom analizom hraniva utvrđeno je da je zrno kukuruza infestirano aflatoksinom B_1 (1,24 µg/kg) i da je to uzrok pojave metabolita AFM_1 u sirovom mleku krava. Na osnovu dobijenih rezultata zaključeno je da je značajno smanjen sadržaj AFM_1 u mleku krava koje su konzumirale hranu sa dnevnim unosom bentonita od 30 i 50 g.

Ključne reči: muzne krave, aflatoksini, bentonit

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