Original scientific paper UDK: 637.065

The use of the two different mycotoxin deactivators in the nutrition of dairy cows

DOI: 10.15567/mljekarstvo.2022.0106

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Received: 03.08.2021. **Accepted**: 01.12.2021.

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Abstract

The aim of this study was to investigate the effect of two different commercial products for mycotoxin deactivation in the dairy cows' nutrition. The research was done on the 18 dairy cows in the different phase of lactation, with the average production of 14 L of milk per day. The diet contained in average 8 kg of concentrate feed, 10 kg of mixed peas and wheat forage, 4 kg hay of alfalfa and cereal straw *ad libitum*. Before starting the trials all components of the cows' ration were analysed for the presence of aflatoxin B1, while cow's milk was analysed for the presence of aflatoxin M1. In the first phase of the trial cows were fed diet containing aflatoxin B1 at level of 5±0.9 μg/kg, which resulted in the average aflatoxin M1concentration of 181±3.5 ng/kg in the milk. In the second phase of the trail 0.5 % of the commercial product 1 (Neozel®) was added into the feed ration of cows and after the period of adaptation, the aflatoxin M1 concentration in milk was measured. In the third phase of the trail 0.3 % of the commercial product 2 (miko-Stop 0.3®) was added into the feed ration of cows and also, after the period of adaptation, the concentration of aflatoxin M1 in milk was measured. The last phase was the control phase in which cows were fed diets with aflatoxin B1 (5 μg/kg) without added mycotoxin deactivators. The use of both commercial products for ten days resulted in the reduction of aflatoxin M1 levels for 35.9 % and 53.6 %, respectively.

Key words: nutrition; dairy cows; mycotoxins; mycotoxin deactivators

Introduction

Aflatoxin B1 was in the centre of attention in 2012 in the Europe and worldwide after the outbreak of these toxins in the corn in the region of Balkan peninsula due to the specific climatic condition (de Rijk et al., 2015; Battilani et al., 2016). The outbreak of aflatoxin B1 in corn resulted in the increased concentrations of aflatoxin M1 in milk and milk products (Kos et al., 2014; Flores-Flores et al., 2015). The legislation regulating aflatoxin M1 occurrence in milk in the EU is one of the most rigorous ones worldwide considering the countries which have the legislation for this mycotoxin, and sets the upper limit to 0.05 μg/kg in milk. The occurrence of aflatoxin M1 in milk and consequently in milk products arose public attention and resulted in significant reduction in the consumption of milk and milk products in the Balkan region. Legislation regarding aflatoxin M1 in milk changed several times in Republic of Serbia during the period of only a few years. First as an attempt to reach the standards of EU, and then as a consequence of the outbreak in 2012 and many problems which were caused by it. The legislation was changed several more times in attempt to protect the dairy production in Serbia by making the compromise between these two legislations (Jajić et al., 2018). Until the outbreak of aflatoxin B1 in corn in the region of Balkan in 2012, the attention regarding the prevention of aflatoxin B1, was more aimed to imported feedstuff. However, it is expected that aflatoxin B1 contamination of feedstuff will be even more increased due to the climate change. The global increase in temperature from +2 °C to +5 °C is projected, whereby the most probable scenario for the temperature increase in Europe is +2 °C which will make aflatoxin B1 a food safety issue in corn (Battilani et al., 2016). Moreover, it seems that the combination of the environment conditions has a little effect on growth but have a strong effect on the aflatoxin biosynthesis genes expression. Medina et al. (2014) investigated the combined effect of water activity, temperature, and elevated CO_2 levels on the expression of aflatoxin biosynthesis genes. It has been suggested that while each factor alone has the effect, their combination impacts the aflatoxin production. In the next few decades, it is expected that the concentration of atmospheric CO_2 will double or even triple (Medina et al., 2014).

It should be noted that corn is not the only a source of mycotoxin contamination in the nutrition of dairy cattle. Forages are also the source of such contamination. Silage made from the whole plant is one of the main forages used in Europe. It is well known that silage can significantly contribute to the daily exposure of mycotoxins in dairy cattle depending on factors such as maturity of silage, aerobic spoilage during ensiling or during the feed-out phase (Richard et al., 2007; Cavallarin et al., 2011). Even the minor feed, ingredient that includes less than 10 % (w/w) of compound cattle feed can significantly contribute to the contamination of aflatoxin contamination of milk (Nordkvist et al., 2009).

The most common situation regarding feedstuffs is the occurrence of multi-mycotoxins contamination. Aflatoxin B1 is one of the most investigated mycotoxins due to the fact that it is carried over from feed to milk and very low concentrations are permitted in milk. Five types of mycotoxins (aflatoxins, deoxynivalenol, zearalenone, fumonisins and ochratoxin A) are regulated through legislation in the feedstuff by reason of their proven effect on animal health and productivity (Pravilnik, 2014). There is a lot of studies reporting low level of co-occurrence more than one of these mycotoxins in the variety of foodstuff (Storm et al., 2014: Krizova et al., 2015; Glamočić et al., 2019; Gallo et al., 2020).

Aflatoxin M1 is a metabolic product of aflatoxin B1 and is a proven carcinogenic precursor (Fink-Gremmels, 2008). Many studies were done to investigate the carry-over from feed to milk. The average carry-over from feed to milk was from 1 % to up 6 % depending on milk yield and on the days of lactation (Veldman et al., 1992; Britzi et al., 2013). Incidentally, caused by the extreme nutrition condition and the change in the cleavage capacity of rumen, ochratoxin A can be occasionally detected in a small amount in the milk (Skaug, 1999). Carry-over of all other regulated mycotoxins from feed to milk in dairy cattle is insignificant and does not present food safety issue (Driehuis et al., 2008; Hashimoto et al., 2016).

Preventing the occurrence of mycotoxins in feed is usually done on the field and in the storages by conforming good agricultural practice principles. However, it is absolutely impossible to completely avoid the contamination. One of the possible solutions is the use of the commercial mycotoxins detoxifying agents. These detoxifying agents are added into animal feed in the function of the reduction of mycotoxins in the gastrointestinal tract of animals and their distribution to organs and blood. Their mode of action can be by absorption or by degradation (Vila-Donat et al., 2018). Some of the commercial products can have one of the modes of action or the combination.

The aim of this study was to investigate the effect of the two different commercial products in the nutrition of dairy cattle feed diet containing 5 μg/kg of aflatoxin.

Material and methods

Experimental design

The study was performed at the experimental farm field of The Agricultural High School, Futog, Novi Sad in which 18 Holstein-Friesian dairy cattle was included. The cows were in different phase of lactation, with the average production of 14 L of milk per day. Before the starting period all components of feed ratio were analysed on the occurrence of aflatoxin B1. All data regarding the components of feed ration were collected in attempt to estimate as accurately as possible the daily intake of B1 trough feed. The feed ratio consisted of 8 kg of concentrate, 10 kg of mixed peas and wheat forage, 4 kg hay of alfalfa and cereal straw *ad libitum*. As mycotoxin deactivators, two commercial products were used. Commercial product 1 was based on clinoptilolite (Neozel®, Gebi, Čantavir, Srbija) while commercial product 2 (Miko-Stop 0.3®, Gebi, Čantavir, Srbija) was based on the mineral and organic component (the extract of yeast).

The trial was carried out in four phases:

- Phase I - cows were fed the diet containing 5 µg/kg of aflatoxin (naturally contaminated) without mycotoxins deactivators added (adaptation period). Three bulk milk samples were taken on the last day of this phase.
- Phase II - cows were fed the diet containing 5 µg/kg of aflatoxin with Neozel® which was added as recommended by producers. Neozel® is commercial product based on clinoptilolite with the added vitamins A, E, B1, B2, B6, C and antioxidans. Bulk milk samples were taken on day 4, 6, and 10.
- Phase III - cows were fed the diet containing 5 µg/kg of aflatoxin with miko-Stop 0.3® which was added as recommended by producers. Miko-Stop is commercial product which contains a mineral and organic component. Components of miko-stops are: vitamins B1, B2, B6, C, selenium, mannan-oligosaccharides, beta-glucans, bentonite, anti-oxidant. Bulk milk samples were taken on day 4, 6, and 10.
- Phase IV - cows were fed the diet containing 5 µg/kg of aflatoxin without mycotoxins deactivators added (control). Three bulk milk samples were taken on the last day of this phase.

Analysis of feed and milk

Feed analysis for aflatoxin B1 content was carried out before the beginning of the first phase. This was done in triplicate using commercial Aflatoxin ELISA test kit (Neogen, UK) and ELISA reader (BioTek ELx800, VT, USA). Aflatoxin B1 was not detected in forages, while its level in concentrate was 5 ± 0.9 µg/kg. Chemical parameters of milk (milk fat, protein, lactose, total solids) were analysed on MilkoScan FT+ (Foss Analytical, Denmark), equipped with a Conveyer 4000 and Pipette case 4000 according to standard method (ISO 9622:2020). Somatic cell count was determined on Fossomatic FT (Foss Analytical, Denmark) using ISO 13366-2:2006 / IDF 148-2:2006 method, while total bacterial count was analysed on BactoScan FC+ (Foss Analytical, Denmark) by applying ISO 16297:2020 / IDF 161:2020 method. Aflatoxin M1 in milk was determined on Agilent 1260 Infinity HPLC system (Agilent Technologies, CA, USA) by applying previously used method (Jajić et al., 2018). All analyses were done in duplicate.

Results and discussion

Table 1 presents the results of the study per days with and without added commercial products. In the first and last phase of study feed ration for dairy cows contained aflatoxin B1 in the concentration of 5 µg/kg without the added commercial product for the reduction of mycotoxins which resulted in the concentration of aflatoxin M1 in milk 181±3.5 ng/kg and 183±48.8 ng/kg. The results of the use of Neozel and miko-Stop 0.3® in concentration recommended by producers are shown per days and are presented in table 1 and in Figure 1. The highest reduction of aflatoxin M1 in milk was achieved on the tenth day with both products. However, a stronger effect on the reduction was noticed when the commercial product 2 (based on the mineral and organic component) was added in the recommended ratio of 0.3 %. The reduction on the tenth day was 53.6 %, and the average reduction for the entire period 44.8 %. The use of the commercial product 1 resulted in the average reduction of 25.3 % for the entire period. However, on the tenth day it was 35.9 %.

Table 1. Aflatoxin M1 content in milk, before, during and after the use of commercial products 1 and 2

Commercial product	%	Phase	Day in each phase	AFM1 content, ng/kg	Reduction of AFM1, %
Adaptation period	-	(I) day 1-10	-	**181±3.5**	-
Neozel®	0.5%	(II) day 11-20	day 4	159	12.2 (87.8)
			day 6	134	26.0 (74.0)
			day 8	132	27.1 (72.9)
			day 10	116	35.9 (64.1)
			average	**135±17.8**	**25.3 (74.7)**
Miko-Stop 0.3®	0.3%	(III) day 21-30	day 4	-	-
			day 6	115	36.5 (63.5)
			day 8	101	44.2 (55.8)
			day 10	84	53.6 (46.4)
			average	**100±15.5**	**44.8 (55.2)**
Control	-	(IV) day 31-40	-	**183±48.8**	-

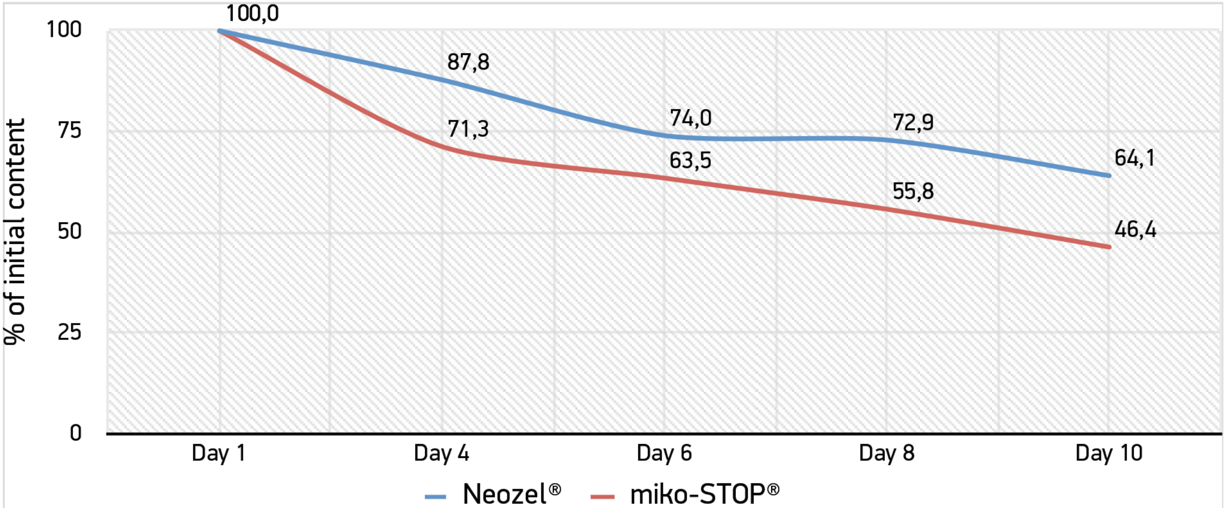

Figure 1. Reduction in the occurrence of aflatoxin M1 (%) as the result of the use two commercial products

The results of carry-over from feed to milk were in the range of the literary data (Veldman et al. 1992; Britzi et al. 2013) as it was expected. The clinoptilolite is the mineral of clay belonging to the zeolites group. The mechanism of action of inorganic absorbents such as clinoptilolite, is the formation of aflatoxin-absorbent complex, which cannot be resorbed in gastrointestinal tract of animals. It is well known for its preventive effect during chronic aflatoxin exposure in broiler nutrition (Oğuz et al., 2000; Oğuz and Kurtoğlu, 2000). In one of the past *in vitro* studies, clinoptilolite bound about 80 % of AFB1 in bovine ruminal fluid (Spotti et al., 2005). However, in the in-field evaluation done in Greece, the use of clinoptilolite significantly reduced the AFM1 concentration in milk at an average rate of 56.2 % (Katsoulos et al., 2016). In our study, the reduction was 35.9 % on the tenth day in the case of the use of commercial product 1 (Neozel®) based on the clinoptilolite. Data from previous studies (Bosi et al., 2002) indicated that clinoptilolite does not adversely affect the basic production parameters, so the use of this inorganic commercial product is justified as the means of the reduction of aflatoxin M1 in milk, as it is shown in our study. The use of the commercial product 2 (miko-Stop 0.3®) based on the mineral component and yeast extract reduced aflatoxin M1 in milk on the tenth day (53.6 %) more than the commercial product 1 (Neozel®) based only on the mineral component. The use of two different products based on the mineral components and yeast reduced the excretion of aflatoxin M1 in milk by 50-60 % (Rodrigues et al., 2019). However, some studies suggested that the addition of 3 sequestering agents had no effect on aflatoxin M1 concentration in milk but they had the effect on time requirement needed to reduce aflatoxin M1 after withdrawal of aflatoxin B1 from the feed of dairy cattle (Ogunade et al., 2016). It seems that the effect of added combination of clay and yeast extract depends on the concentration of aflatoxin B1 in feed. The effect is more pronounced when the concentration of aflatoxin B1 in feed was lower (Xiong et al., 2015).

The results of the use of two different commercial products on some milk quality parameters are presented in the Table 2. The use of both products did not affect any parameters of milk as fat, protein, lactose, or total solids. These findings are in agreement with the findings of some other authors (Maki et al., 2016; Rodrigues et al., 2019). The clinoptilolite addition in concentrations of 200 g/day did not affect protein or fat content in milk of dairy cows (Bosi et al., 2002). Addition of another product based on clay also did not affect the milk composition

Table 2. Results of some milk quality parameters determined during the use of commercial products 1 (Neozel®) and 2 (miko-Stop 0.3®)

Phase	Milk fat (%)	Protein (%)	Lactose (%)	Total solids (%)	Somatic cell count (x1000/mL)	Total plate count (x1000 CFU/mL)
(I) day 1-10	3.29±0.49	3.15±0.28	4.66±0.07	11.87±0.88	488±204	17.00±5.66
(II) day 11-20	3.24±0.11	3.09±0.08	4.67±0.05	11.72±0.18	269±18	25.43±16.87
(III) day 21-30	3.19±0.26	3.21±0.13	4.55±0.10	11.74±0.47	405±143	12.73±3.00
(IV) day 31-40	3.34±0.47	3.03±0.08	4.55±0.08	11.72±0.56	688±407	21.00±2.16

(Maki et al., 2016). In the study of Rodrigues et al. (2019) two different commercial products containing mineral component and yeast extract were used and neither of these did not affect the milk composition.

Conclusion

Mycotoxins, especially aflatoxin B1, are and will remain to be the problem in animal nutrition. Some of the reasons are climate changes, inadequate storage of crops, unsatisfactory agricultural practice. However, even with all measures taken to prevent mycotoxin contamination, it is still unavoidable and always present to some extent. Aflatoxin B1 is the mycotoxin that is the most important from the point of view of food safety because milk and milk products are more consummated by the younger population. Some of the commercial products for binding mycotoxins are imposed as the potential solution of the problem. Both products had an effect on the reduction of aflatoxin M1. The commercial product 2 based on the mineral and organic component reduce aflatoxin M1 (53.6 %) more than commercial product 1 based on clinoptilolite (35.9%). In conclusion, both commercial products are a useful tool in the action against the occurrence of mycotoxins in feed for dairy cattle.

Primjena dva različita deaktivatora mikotoksina u hranidbi mliječnih krava

Sažetak

Ovo istraživanje je provedeno kako bi se ispitao učinak dva različita komercijalna proizvoda za deaktivaciju mikotoksina u hranidbi mliječnih krava. Istraživanje je provedeno na 18 mliječnih krava u različitoj fazi laktacije, s prosječnom proizvodnjom od 14 L mlijeka dnevno. Hranidba krava sastojala se od 8 kg koncentrata hrane, 10 kg smjese graška i pšenice, 4 kg sijena lucerne i slame žitarica davane *ad libitum*. Prije početka provedbe istraživanja, sve komponente obroka analizirane su na prisutnost aflatoksina B1, a mlijeko je ispitano na prisutnost aflatoksina M1. U prvoj fazi pokusa krave su hranjene krmom koja je sadržavala aflatoksin B1 u koncentraciji od 5±0,9 μg/kg, što je rezultiralo koncentracijom aflatoksina M1 od 181±3,5 ng/kg u mlijeku. U drugoj fazi pokusa u krmni obrok je dodano 0,5 % komercijalnog proizvoda 1 (Neozel®) te je nakon razdoblja adaptacije određivana koncentracija aflatoksina M1 u mlijeku. U trećoj fazi pokusa u krmnu smjesu je dodano 0,3 % komercijalnog proizvoda 2 (miko-Stop®) te je također nakon razdoblja adaptacije određivana koncentracija aflatoksina M1 u mlijeku. Posljednja faza bila je kontrolna faza u kojoj su krave hranjene krmivom koje je sadržavalo aflatoksin B1 (5 μg/kg) bez dodanih deaktivatora mikotoksina. Korištenje oba komercijalna proizvoda tijekom deset dana rezultiralo je smanjenjem koncentracije aflatoksina M1 za 35,9 %, odnosno 53,6 %.

Ključne riječi: hranidba; mliječne krave; mikotoksini; deaktivatori mikotoksina

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