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Original paper

Corn silage as a source of aflatoxin B1 in feed for dairy cattle

DRAGAN GLAMOČIĆ[1], MIROSLAVA POLOVINSKI HORVATOVIĆ[1], IGOR JAJIĆ[1], SAŠA KRSTOVIĆ[1], MIRKO IVKOVIĆ[1], NOEMI ČUZDI[1]

[1]Department of Animal Science, Faculty of Agriculture, University of Novi Sad, Serbia

Abstract Nutrition of dairy cattle is based on two components, concentrates and forages. The main forages in Vojvodina, north province of Serbia is silage made from the whole plant of corn. After the outbreak of aflatoxin B1 in corn in 2012, the occurrence of aflatoxin B1 in corn as a source of contamination of aflatoxin M1 in milk was very broadly investigated. There is no data regarding the occurrence of aflatoxin B1 in silage and how much silage can contribute to the overall intake of aflatoxin B1 in this region. This work is an attempt to estimate how much silage, in condition and practice used in Vojvodina, contributes to the intake of aflatoxin B1, and consequently aflatoxin M1 in milk. In total, 82 samples of corn grain and 72 samples of corn silage were analyzed on the occurrence of aflatoxin B1 during 2017-2018 period. Aflatoxin B1 was found in 13.41% of corn samples in the range from 6.82 to 187.5 ppb (average 63.5 ppb). All positive samples were from 2017, while no positive samples were found during 2018. Incidence of aflatoxin B1 in silage was 54.17% in the range of 3.5-58.0 ppb (12% moisture content) or 0.95-16.1 ppb in the fresh matter. Results suggest that silage can be a significant factor to overall intake of aflatoxin B1 and that further research is needed.

Keywords Aflatoxin B1, silage, cow, nutrition.

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✉ *Corresponding author: MIROSLAVA POLOVINSKI HORVATOVIĆ, Dr., Department of Animal Science, Faculty of Agriculture, University of Novi Sad, corresponding author, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
E-mail: miroslavapolovinski@yahoo.com Tel.: +381 061 230 71 50, Fax: +381 021/63-50-019

Introduction

Aflatoxin B1 is one of the most well-known mycotoxins (BLOUNT, 1961). It is possible contaminant of the dairy cattle feed (FINK-GREMMELS, 2008) and can cause two types of problems. First type of problem is food safety concern because of the presence of aflatoxin M1 in milk (PRANDINI et al, 2009; FLORES-FLORES et al, 2015). Aflatoxin M1, like aflatoxin B1, is proven carcinogen, however in lesser degree (IARC, 1993). The most often in animal feed occur several mycotoxins due to the fact that one fungi can produce several mycotoxins, and in the majority of the feed are usually contaminated with more than one species of fungi (GARON et al, 2006; DRIEHUIS et al, 2008). Secondly, apart from being a food safety concern, mycotoxin cause the effect on animal productivity by reducing it (BRYDEN, 2012).

Sure indicator of dairy cattle exposure to aflatoxin B1 through the feed is aflatoxin M1 in milk which is a metabolite of aflatoxin B1 (FINK-GREMMELS, 2008; BRITZI et al, 2013). After the outbreak of aflatoxin B1 in corn in 2012 in Balkan region (KOS et al, 2013), it is even more rigorously tested on the presence of the aflatoxin B1, especially if intended for export. Situation from the 2012 is rare (KOS et al, 2013; KOS et al, 2018), however it left huge consequence on the dairy industry. There was reduction in the number of the dairy cattle as the side effect of the reduced demand for milk and milk products on the market.

Serbia as a country tried to solve the problem caused by the aflatoxin B1 outbreak and its consequences on different ways. One of the approaches was the changes in the regulations regarding aflatoxin M1 in milk on several occasions (JAJIĆ et al, 2019). Dairy industry in Serbia has few specificities. Vojvodina, northern province of Republic of Serbia, have the most developed dairy cattle production and it was mainly affected by the aflatoxin B1 outbreak. The most prominent effect was on primary producers. Previously, dairy plants in Vojvodina, and in Serbia were state owned. After the transition from the state owned to privately owned, majority of dairy plants are now the property of only a few corporations. The primary production of milk is divided between a large number of small producers who have up to 5-10 cows per farm (26%) and up to 5 cows per farm (32%) (Chamber of Commerce of Vojvodina and Agriculture, 2013). The interest of dairy processing plants is to produce the best quality of milk and milk products. They regularly test milk for the occurrence of aflatoxin M1. There is huge difference in the economic power between the primary producers of milk and dairy processing plants. The primary producers are in the struggle to achieve the desirable level of quality of milk. The usual practice for the producers is to regularly test corn for the occurrence of aflatoxin B1 especially after the outbreak 2012, while forages, as a source of aflatoxin in cattle feed are usually overlooked. One of the most often used forages in nutrition of dairy cattle in Vojvodina is silage made from the whole plant of corn. However, there is not enough data to indicate to which extent the forages or the silage made from the whole corn are the source of aflatoxin B1 in nutrition of dairy cattle. Although the current legislation for the aflatoxin M1 in the Republic of Serbia is 0.25 µg/kg, some milk processing plant are encouraging the producers to produce the milk with the lower level than 0.05 µg/kg of the milk. Although the outbreak of 2012 was the extreme, the producers are struggling during the "normal years" to produce milk in accordance with the quality desirable by dairy plants. Silage made from the whole plant of corn are made on each farm separately, in different conditions, and therefore its quality varies greatly. Also, usage time, technology of cutting the edge of the silage are different, as well as other numerous factors on each farm which can and have effect on the mycotoxicological quality of silage.

This study was done on the farms in Vojvodina. Samples of corn (82) and samples of silage made from the whole plant of corn (72) were analyzed on the occurrence of the aflatoxin B1 from the randomly chosen farms in Banat, Bačka and Srem (Vojvodina) during two years period (2017-2018). At the same time, a survey regarding feeding management was conducted to obtain data regarding the average use of silage on the daily bases in the nutrition of dairy cows in the region of Vojvodina, Serbia.

Material and Methods

During the two-year period (2017-2018), 72 samples of silage (made from the whole plant of corn) and 82 samples of corn from the Serbian province of Vojvodina were analyzed. The analyses of samples were done in the Laboratory for Quality Control of Feed and Animal Products at the Department of Animal Science, Faculty of Agriculture, University of Novi Sad.

Silage

One sample of silage was taken from each farm. Sampling was done by taking several sub-samples from the cut edge, from the upper, middle, and low section. Sub-samples were homogenized and quartered to get 500-1000 g of laboratory sample. Immediately after sampling, 200 g of each sample were dried at 60°C overnight and then prepared for analysis by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. Then, the sample was homogenized by mixing and packed in plastic bags. Samples were stored in a freezer at -20°C until analysis. Prior to each analysis, the samples were allowed to reach room temperature. A portion of sample was used for the determination of moisture content at 105°C.

Corn

Samples. 82 samples of corn were randomly collected after 2017 and 2018 harvests from different locations in Serbia. After sampling, approximately 300 g of each sample was prepared by grinding in a laboratory mill (0.5 mm sieve). After that, the sample was homogenized by mixing. Samples were then packed in plastic bags and stored in a freezer at -20°C until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

Extraction

Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol in the water on an Ultra Turrax T18 homogenizer (IKA, Germany) for 3 min at 11,000 rpm. The crude extract was then filtered through a quantitative slow filtration filter paper (Filtros Anoia, Spain). Analysis and quality control. The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 μg/kg).

The analytical procedure was carried out according to the manufacturer's procedure. Aflatoxin quantification was done on ELISA reader equipped with a 630 nm filter (BioTec Instruments, USA). Validation parameters of a method for aflatoxin determination were evaluated according to the European Commission (2006). The limit of quantification (LOQ) was 1.37 μg/kg and the average trueness value equaled 106.2%. The method was found to be precise in terms of repeatability and reproductivity. The obtained results showed that the proposed analytical method fits well for control purposes of aflatoxin in feed samples.

Survey

A survey, regarding feeding management, taking into consideration diversity of farms in the Vojvodina was conducted. The majority of the producers have small number of cattle per farm. Furthermore, some of the producers did not even have the basics data about how much they use different type or the amount of the feed in the nutrition.

Results

The results on the occurrence of aflatoxin B1 in corn and silage are shown in table 1. In the two-year period (2017-2018), aflatoxin B1 was detected in corn only in samples from 2017, at the incidence of 22.45%. The maximum level found in corn was 187.5 ppb. In silage, 54.17% of samples originating from the both years, contained aflatoxin B1. In each year, the occurrence of aflatoxin B1 in silage was similar (56% and 53.19%). During the observed period, aflatoxin content in silage was ranged from 3.5 to 58.0 ppb (12% moisture), or 0.95-16.1 ppb (fresh matter basis). Average value of aflatoxin B1 in all positive samples was 17.37 ppb (12% moisture) or 6.18 ppb in fresh matter.

Table 1. Occurrence of aflatoxin B1 in corn (12% moisture content) and silage (12% moisture content and in fresh matter) in the period from 2017-2018 in the region of Vojvodina

			No. of samples	Positive samples, %	Concentration of samples, ppb		
					Average	Range	Median
corn	2017		49	22.45	63.53	6.82-187.5	14.77
	2018		33	0	0	0	0
∑			82	13.41	63.42	6.82-187.5	14.77
silage	2017	12% moisture	25	56.00	27.27	7.90-58.00	25.05
		Fresh matter			9.63	3.41-20.00	9.47
	2018	12% moisture	47	53.19	11.82	3.50-44.00	7.00
		Fresh matter			4.25	0.95-16.11	3.02
∑		12% moisture	72	54.17	17.37	3.50-58.00	13.2
		Fresh matter			6.18	0.95-16.11	4.38

A survey regarding feeding management on dairy farms in region of Vojvodina was done on 20 farms with 8 to up 380 cows per farm. The care was taken that all kind of farms were a part of the research. Some of the farmers were reluctant to give information or they gave incomplete information regarding feeding management for the reason of not knowing how much they use concentrate or forages in nutrition (excluded from the questionnaire). Majority of the producers were making the concentrate on the farm (71%), while only 29% were buying them from the manufacturer. They used corn, wheat, and barley as a source of energy and soya bean meal and sunflower meal as a protein source. Beside concentrate and silage, all producers used alfalfa hay or dehydrated alfalfa. The results of the survey are presented in the Table 2 with the average consumption of concentrate and silage on the farms which took part in the survey.

Table 2. Daily intake (kg) of silage and concentrate in dairy cows in the region of Vojvodina based on the questionnaire

	Number of farms	Min	Max	Average
Silage	20	10	35	18.7
Concentrate	20	4	15	9

Discussion

It was estimated that daily intake of aflatoxin B1 should be less than 40 µg/animal/day, for the cows producing 30 L milk per day, to avoid aflatoxin carry over from feed into milk at levels above 50 ng/kg aflatoxin M1 in milk (VELDMAN et al, 1992). The average value in all positive samples of silage in the fresh matter was 6.18 ppb, median 4.38 ppb. Daily consumption of silage is about 19 kg per cow. It is relatively easy to estimate that in some cases daily intake of aflatoxin B1 through silage can be significantly more over and can be responsible for producing milk with aflatoxin M1 above the desirable level. Some of the researchers pointed out that silage made from the whole plant is dangerous, if the concentration of aflatoxin B1 is above 4 ppb (CAVALLARIN et al, 2004).

Commercial feed is rarely the source of mycotoxin contamination, due to the regular testing by the authorities for official control. Corn and silage produced on the farm are more probable source of contamination. Also, there is a link between the feeding management and likelihood of having a problem with mycotoxin contamination (BOUDRA et al, 2007). Majority of milk in Serbia is produced on the small farms with up to 10 cows per farm (Chamber of Commerce of Vojvodina and Agriculture, 2013), with the very modest feeding management. Pasture is rarely an option in the Vojvodina for the dairy cattle, the forage is mostly based on hay, silage and haylage. Silage made from the whole plant of corn is most often used silage in this region.

Occurrence of aflatoxin M1 in milk in Serbia due to the 2012 aflatoxin B1 outbreak in corn is well documented and broadly studied (KOS et al, 2014; UDOVICKI et al, 2018; KOS et al, 2018; JAJIĆ et al, 2019). However, aflatoxin B1 in some years was not detected in corn (KOS et al, 2013). Even after the outbreak 2012, in years after, there were years without aflatoxin B1 occurrence in corn (KOS et al, 2018). However, there were samples of milk with the aflatoxin M1 during years with the low or no occurrence of aflatoxin B1 in corn (JAJIĆ et al, 2019).

It is well known fact that silage can contribute significantly to the intake of mycotoxins (RICHARD et al, 2007; ALONSO et al, 2012). Even in some cases, depending on the feeding management, it can be even greater source than the concentrate (DRIEHUIS et al, 2008). Also, during the use of silage, an increase in mycotoxin contamination may occur, especially aflatoxin M1 (PEREYRA et al, 2008; CAVALLARIN et al, 2011; GONZÁLEZ PEREYRA et al, 2011).

Aflatoxin B1 and consequently aflatoxin M1 will be the problem in Serbia and region in years to come due to the climate changes (MEDINA et al, 2014, HORVA-TOVIC et al, 2018). Especially in the case of average increase in temperature of +2°C, which is the most probable scenario for the years to come (BATTILANI et al, 2016). Silage should not be ignored as a potential source of aflatoxin B1 in nutrition of dairy cattle. Results of the survey showed that average consumption of silage in the region of Vojvodina is about 18.7 kg per day per cows. Even minor feed ingredients included in less than 10% (w/w) in the compound cattle feed can significantly contaminate milk with aflatoxin M1. It remains to be seen how much silage can contribute to overall intake of mycotoxins in the condition of the agricultural practice in Vojvodina, Serbia. It is possible that during the years when the occurrence of aflatoxin B1in corn are not notable, the silage can be an important aflatoxin B1 source, even with small amounts, since its daily consumption is quite significant. It is well known that type of silos, management of silage or duration of the use can increase the occurrence of mycotoxin in it (RICHARD et al, 2009; GONZÁLEZ PEREYRA et al, 2011; SHIMSHONIA et al, 2013). All these factors can and are quite different from farm to farm and are much less under climatic influence and more under the influence of individual farmers. Conditions, where the majority of farmers are small farmers with up to 10 cows per farm can be an aggravating factor.

Conclusion

Dairy industry is very intense and is under huge pressure by the market and the demand of consumers to obtain the milk with the desirable quality. Outbreak of aflatoxin B1 in corn 2012 had a huge effect on the producers, especially small producers with small economic power which had small chances to overcome the crisis like that. There is huge pressure to produce the milk with the desirable quality and the producers quite challenged to achieve that. This study suggests that silage can be part of problem. Consumption of silage per cow is about 19 kg. Farms are small with the very different agricultural practice so the quality of silage can vary greatly. It is possible that during the years when occurrence of aflatoxin is very rare

in corn that silage can be source of problems with the low concentration per kg but with high consumption of kg per day. It remains to be seen in the years to come how much silage made from the whole plant will be problem.

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Conflict of Interest

The authors has no conflict of interest to declare.

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