Aflatoxin in Maize Silage Collected from AP Vojvodina, Serbia

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Abstract

The aim of this work was to investigate aflatoxin (AF) occurrence in maize silage samples from 2017 harvest. In total, 30 samples of maize silage (whole plant) for nutrition of milking cows were inspected for AF occurrence. The samples originated from three regions (Bačka, Banat and Srem) in autonomous province (AP) of Vojvodina, Republic of Serbia (10 samples collected from each region). In AP Vojvodina, overall AF presence was 67% with its levels ranged from 3.52 to 24.64 µg/kg. Levels were standardized to 12% moisture content in all samples. Regarding each region, the highest presence was observed in Srem (80%) followed by Banat (70%), while the lowest presence was found in Bačka region (50%). However, the average AF contamination levels were not correlated to its presence. The highest AF level of 12.76 µg/kg (ranged from 6.16 to 24.64 µg/kg) was found in Srem region, whereas its lowest level was observed in Banat region (average of 4.40 µg/kg, ranged from 3.52 to 5.28 µg/kg). In the region of Bačka the average AF level was 8.45 µg/kg. Despite high presence, none of the samples contained AF above maximum level set by Serbian regulation (30 µg/kg), while 2 samples from Srem region exceeded the EU maximum level of 20 µg/kg. These results may be a result of warm and dry weather conditions during summer months of 2017, that prevailed on almost entire territory of AP Vojvodina.

Keywords: aflatoxin, AP Vojvodina, maize silage, Serbia.

1. Introduction

Recently, aflatoxin M1 became the center of the attention for the researchers in Serbia and the region. More precisely, since 2012 when a huge outbreak of aflatoxin B1 hit the whole region including Serbia. Common source of aflatoxin B1 contamination in the dairy cattle feed is considered the maize, which was the case in 2012. The result of the outbreak in 2012 was the public fear of consuming milk and milk products which caused sale drop. For example, in Romania sale dropped by 45% in a few weeks after the media cover of the outbreak [1]. Besides maize, forage or silage in dairy cattle feed can be a potential source of contamination with aflatoxin B1, but it is rarely considered as such.

Carry over from feed to milk is relatively small and it can be from 0.6 to 6% [2]. However, the carry over in high yielding cows can be significantly higher and up to 11.4% [3]. The legal limits regarding aflatoxin B1 in the feed for dairy animals is set for the prevention of the aflatoxin M1 undesired levels in milk. Britzi et al. [3] suggested that even when aflatoxin B1 in feed is within legal limits, aflatoxin M1 in milk can occur at levels that exceed the legal limits due to higher carry over in high yielding cows. It should be taken into consideration that maize is not the only source of aflatoxin B1 in animal diet. Other ingredients in feeding mixtures can be the sources

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of aflatoxin B1 and may contribute to the overall intake of this mycotoxin.

Serbia have one more specificity relating to aflatoxin M1 legislation. The EU countries have the legislation on aflatoxin M1 in milk which is obligatory for the all EU members and it is 50 ng/kg [4]. Even though Serbia is not EU member, its legislation was the same as in the EU as an attempt to achieve the EU standards of food safety [5]. After the aflatoxin B1 outbreak in 2012 in maize and 2013 in milk, legislation has been changed several times from 500 ng/kg [6] to 50 ng/kg [7] and finally to 250 ng/kg [8]. Many dairy plants in Serbia have internal control of milk on aflatoxin M1 level. So, although the legal limit is 250 ng/kg at the moment, management of dairy plants are paying higher price for milk containing aflatoxin M1 below 50 ng/kg. This puts primary producer under even higher pressure in already harsh market condition. Most producers regularly check maize on aflatoxin B1, while silage is hardly tested.

During autumn of 2015, considerable aflatoxin M1 levels were found in milk samples collected in Banat region of AP Vojvodina. However, data on aflatoxin B1 occurrence in maize appeared not to be sufficient to induce higher levels of aflatoxin M1 in milk. The unofficial data of Ministry of Agriculture, Forestry and Water Management indicated maize silage as a potential source of contamination in this region.

Toxigenic fungi found on the maize plant, can be the source of the mycotoxins in silage, and thus aflatoxin B1. Ensilaging process stop fungal growth and mycotoxin production. In silage, numerous mycotoxins can be found, and may have cumulative and additive effect on animals and their production [9]. Some researchers suggested that silage can be significant source of mycotoxin contamination in dairy cattle diets, much more than previously considered [10, 11]. Also, during the use of silage, the inadequate agricultural practice can cause elevation of aflatoxin B1 levels due to aerobic exposure [12].

This data arouses a need to investigate the quality of the silage as one of the possible source of contamination with the aflatoxin B1 of dairy cattle feed in Vojvodina.

2. Materials and methods

Samples. Thirty samples of maize silage (whole plant) for nutrition of milking cows were collected from three regions (Bačka, Banat and Srem) in AP Vojvodina, Serbia. Samples were taken from ten households in each region. Most households were milking between 20 and 30 cows. Samples were collected in the period from November 2017 to January 2018. 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, sample was homogenized by mixing and packed in plastic bags. Samples were stored in a freezer at -20ºC 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.

Extraction. Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper.

Analysis. 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). Analytical procedure was carried out according to manufacturer's procedure. Optical densities were obtained using the reader of microtiter plates using a 630 nm filter (BioTec Instruments, USA). All results are standardized to 12% moisture content. Moisture content was determinated using NFTA reference method [13].

Quality control. To ensure quality of obtained results, method for determination of aflatoxin was previously validated [14]. Validation parameters of the method were estimated according to European Regulation [15] and the method was suitable for the determination of aflatoxin in animal feed.

Statistical analysis. Statistical analysis was performed using Statistica 13.2 software package (Dell Software, Inc., Round Rock, Texas, USA).

3. Results and discussion

Results of aflatoxin analysis in maize silage samples are presented in Table 1. Aflatoxin levels were standardized to 12% moisture content in all samples, to be comparable with legal limits. In AP Vojvodina, overall AF presence was 67% with its levels ranged from 3.52 to 24.64 µg/kg. Regarding each region, the highest presence was observed in Srem (80%) followed by Banat (70%), while the lowest presence was found in Bačka region (50%). However, the average AF contamination levels were not correlated to its presence. The highest AF level of 12.76 µg/kg (ranged from 6.16 to 24.64 µg/kg) was found in Srem region, whereas its lowest level was observed in Banat region (average of 4.40 µg/kg, ranged from 3.52 to 5.28 µg/kg). In the region of Bačka the average AF level was 8.45 µg/kg. Despite its high presence, none of the samples contained AF above maximum level set by Serbian regulation (30 µg/kg). On the other hand, 2 samples from Srem region exceeded the EU maximum level of 20 µg/kg. Robust parameters (median and median absolute deviation) were also calculated but appeared not to be noteworthy.

Results were also analyzed using one-way ANOVA and post-hoc Tukey test. Least square means with minimum (-95%) and maximum (+95%) values are presented in Figure 1. Tukey test revealed significant difference between results in Banat and Srem region ($p<0.05$) (data not shown). These results may be an outcome of hot and dry weather conditions during summer months of 2017, that prevailed on almost entire territory of Serbia and AP Vojvodina.

Table 1. Aflatoxin in maize silage samples (whole plant) in AP Vojvodina, Serbia (µg/kg)

	Vojvodina	Bačka	Banat	Srem
Number of samples	30	10	10	10
Positive samples	67%	50%	70%	80%
Average±standard deviation	8.76±6.42	8.45±4.68	4.40±0.88	12.76±7.83
Range	3.52-24.64	3.52-14.96	3.52-5.28	6.16-24.64
Median	6.16	7.04	4.4	9.68
Median absolute deviation	6.16	7.04	0.88	3.96

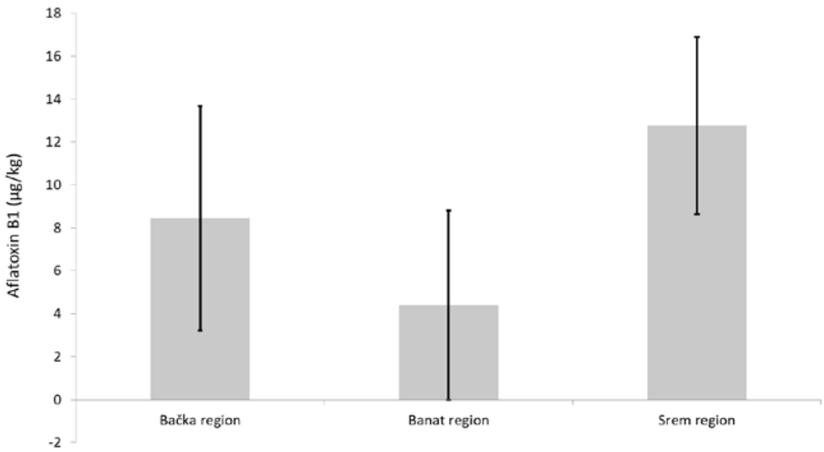

Figure 1. Least square means (±95%) of aflatoxin content in maize silage samples.

According to the Serbian hydrometeorological service [16], vegetation period of 2017 (April-September) was warmer and drier in comparison with the average conditions. In the period April-September an average precipitation of about 310 mm was recorded for Serbia, which is about 20% less than the average values. Standardized precipitation index (SPI-6) showed normal humidity conditions on most of the territory of Serbia. During the summer (June-August), unfavorable temperature and humidity conditions were recorded on most of the territory of Serbia, which in some parts of the country resulted in a significant reduction in yields, especially maize. The precipitation was uneven both, in terms of quantity and territorial distribution. The rainfall did not meet the needs of the plants for moisture, especially in periods of hot waves when the maximum temperatures were higher than 35°C for several days, and some values measured at about 40°C. Regarding agrometeorological conditions, the production year of 2016/2017 was unfavorable for many agricultural crops, particularly maize. The lower quality and quantity of yields of certain agricultural crops was a result of climatic extremes which took place during very important vegetative and generative processes in agricultural crops, as well as insufficient application of appropriate agrotechnical measures. Extreme climatic events did not equally affect the entire territory of the country and did not last equally long. Among these, the drought was predominant, and it caused the greatest damage, especially to maize (30% lower yields compared to the average). The consequences of drought were more pronounced in the southern and central Banat, part of the Srem region, while in Bačka region the excellent yields of this culture were recorded.

By comparing weather conditions with our results among regions, it can be said that no firm evidence of correlation can be found since the regions are close geographically. However, the different results among Banat and Srem region might be explained by different silage preparation techniques, as well as its utilization at households.

In Serbia, a few researchers investigated aflatoxin occurrence in maize silage. Bočarov-Stančić [17] examined silage samples for mycotoxins in Vojvodina region in the period 2000-2004. It was found that 25% of the samples were contaminated with aflatoxin, although at low levels (average of 3 µg/kg). A few years later, Lević et al. [18] reported considerable presence of aflatoxins in maize silage (38%).

In Europe, silage was mostly analyzed for the presence of fusariotoxins [19-23]. On the other hand, aflatoxins were investigated in silage from Argentina [24, 25]. Namely, Gonzales Pereyra et al. [24], found that 17% of Argentinian maize silage samples were highly contaminated (aflatoxin levels ranged from 1.43 to 155.78 µg/kg), whereas Signorini et al. [25] detected aflatoxin in even 73% of investigated samples. Furthermore, Egyptian authors [26] found aflatoxins in 9% of silage samples, although they did not specify whether it was maize silage. These reports demonstrate that aflatoxin problem in silage is not recognized sufficiently, since dairy cows usually consume almost the same amount of dry matter from silage, as they do from feeding mixtures. It is necessary that more systematic monitoring of aflatoxin in maize silage needs to be done to reduce the risk of aflatoxin M1 contamination in dairy cow's milk.

4. Conclusions

Based on the results, it can be concluded that aflatoxin occurrence in maize silage samples was the outcome of hot and dry weather conditions during summer months of 2017 in AP Vojvodina. On the other hand, the differences in aflatoxin contamination among the observed regions are probably a result of different silage preparation techniques and its utilization at households.

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