DETERMINATION OF THE PRESENCE OF AFLATOXIN B1 IN FOOD AND FEED IN REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA) IN THE PERIOD BETWEEN 2014 AND 2016

ABSTRACT: Aflatoxins are a mixture of related chemical compounds of bisfokumarine type. They are synthesized in a variety of agricultural and food products such as oilseeds, spices, cereals and other products. In the period of 2014–2016, 418 samples were analyzed for the presence of aflatoxin B1, using the ELISA method. The analysis included samples of silage (75 samples), concentrated feed (272 samples), dry fruits (16 samples), nuts (15 samples), flours (5 samples) and other types of samples (35 samples). The presence of aflatoxin B1 was determined in all of the analyzed samples. The highest detected concentration of aflatoxin B1 was 4 μg/kg in the silage samples, 30 μg/kg in the concentrated feed samples, 0.40 μg/kg in samples of dried fruit, 0.81 μg/kg in nuts, 0.5 μg/kg in flour samples and 0.5 μg/kg in the other analyzed samples. Higher concentration of aflatoxin B1 from maximum residual level specified in Regulation was detected in samples of concentrated feed (2.57%).

KEYWORDS: Aflatoxin B1, ELISA, food, feed

INTRODUCTION

Mycotoxins in food and feed are recognized as a public health problem. Many researchers dealt with fungal toxins establishing their carcinogenicity. Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2) are potential teratogenic and carcinogenic metabolic products of Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius (Kurtzman et al. 1987). Fungi are commonly found on the grains, almonds, walnuts, peanuts.

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Aflatoxins are a mixture of related chemical compounds. Series of aflatoxin B has the structure of molecules, where a cyclopentane ring is replaced by a G in the series of the lactones. Three structural variations give 18 molecules of aflatoxin, 8 of which are toxic and so far known aflatoxin B1 is the most toxic (Sinovec et al. 2006). Clinical signs of an acute aflatoxicosis include loss of appetite, lethargy, weight loss, neurological disorders, jaundice mucous membranes and cramps. High doses of aflatoxins are acutely toxic, causing substantial damage to the liver and intestinal and peritoneal bleeding, which can be lethal (Marriott and Gravani, 2006).

MATERIAL AND METHODS

As the material in our testing, we used a feed: 103 samples in 2014; 155 samples in 2015; 86 samples (silage, maize, concentrate feed) and 71 samples (dried fruit, nuts, flour and other foods (pasta, etc)) in 2016. Analyses were performed by ELISA method, using test kit produced by manufacturer Bioscinetific, Max signal (Austin USA).

Test protocol

5.0 g of representative and ground samples were extracted with 25 mL of 70% methanol. Extraction was performed by combination of shaking, vortex (10 min.) and centrifuge (4,000 rpm during 10 min.) of the samples. The obtained supernatants were diluted with solution C and vortexed. 50 μL of the diluted samples was used for the analysis.

RESULTS OF ANALYSIS

Feed samples

The obtained results for feed samples are shown in Table 1

<table>
<thead>
<tr>
<th></th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
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<tbody>
<tr>
<td></td>
<td>&lt;5 μg/kg</td>
<td>5 μg/kg – 20 μg/kg</td>
<td>&gt;20 μg/kg</td>
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<tr>
<td></td>
<td>&lt;5 μg/kg</td>
<td>5 μg/kg – 20 μg/kg</td>
<td>&gt;20 μg/kg</td>
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<tr>
<td></td>
<td>&lt;5 μg/kg</td>
<td>5 μg/kg – 20 μg/kg</td>
<td>&gt;20 μg/kg</td>
</tr>
<tr>
<td>Concentrated feed</td>
<td>90</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Silage</td>
<td>2</td>
<td>nd</td>
<td>nd</td>
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</tbody>
</table>

Table 1 shows review for period 2014–2016 where we analyzed a total of 418 samples. 2.57% (7 samples) were contaminated by aflatoxin B1 at concen-
trations higher than allowed by valid regulation (>20μg/kg). The greatest number of samples (316) had a concentration of aflatoxin B1 less than 5 μg/kg and 20 samples in the concentration range from 5μg/kg – 20μg/kg. The highest detected concentration of aflatoxin B1 in silage was 4 μg/kg and at concentrate feed it was 30 μg/kg.

Food samples

In all analyzed samples of food obtained concentrations were below 2 μg/kg. The largest number of analyzed samples (35 samples) was food like pasta, spices and grains. There were also 16 samples of dry fruits, 15 samples of nuts and 5 samples of flour. The highest recorded concentration was 0.81 μg/kg detected in samples of nuts.

All analyzed samples of food were within range of maximum residual level of valid regulation.

DISCUSSION

Results of our studies are compared to results of available literature. Kos et al. (2013) in their studies found that of the 78 analyzed samples, presence of aflatoxin B1 was detected in 44. The most contaminated samples had a concentration (17.9%) of 1–10 ppb. Škrinjar et al. (2013) reported that in 12 of the tested samples the concentration aflatoxin B1 in feed material was from 6 μg/kg to 145.8 μg/kg, while we in our studies recorded a highest concentration of 30 μg/kg. Nedić et al. (2014) in their work detected 7.21% of positive samples in concentrate feed analyzed in 2013, which is slightly higher than the results of our examination. Increased values of concentration of aflatoxin B1 (51%) were found by Škrinjar et al. (2013) in their studies. Almeida et al. (2013) in their research reported that the largest number of analyzed samples had a concentration of aflatoxin less than 5 μg/kg, which would correspond to our test results. Results of the analysis by Nedić et al. (2014) and Škrinjar et al. (2013) indicate an increased concentration of aflatoxin B1 in feed in 2012 which is direct consequence of drought that had happened that year in this region. Results of our examination show that in the following period (2014, 2015 and 2016), concentration of aflatoxin B1 was smaller than in 2012.

According to official data (FAO 1995), the average content of aflatoxin B1 varied between 4 and 8 μg/kg with a maximum value of 30 μg/kg. In worldwide scale in the period 1986–1997 from 2,460 tested samples of grain, 1,273 of the samples contained B1 at concentrations of 7–44 μg/kg. In Brazil, from 2,546 samples of corn, 51% were contaminated with aflatoxin B1, a maximum quantity amounted to 2,440 μg/kg (Sinovec et al. 2006), which is more than what we have found in our tests. Higher concentrations of aflatoxin B1 (251 μg/kg) are recorded in researches in China.
Researchers in Spain, by analyzing the rice from different areas, have found that the rice is contaminated by aflatoxin B1 in the range from 0.8 μg/kg to 91.7 μg/kg. The same researchers examined rice in Mexico where they found concentration of aflatoxin B1 in range from 4.5 μg/kg to 8.1 μg/kg (Bonnet at al. 2013). The mean concentration of aflatoxin B1 in peanut samples, in season 2014/2015 amounted to 38.24 μg/kg (Villers, 2017), which is greater than the value that we have got we in our tests.

Conclusion

Based on the results obtained in this experiment, it is possible to conclude that it is necessary to do monitoring of concentration of aflatoxin B1. Concentration of aflatoxin B1 depends on climate changes. Thus, it is necessary to apply agro-technical measures and make selection of corn species and other food and feed, which are more resistant to contamination of aflatoxin B1.

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ОДРЕЂИВАЊЕ ПРИСУСТВА АФЛАТОКСИНА Б1 У ХРАНИ И ХРАНИ ЗА ЖИВОТИЊЕ У РЕПУБЛИЦИ СРПСКОЈ (БИХ) У ПЕРИОДУ 2014–2016

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РЕЗИМЕ: Афлатоксини су мјешавина повезаног хемијских јединиња типа бисфокумарине. Синтетисани су у различитим пољопривредним и прехрамбеним производима, као што су уљарице, зачини, житарице и други, производима с ниском активношћу воде. У периоду од 2014. до 2016. године, методом Елиса анализирано је 418 узорака на присуство афлатоксина Б1. Анализа је обухватала узорке силаже (75 узорака), концентроване хране (272), сувог воћа (16), језгру ораха (15), брашна (5) и других врста узорака (35). Присуство афлатоксина Б1 утврђено је у свим анализираним узорцима. Највећа детектована концентрација афлатоксина Б1 била је 4 ppb у узорцима силаже, 30 ppb у узорцима концентрисане хране, 0,40 ppb у узорцима сувог воћа, 0,81 ppb у језгру ораха, 0,5 ppb у узорцима брашна, и 0,5 ppb у осталим анализираним узорцима. Већа концентрација у односу на максимално дозвољену концентрацију афлатоксина Б1 прописана важећим правилником детектована је у узорцима концентратара.

КЉУЧНЕ РЕЧИ: афлатоксин Б1, ЕЛИСА тест, храна, храна