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COMPARATIVE ANALYSIS OF MILK SAMPLES FROM BOSNIA AND HERZEGOVINA CONTAMINATED WITH AFLATOXIN M1

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ABSTRACT: *Mycotoxins are toxic metabolites of extracellular different molds in which living organisms (plants, animals, people) cause different diseases, known collectively as mycotoxicoses. Molds, potential producers of toxic metabolites, are constantly present. Aflatoxins are the most known and most toxic mycotoxins. Aflatoxin M1 is highly toxic 4-hydroxylated metabolite of aflatoxin B1 and B2. Given that occurs in the milk of mammals that were fed food contaminated mentioned aflatoxin, a mark M is received from the English word milk. Aflatoxin M1 is a relatively stable compound in raw and processed milk that has no effect on the process of pasteurization or processing into cheese. According to the Regulations on maximum levels for certain contaminants in foodstuffs maximum permissible limit of aflatoxin M1 in milk and dairy products was 0.05 mg/kg, which is in accordance with the current regulations in force in the EU. Made many studies aflatoxin M1 in different areas and found that the presence of this toxin can affect the climate or season. As one of the tests for the detection of pathogens in food on the basis of immunological characteristics are widely applied imunoadsorpcioni enzyme assay - ELISA (The enzyme-linked immunosorbant assay). The method is designed to replace the detection and isolation of the solid phase is relatively easy to perform, can be applied to a larger number of pathogens, can be semi-automatic and gives quick results. To use the ELISA method researchers are usually decided by the fact that the ELISA is simple, sufficiently accurate, inexpensive and reliable method for the analysis of samples in a short period of time. In interpreting the results, it should be noted that the percentage of absorbance is inversely proportional to the concentration of the toxin, respectively, as a percentage of absorbance higher concentrations of not aflatoxin M1 in milk sample is lower. Although it is known that the presence of mycotoxins can not be completely avoided, the recommended preventive measures in order to prevent the occurrence of mycotoxins in animal feed refer to the quality control of production conditions and storage conditions (propionic acid to grains, hay and silage, CO2, cold storage, dry cereal, improving fermentation in silage-enzyme supplements, probiotics, bacteria).*
KEYWORDS: Milk, mycotoxins, aflatoxin M1, ELISA

INTRODUCTION

Modern mass production of food aims to provide large quantities of food that will meet the criteria for high nutritional quality with the aim of producing safe food without biological, physical and chemical contaminants. Mycotoxins are one of the most well-known and common chemical food contaminants. As secondary products of mold

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metabolism, they can contaminate a large number of different agricultural and food products, as well as animal feed. Scientific literature data show that about 25% of the world's total cereal production is contaminated with at least one mycotoxin. The frequent occurrence of mycotoxins, in addition to the proven multiple negative effects on human and animal health, also causes great economic losses. The reasons for this are climate change, but also more frequent and more modern control of their presence, supported by sensitive analytical methods. (Scudamore, 2008). The aim of the study was primarily to determine the influence of the seasons on the contamination of milk samples with aflatoxin M1. That is, which samples were more contaminated, those analyzed during spring and summer compared to those analyzed during autumn and winter. Then, the aim of the paper was to show the impact of enhanced control of animal feed and raw milk samples by the inspection or the dairies themselves from the last affair in 2013 and over a number of years. Also, this paper created the validation of the method to determine whether the ELISA method meets the eligibility criteria set by the applicable Regulations.

LITERATURE/THEORETICAL UNDERPINNING

Mycotoxins

Mycotoxins are secondary products of mold metabolism. Of the hundreds of thousands of different types of molds, about 200 to 250 are toxigenic, that is, they have the ability to synthesize mycotoxins (Pitt, 2000; Hussein and Brasel, 2001). Mold toxins most commonly belong to the genera *Aspergillus, Fusarium, Penicillium, Mucor, Alternaria*, and *Cladosporium* (Scudamore, 2008). They are very difficult to systematize uniquely because of their different chemical structure, biochemical pathway of synthesis, origin, and biological effects (Bennett and Klich, 2003). Mycotoxins are highly toxic food contaminants for humans and animals and pose a serious problem to food producers around the world. As almost all plants can be substrates for the growth of toxigenic molds, the synthesis of mycotoxins can occur in the field, during harvesting, transport, storage of raw materials, processing, storage of finished products and their distribution. Mycotoxins in foods of animal origin most commonly reach through contaminated feed. If animals consume food contaminated with mycotoxins, there is a high probability that they will accumulate in various tissues and organs (Scudamore, 2008; Bailly and Guerre, 2009). Since 1960, more than 400 different mycotoxins of various chemical structures and biological effects have been discovered, and it is estimated that there are about several tens of thousands of them. They differ from each other by the type of mold that synthesizes them, chemical structure, mechanism of action and toxicity. Although a large number of different mycotoxins have been identified, the greatest attention is paid to those that most commonly occur as food and feed contaminants (Marasas et al., 2008): aflatoxins, ochratoxins (OTA), zearalenone (ZEA), fumonisins, trichothecenes (deoxynivalenone), patulin. Molds such as *Aspergillus spp., Alternaria spp., Claviceps spp., Fusarium spp., And Penicillium spp.*, are among the most important producers of mycotoxins. Since the main source of mycotoxins in the food chain of humans and animals are agricultural products, ie. cereals and oilseeds and products of animal origin, prevention of mold growth as well as the formation of mycotoxins can be achieved by applying a number of measures of

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good agricultural practice, good production practice and HACCP principles (Duraković and Duraković, 2000.).

Aflatoxins

Aflatoxins are the best known and most toxic mycotoxins. Aflatoxins are named after a combination of the letter "a" derived from the genus *Aspergillus*, the word "fla" derived from the species *A.flavus* and the word "toxicum" meaning poison (Ellis et al., 1991). Aflatoxins are metabolic products of *Aspergillus flavus* and *A. parasiticus* that synthesize them already in the field, as well as during harvest and storage and processing of cereals, at a temperature between 24 and 35 °C and humidity above 7% (10% in a ventilated area). 18 aflatoxins are known, and the most important representatives are aflatoxins B1, B2, G1, G2, found in food and feed, and M1, M2 as metabolic products of aflatoxins B1 and B2 found in milk and dairy products (Heshmati and Milani, 2010). Aflatoxins are colorless to pale yellow crystalline substances that fluoresce intensely in the ultraviolet region. AFB1 and AFB2 emit blue, while AFG1 and AFG2 emit green, which is why they got their name. AFM1, as a derivative of AFB1, also emits blue (Mejía-Teniente et al., 2011).

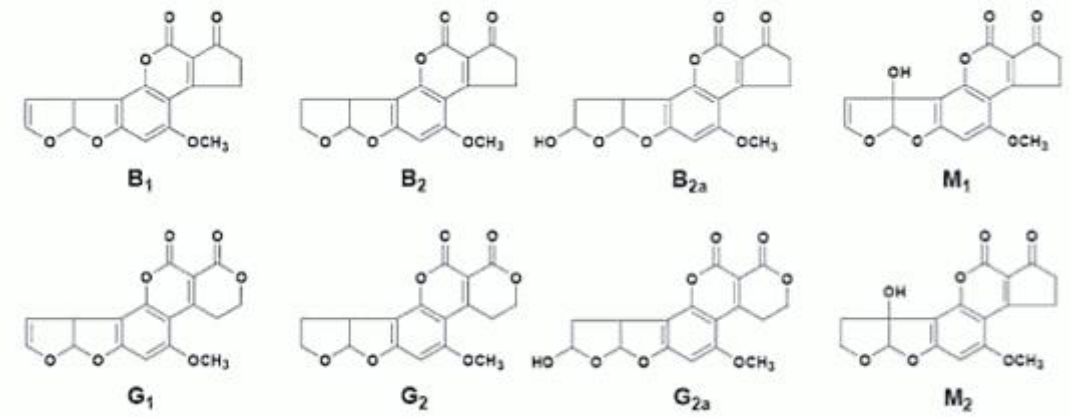

Figure 1. Structure of aflatoxins

Aflatoxins are very poorly soluble in water (10-30 µg/ml), are insoluble in non-polar solvents, and are best soluble in polar organic solvents such as methanol, acetone and acetonitrile. They are unstable at pH values less than 3 and higher than 10 (Cole i Cox, 1981).

Aflatoxin M1

Aflatoxin M1 is a highly toxic 4-hydroxylated metabolite of aflatoxins B1 and B2. Since it occurs in the milk of mammals fed food contaminated with said aflatoxins, the designation M is derived from the English word milk. Given that it is one of the most potent hepatokarcinogens, mutagens, teratogens and immunosuppressants, and animal feed is often contaminated with aflatoxinogenic fungi and aflatoxins, contamination of milk and dairy products with aflatoxin M1 is also possible. Therefore, its timely detection and determination of concentration in milk and dairy products for human

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consumption is very important (Prandini et al., 2009). Some authors claim that the appearance of AFM1 in milk also depends on the type of animal, and that AFM1 is most often present in cow's milk. They cite a different digestive tract in ruminants as a possible reason, as well as a higher amount of food intake, and a higher presence of nutrients (corn) that are more frequently contaminated with AFB1 (Barbiroli et al., 2007; Hussain et al., 2010). In addition, the season has a great influence on the appearance of AFM1 in milk. Namely, during the autumn and winter periods, the largest share in the diet of dairy animals is occupied by bulky food (corn, corn silage, mixtures) which is more often contaminated with AFB1, compared to plants that graze animals during the spring and summer months (Anfossi et al., 2011; Fallah et al., 2011). The European Food Safety Authority's (EFSA) CONTAM Panel in its 2004 document emphasizes that aflatoxin B1 is a particularly undesirable substance in animal feed, especially for dairy cows. The European Commission has set an upper level for aflatoxin M1 in milk, which is 0.05 μg / kg, based on an expert report from the Scientific Council for Food of the European Union. Namely, based on many scientific reports on risk assessment, it can be concluded that even very low levels of aflatoxins, ie. 1 ng / kg or even less can significantly increase the risk of developing liver cancer. According to the Ordinance on maximum permitted amounts of certain contaminants in food (February 12, 2009 "Official Gazette of BiH", No. 37/09 - Part 2. Mycotoxins, 2.1 Aflatoxin, 2.1.8 Fresh milk, heat-treated milk and milk for production milk-based products) the maximum permitted level of aflatoxin M1 in milk and dairy products is 0.05 μg / kg (MDK), which is in line with current EU regulations. Strict controls on the production of animal feed and monitoring plans for Aflatoxin M1 in milk and dairy products are being implemented in European countries, which is the reason for low levels of aflatoxin contamination. Countries with poorly developed food control systems should increase the frequency of sample controls through competent authorities and educate feed manufacturers and livestock farmers about the potential adverse effects of aflatoxins. (Bilandžić et al, 2013).

Milk

Milk is the most complete natural liquid because it contains all the substances necessary for maintaining health and normal function of the human body. It has a complex and variable composition, white to yellowish-white color, characteristic taste and smell. It is secreted from the mammary gland of female mammals for some time after birth, primarily for the purpose of feeding the young. Milk is a basic food product, which, in addition to energy-valuable substances, also provides the body with protective substances, which are essential for human health. Milk contains a number of physico-chemical ingredients, and in practice the usual data on the content of water, milk fat, protein, milk sugar (lactose) and ash content, as well as the total non-fat dry matter are used. Milk is characterized by density, viscosity, boiling point, freezing point and acidity, and as a summary, the content of fat-free dry matter. Milk contains an average of 87.40% water, with oscillations in the possible range of 77.5% to 91.9%, and water comes in two forms: free or bound water. Milk fat has the highest energy value in milk, it mainly consists of triglycerides (97% - 98%), while other ingredients are found in small amounts. The milk fat content is the most variable ingredient (Stojanović and

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Katić, 1998). Aflatoxin B1 and B2 are metabolized in the mammary glands of mammals in the animal's body to aflatoxin M1 and M2, and excreted in the mammary glands and can be found in milk and dairy products (Knežević, 2007; Diener and Davis, 1996; Valpotić and Šerman, 2006). AFM1 can be detected in milk 12 to 24 hours after aflatoxin B1 consumption, and peak levels are reached after a few days. After cessation of AFB1 intake, aflatoxin M1 concentration decreases within 72 hours to an amount when it can no longer be detected. If the level of aflatoxin M1 in milk is higher than allowed, the product must not be used for human consumption or for the production of dairy products. Studies have shown that there is no significant difference in aflatoxin M1 concentration between raw and boiled milk samples. The results of quantitative analysis of aflatoxin M1 show that boiling milk has no effect on the presence of this mycotoxin in milk. These results are in accordance with the literature data on the thermal stability of this molecule, which cannot be disturbed in the process of pasteurization and sterilization. (Govaris et al, 2002).

METHODOLOGY

As test materials we used milk samples from Bosnia and Herzegovina. In doing so, samples that can be found in free sale or UHT (ultra high temperature) milk are taken into account, but also samples from independent producers, ie. raw milk that can be preserved with azidiol tablets or bronopol, which can come into contact with a large number of people and thus pose a danger to their health. The analysis was performed in the Laboratory for chemical safety of food and residue control in the premises of the Institute of Veterinary Tuzla Canton using ELISA technique. Raw and UHT milk samples can be used directly without preparation. High-fat milk is centrifuged for 10 minutes at 4,000 rpm at room temperature (20 °C to 25 °C). After centrifugation, the upper layer of fat is removed and such milk is further used for analysis. For the purposes of the experiment, a soldered sample was prepared and used as a certified reference material (CRM). This sample is prepared from a 10 ppb solder standard, which is an integral part of a commercial putty. The ELISA method for the determination of aflatoxin M1 is a non-standard method and as such must be validated. The parameters determined for the validation of the method are repeatability, accuracy, CCβ detection ability and lower limit of LOD detection. Repeatability is the absolute value of the difference between two results obtained under repeatable conditions (same sample, same tester, same instrument, same laboratory and short time interval) with a probability of 95% expected to be less than r ($| x_1 - x_2 | < r$) where $r = 2.8 \cdot sr$. Accuracy is the quantitative value of the ratio of the results evaluated by the ELISA test and the results determined by the reference HPLC method. It is expressed as the degree of utilization (%). Detectability (CCβ) is defined as the lowest content of a substance that can be detected, identified and quantified in a sample with an error probability of β (5%). We selected 20 samples of UHT milk fat 2.8% (unenriched samples), replicates of these samples were enriched / soldered to an orientation concentration (half the legal limit of 0.05μg/kg) of 0.025μg/kg. The same samples were analyzed. The lower limit of detection is the analytical sensitivity, ie the lowest concentration that can be measured. The lower limit of LOD detection was determined in the laboratory by

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performing 10 consecutive measurements of the sample, which was prepared from a 10 ppb splice standard, which is an integral part of a commercial kit. It was prepared by first checking the matrix (UHT milk fat 1.5% milk fat) and determining that it does not contain aflatoxin M1, then made a dilution in the matrix to a concentration of 0.005 ppb (LOD from the manufacturer's instructions). After 10 consecutive measurements, determine the mean value, standard deviation and measurement uncertainty.

For the determination of aflatoxin M1 in milk, the Bioo Scinetific kit was used, which is stored in the refrigerator at a temperature of +2 ºC to +8 ºC. Prior to work, the putty and samples need to reach room temperature. Standards and samples are applied to a microtiter plate in double wells. Add 200 µl using a micropipette of each Aflatoxin M1 standard to the double wells of the microtiter plate (standards are added in order from lowest to highest concentration), taking care to use a new micropipette tip when applying each standard. Add 200 µl of the soldered sample to the double wells and add 200 µl of each sample also to the double wells of the microtiter plate and incubate the microtiter plate for 45 minutes at room temperature (20 ºC to 25 ºC). After incubation, wash the microtiter plate 3 times with 250 µl of previously prepared 1x Wash Solution and dry the wells well. After washing, 100 µl of Aflatoxin M1-HRP conjugate was added to each well and the microtiter plates were incubated for 15 minutes at room temperature (20 °C to 25 °C). After incubation, rinse again in the manner previously described and take the next step immediately after rinsing and do not allow the plate to air dry. Add 100 µl TMB of substrate, carefully manually stir the microtiter plates for 1 minute and incubate for 15 minutes (including this minute) at room temperature (20 ºC to 25 ºC). Immediately after incubation, add 100 µl of Stop Buffer to stop the enzymatic reaction and read the optical density at 450 nm on an ELISA reader, as soon as possible after the addition of Stop Buffer. The read values are entered into the appropriate software that has been validated. Based on the value of the standard, a calibration curve is formed, on the basis of which the sample concentrations expressed in µg/kg are calculated.

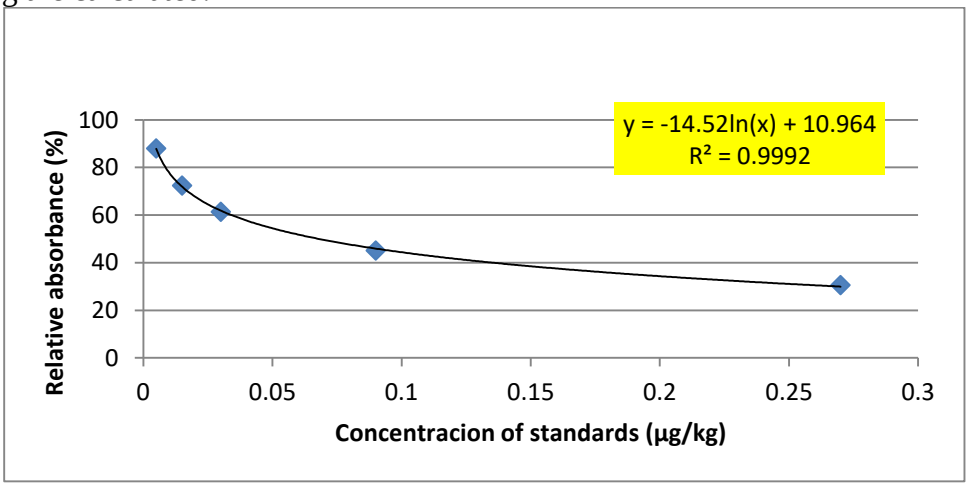

Figure 2. Calibration curve

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RESULTS/FINDINGS

The results of the research for the repeatability parameter during the validation of this method are shown in Table 1.

Table 1. Repeatility

Measurement	Concentration (µg/kg)
1.	0,008
2.	0,007
Average value	0,0075
Sr	0,000707
r	0,0019
$\lvert x_1 - x_2 \rvert$	0,0010

The results of the research for the accuracy parameter during the validation are shown in Table 2.

Table 2. Accuracy

No of samples	Reference value	Lab. value	Difference (Xlab-Xref)	Cv (Xlab-Xref)/ Xref · 100	Recovery % (Xlab/Xref) · 100
1	0,057	0,055	-0,002	-3,508772	96,49
2	0,005	0,006	0,001	20,000000	120
3	0,015	0,011	-0,004	-26,666667	73,33
4	0,03	0,034	0,004	13,333333	113,33
5	0,090	0,108	0,018	20,000000	120
6	0,270	0,245	-0,025	-9,259259	90,74

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The results for determining the ability to detect CCβ are shown in Figure and Table 3.

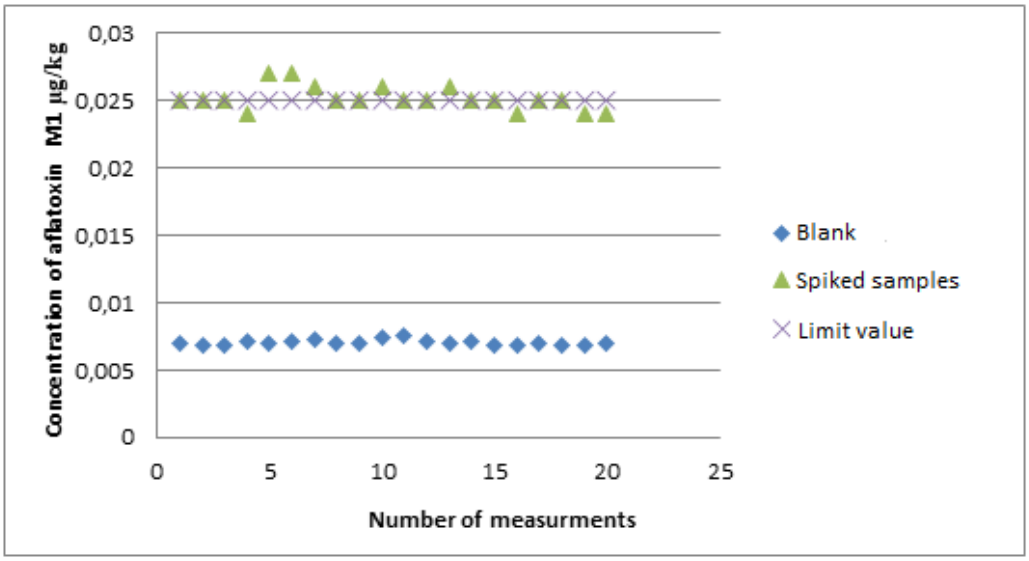

Figure 3. Graph of CCβ determination by concentrations

Table 3. Determination of detection capability

CALCULATION		
Mean value of blank		0,00704
Mean value of the soldered sample		0,0251
Sd blank		0,000198415
Sd solderd sample		0,000660144
a)	*T=*	B+1,64·Sdb
		0,00704+1,64·0,0001984
		0,0073654
b)	*Fm=*	M+1,64·Sd
		0,0251+1,64·0,000660144
		0,0262

Fm > T; 0,0262 > 0,0073654; CCβ is under 5%

The results of the research for the LOD are shown in Table 4.

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Table 4. LOD

No. of measurment	Results
1.	0,006
2.	0,006
3.	0,006
4.	0,007
5.	0,006
6.	0,006
7.	0,006
8.	0,006
9.	0,007
10.	0,006
Sr. Vrijednost	0,0062
Sd	0,00042
	1637
Cv	6,80059
	7119

The results of the research by the analyzed years are shown in Figures 4, 5 and 6.

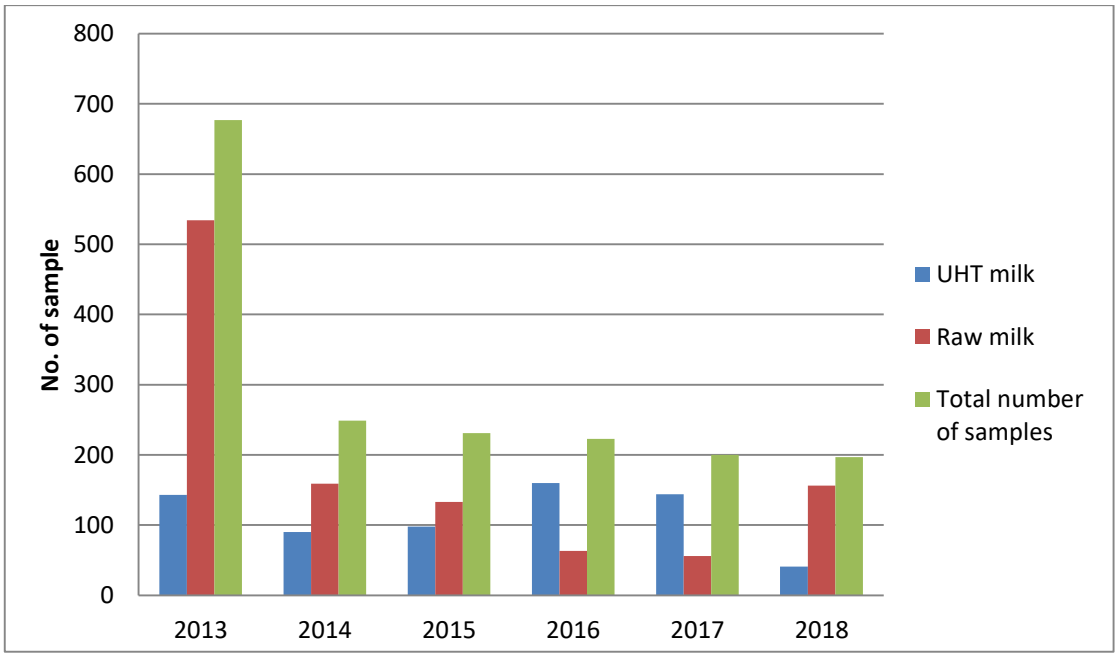

Figure 4. Number of samples in the period from 2013-2018

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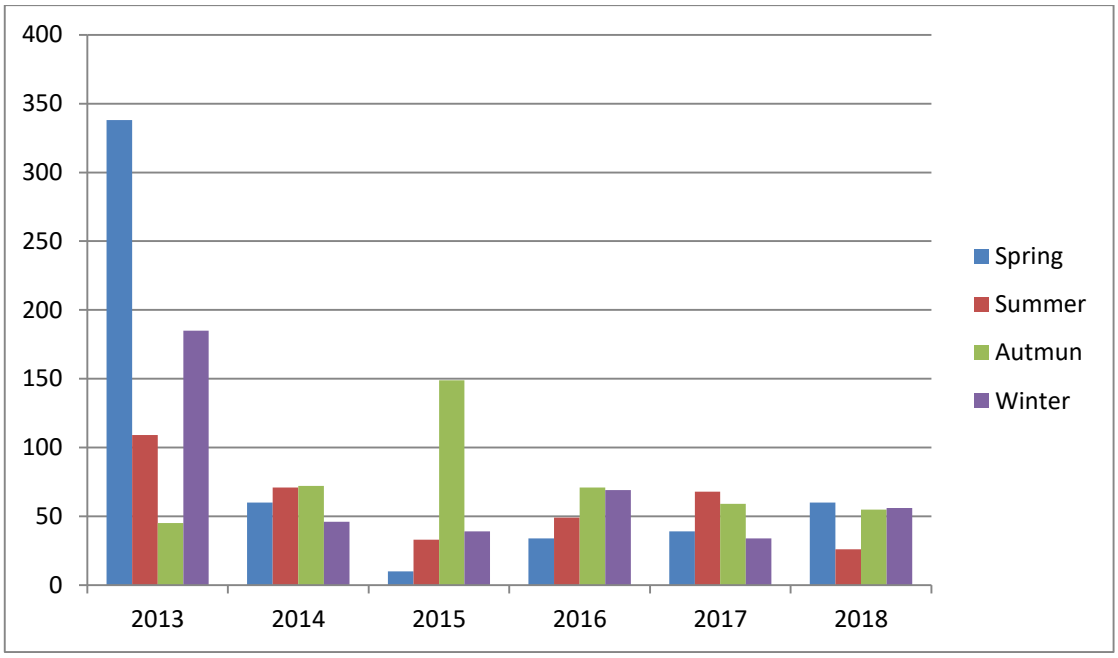

Figure 5. Number of milk samples by seasons in the period from 2013-2018

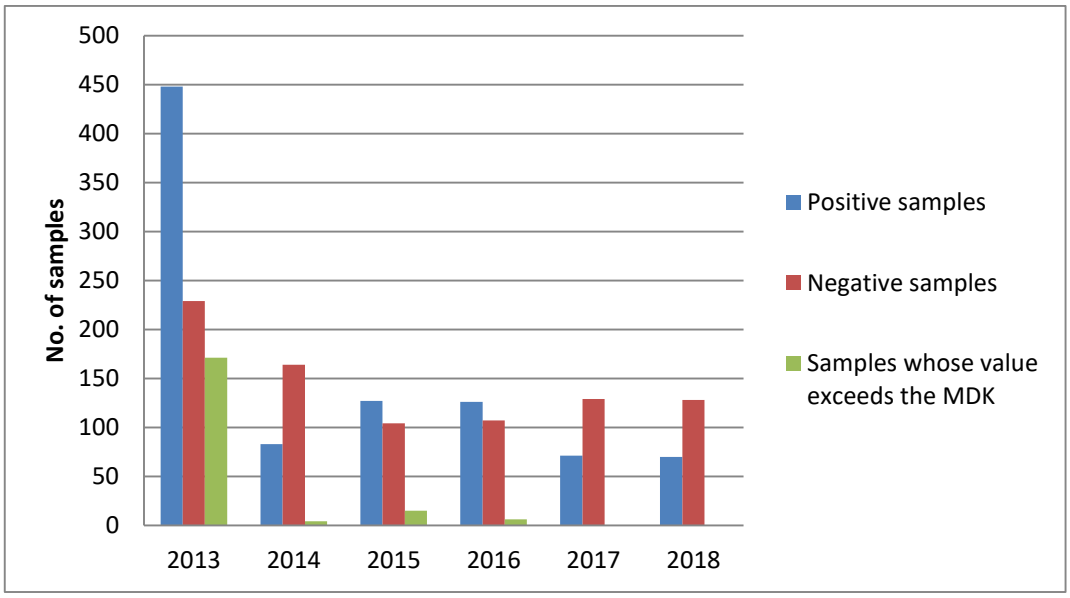

Figure 6. Number of milk samples analyzed for the presence of Aflatoxin M1 in the period 2013-2018

DISCUSSION

Calculations for validation of the method for determination of Aflatoxin M1 in soft ELISA technique were performed according to the Rulebook on methods of sampling and analysis for official control of mycotoxins in food (Official Gazette of BiH No. 37/2009) and based on established values for repeatability can be concluded that the

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method met the prescribed repeatability criterion $|x_1 - x_2|<r$. Accuracy, ie utilization satisfies the criterion of acceptability in the ratio of 60-120%. The CCβ parameter satisfies the acceptability criterion. Based on the determined values and according to the data from Tables 1, 2, 3 and 4, for the parameters repeatability and accuracy (degree of utilization%) and based on the determined values for CCβ = 0.026 and LOD = 0.0062, it can be concluded that the method for Determination of aflatoxin M1 in milk by ELISA test met the prescribed criteria for the mentioned parameters from the Ordinance on methods of sampling and analysis for official control of the amount of mycotoxins in food (Official Gazette of BiH, No. 37/09).

The largest number of analyzed milk samples in 2013 was a total of 677 and the lowest in 2018 a total of 197, which is 70.90% less, which can be related to the so-called. "Affair" of increased concentrations of Aflatoxin M1 in milk, where in 2013 a large number of samples were found that contained this mycotoxin and far above the MDK. The appearance of Aflatxin M1 in the analyzed cow's milk samples from 2013 can be related to dry weather conditions and the appearance of aflatoxins in corn (Kos et al., 2013). Based on the presented graph (Figure 4), a decrease in the number of samples can be observed from 2013 to 2018. It is assumed that the decline in samples by 2018 is associated with increased control of raw materials and finished products by dairies and purchase organizers. When it comes to raw milk samples, the following series of samples can be determined by years in 2013. > 2018 > 2014 > 2015 > 2016 > 2017 The number of UHT milk samples by years has the following series in 2016. > 2017 > 2013 > 2015 > 2014 > 2018 Most of the published scientific data on the occurrence of Aflatoxin M1 in milk comes from the Mediterranean and the Middle East, where weather conditions are favorable for the development of Aspergillus species, aflatoxin synthesis, and thus the occurrence of Aflatoxin M1 in milk (EFSA, 2010). Published papers indicate that frequent occurrences of Aflatoxin M1 in milk and dairy products have been reported in Iran, India, Thailand, Brazil, Syria, and Kenya (Duarte et al., 2013). In contrast, literature data confirm that the occurrence of Aflatoxin M1 in milk from European countries is significantly rarer, or that this mycotoxin is mainly present in concentrations below the MRL (0.05 μg/kg).

In the spring period in 2013, the largest number of milk samples was analyzed, 338 of them, in the same year, the largest number of samples in the winter period was analyzed in relation to other years, more precisely 185 samples. When it comes to the autumn period, the largest number of samples was analyzed in 2015, 149 samples. The largest number of samples, 109 of them in the summer period, was analyzed in 2013. Numerous literature data indicate the influence of weather conditions on the occurrence of aflatoxins in cereals, which are used as components of animal feed and directly affect the occurrence of Aflatoxin M1 in milk (EFSA, 2010). Aflatoxin pollution is affected by the cold and warm seasons by making fresh food available for livestock in spring and summer, such as grazing, grass, weeds and raw nutrients, while in the cold months, animals are much more likely to consume dry, prepared nutrients or concentrates. . In Bosnian rural areas, a diet of dry hay is common, which, if stored improperly under inadequate conditions, can result in the appearance of aflatoxins. Important factors on

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which the level of aflatoxin pollution depends are temperature and humidity. Molds such as Aspergillus flavus and Aspergillus parasiticus grow easily at 28 °C on nutrients with a moisture content between 13-18% and a suitable relative humidity between 50 and 85%. Also, if soil moisture is present and nutrient damage caused by insects also increases the possibility of developing such molds. However, the most common cause of pollution is still inadequate storage conditions. That is why controls are carried out in the European Union and around the world to monitor the production of animal feed and the content of Aflatoxin M1 in milk. Bilandzic et al. (2010) determined the concentration of Aflatoxin M1 on 61 milk samples on dairy farms in the Republic of Croatia in the period winter-spring and summer.

On milk samples from the winter-spring period, the content was on average 0.018 μg/l, and from the summer period 0.04 μg/l. The largest number of positive samples appeared during 2013, and the smallest in 2018. During 2017 and 2018, there was not a single sample whose value was above the MDK, which is proof that more is being done to prevent the occurrence of Aflatoxin M1. A worrying fact is that in 2013, as many as 177 samples appeared whose value was above the MDK. The maximum concentration of Aflatoxin M1 found in the period from 2013 to 2018 was 0.556 μg/kg, which is 11 times higher than the limit value (0.05 μg/kg), while the minimum concentration found was 0.006 μg/kg. Bilandžić et al. (2015) especially emphasize 2013 as a turning point year with the maximum measured concentrations of Aflatoxin M1 in raw milk. In their 2015 paper, Bilandžić et al. examine milk samples from October 2013 to September 2014. Special emphasis is placed on milk taken in 2013, primarily in the eastern part of Croatia, where maximum concentrations of Aflatoxin M1 in milk of 0.764 μg / kg were measured. This research also states, but also in others related to that period, that such high contents led to an alarming situation and that after that a more careful approach was taken to control primarily animal feed, but also milk as a product containing Aflatoxin M1. Bilandžić et al. Have determined in their further research that the elevated content of Aflatoxin M1 has been constantly maintained in fresh milk samples for the last few years (2016). The authors examined 548 milk samples taken during February and March 2015 and determined the average content of Aflatoxin M1 of 0.0369 μg/kg in the western, 0.0311 μg/kg in the central and again the highest in the eastern region of Croatia where the average content of 0.0414 μg/kg. The average content indicates the permissible content of Aflatoxin M1 in milk. However, this does not mean that individual samples were not above the NDK for milk. It is generally believed that approximately 1-3% of Aflatoxin B1 present in animal feed occurs as Aflatoxin M1 in milk (Bilandžić et al., 2013). After consuming food in which Aflatoxin B1 is present, the milk is contaminated with the hydroxyl metabolite Aflatoxin M1. In cows with high milk yield, due to significantly higher feed consumption, carry-over can occur up to 6.2% (EFSA, 2004). If food contaminated with Aflatoxin B1 is consumed, Aflatoxin M1 will appear in the milk two to three days after ingestion. Also, two to three days after consuming aflatoxin-free foods, there is a decrease in Aflatoxin M1 concentrations in milk. Humans can be exposed to Aflatoxin M1 through endogenous production or intake of dairy products. It is assumed that children are most exposed because they are the largest consumers of milk and dairy foods, and infants should not

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be neglected due to the excretion of toxins in the milk of breastfeeding women. The process of pasteurization or processing into cheese is considered to act on Aflatoxin M1 in raw and processed milk. Nevertheless, due to its poor solubility in butter and good absorption in cottage cheese, products such as butter, cottage cheese and whey show deviations in the content of Aflatoxin M1 compared to the original milk. Due to their thermal stability, aflatoxins also occur in milk treated with ultra-high temperatures and fermented milk products. The transmission of aflatoxins from animal feed to milk in dairy cows is influenced by various physiological and nutritional factors, including feeding regime, degree of digestion, animal health, biotransformation capacity of the liver and milk production.

Chemical processes can be used to reduce, destroy or inactivate aflatoxins in milk. Big number chemicals such as acids, bases and oxidants tested for degradation or inactivation aflatoxin (Varga et al, 2020.)
Antioxidants such as vitamins C and E can be added to food to prevent oxidation reactions with other substances which are dangerous to human cells. Others in words antioxidants can help detoxifying toxins in the liver and others cells and consequently reduce the occurrence mycotoxicosis (Naeimipour et al., 2018).

CONCLUSION

Based on the established values, for the parameters of repeatability, accuracy, CCβ and lower limit of detection, it can be stated that the method for Determination of aflatoxin M1 in milk by ELISA test met the prescribed criteria for the mentioned parameters from the Ordinance on sampling and analysis methods for official control of mycotoxins in food (Official Gazette of BiH, No. 37/09), Guide for Validation of Orientation Methods for Residues and Instructions to Producers. The largest number of analyzed samples was in 2013, a total of 677 and the lowest in 2018, a total of 197, which is 70.90% less, which can be related to the so-called. "Affair" of increased concentrations of aflatoxin M1 in milk, where in 2013 a large number of samples were found that contained this mycotoxin and far above the MDK. When it comes to samples of raw milk, the following series of samples can be determined by years in 2013 > 2018 > 2014 > 2015 > 2016 > 2017 The number of UHT milk samples by years has the following series in 2016 > 2017 > 2013 > 2015 > 2014 > 2018 In the spring period in 2013, the largest number of milk samples was analyzed, 338 of them, in the same year, the largest number of samples in the winter period was analyzed in relation to other years, more precisely 185 samples. When it comes to the autumn period, the largest number of samples was analyzed in 2015, 149 samples. The largest number of samples, 109 of them in the summer period, was analyzed in 2013. The largest number of positive samples appeared during 2013, and the smallest in 2018. During 2017 and 2018, there was not a single sample whose value was above the MDK, which is proof that more is being done to prevent the occurrence of aflatoxin M1. The maximum concentration of aflatoxin M1 found in the period from 2013 to 2018 was 0.556 µg/kg, which is 11 times higher than the limit value (0.05 µg/kg), while the minimum concentration found was 0.006 µg/kg.

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