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DETECTION OF AFLATOXIN B1 IN DIFFERENT ANIMAL FEEDSTUFFS IN THREE REGIONS OF BEKAA VALLEY–LEBANON

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ABSTRACT

Present study aims to determine the levels of AFB1 in different animals' feedstuffs (Soybean, Corn, Cotton seeds, Barley bran, Wheat bran and Hay). For this purpose 90 samples were collected from 15 small farms of three regions of the Bekaa Valley Baalbeck (R1), Zahleh (R2) and West Bekaa (R3) between June 2022 and July 2022. Samples were placed in plastic bags and transported to the laboratory within 12 hours. Samples were stored at 4℃ and analyzed for AFB1 within seven days after collection using a competitive Enzyme–Linked Immunosorbent Assay (ELISA) technique. Mean of AFB1 levels were exceeded the maximum for European Union accepted levels for all samples and USA and Lebanese permissible levels for Soybean, Cotton seed, Corn and Hay but showed no statistical significance when compared to this limit (p>0.05). This finding shows that feed producers and dairy farmers should maintain sustainable good procedures for all feed harvesting, storage, and feeding techniques in order to avoid aflatoxin contamination.

Key words: Aflatoxin; AFB1; AFM1; ELISA; Feed.

Introduction

Detection of aflatoxin B1 in animal feed is very important. Therefore, the level of AFB1 in the feed for dairy animals is directly correlated with the presence of AFM1 in the milk (Ismail *et al.*, 2016). In the aflatoxin group, there are 16 compounds are known (aflatoxin B1, B2, G1, G2 and M1 are monitored routinely) and the most important toxic member is AFB1 (Bennet and Klich, 2003). According to studies (Iqbal *et al.*, 2012; Hammami *et al.*, 2014), the AFB1 has the most hazardous and cancer–causing properties for both people and animals. It has also been linked to the emergence of human hepatic and extra–hepatic carcinogenesis. Additionally, it slows the animal development rate, lowers milk quality, and reduces milk production (Whitlow and Heagler, 2004; Akande *et al.*, 2006). AFB1 is biodegraded in the liver and transported into milk as aflatoxin M1 (AFM1) after being present in dairy animal meals. According to an animal model, the conversion factor of AFB1 into AFM1 ranges from 0.3 to 6% (Var and Kabak, 2009). According to the Food and Agriculture Organization (2004) the maximum allowable amount of AFB1 in feed was established at 20 ppb. United State of America (USA) Food and Drug Administration (FDA) imposed an aflatoxin limit of 20 parts per billion (ppb) for foods, as well as for the majority of feeds and feed ingredients. On the other hand, the European Union (EU) set a 20 µg/kg maximum limit for AFB1 in all feed materials, complete and complementary feeding stuff for cattle, sheep, goats, pigs, and poultry (except for young animals), and 5 µg/kg for complete feeding stuff for dairy animals (Food Standard Agency (FSA), 2004)). The aim of this study is to detect the presence of AFB1 in animal feed in order to know the origin of AFM1 in raw milk samples or to find out the most common source of milk contamination.

Materials and Methods

Sample collection and preparation

In the study, 15 farms from three regions (5 farms from Baalbeck (R1), 5 from Zahleh (R2), and 5 from West Bekaa (R3) were selected, and from each farm 6 feeds ingredients were collected (**Total 90 samples**). Those ingredients are Barley bran, Soyabean, Corn, Cotton seeds (imported), wheat bran and Hay (local origin). For all ingredients, the sampling is from the farm not from the origin source. All samples were collected between the end of June 2022 and the beginning of July 2022. Most of the farms from which collected the sample were small–scale farms from the three regions. Samples were placed in plastic bags (200 g of each ingredient) and transported to the laboratory within 12 hours. The samples were stored at 4 ℃ and analyzed for AFB1 within seven days after collection. Sample preparation (extraction, filtration, and dilution), test preparation, and procedure were done according to RIDASCREEN®FAST Aflatoxin SC Kit (Art. No.: R9002) (R–Biopharm AG, Darmstadt, Germany) which is a competitive enzyme–linked immunosorbent assay for the quantitative analysis of aflatoxin in feed. Special software the RIDASOFT®Win.NET Food & Feed (Art. No. Z9996FF) (R–Biopharm AG, Darmstadt, Germany) was used to calculate the content of aflatoxin in the samples.

Data evaluation and Statistical analysis

All statistical analyses were performed with software GraphPad Prism 8.4.2. We applied a One–sample t–test to compare the mean concentration of AFB1 in feedstuffs to the USA and Lebanese legal limit (20 µg/kg), and to determine if they are significantly different. Analysis of Variance (One–Way ANOVA) was employed to investigate for statistical differences among feedstuffs AFB1 mean levels. A probability of $p<0.05$ was considered significant.

Results

The determined levels of AFB1 in the samples, collected from 15 farms, were variable and found in all samples. One–sample t–test results, minimum, maximum, and mean values of AFB1 have been shown in Table 1.

Table 1: Mean levels of AFB_1 (µg/kg) in feedstuffs ($\bar{X}$ ± SD).

FEEDSTUFFS	N	Number of sample above limit of 20 µg/kg	% above limit of 20 µg/kg	($\bar{X}$)	SD	Min	Max	*p–value*
Barley bran	15	5	33.33	16.4	7.05	8.30	29.3	0.069
Wheat bran	15	6	40	16.4	9.10	4.32	32.5	0.150
Soybean	15	7	46.67	20.5	15.3	2.56	46.5	0.908
Corn	15	8	53.33	24.7	15.5	5.90	58.5	0.262
Cotton seed	15	9	60	25.1	15.6	5.81	50.3	0.229
Hay	15	9	60	27.0	12.8	10.5	49.6	0.052

N – *Number of samples*; ($\bar{X}$) – *Mean* (*µg/kg*); SD – *Standard Deviation*; Min – *Minimal levels* (*µg/kg*); Max – *Maximal levels* (*µg/kg*).

Out of 90 samples, 44 samples (48,88%) showed contamination exceeding the limit accepted by USA and Lebanese limit (20 µg/kg).The six type of feedstuffs as cited above: Barley bran, Wheat bran, Soybean, Corn, Cotton seed, and Hay revealed from 33.33 to 60% contamination rate, respectively (Table 1). The maximum level of AFB1 in those samples can be presented in this order: Corn > Cotton seed > Hay > Soybean > Wheat bran > Barley bran. The highest (maximum) level was detected in a sample of Corn, and the lowest (minimum) level was detectd in a sample of Soybean (Table 1). The lowest mean of AFB1 was detected in Barley bran and Wheat bran , while in the Hay aflatoxin AFB1 showed the highest mean levels (Table 1). AFB1 mean concentrations in Barley bran and Wheat bran were higher than the European Union legal limit level (5 μg/kg), but lower than the USA and Lebanese legal limit (20 μg/kg). Interestingly, AFB1 mean levels in the samples of Soybean, Corn, Cotton seed, and Hay were higher than the US and Lebanese legal limit (20 μg/kg), but showed no statistical significance when compared to this limit (Table 1). Mean value of AFB1 levels in Hay were close to be significantly higher than the USA and Lebanese limit. Notably, AFB1 mean concentrations exhibited an upward trend when comparing samples of Soybean, Corn, Cotton seed, and Hay, but no significant difference was detected among means.

Discussion

Aflatoxine contamination in several types of food and feed is inevitable. As a result, much study is conducted to identify this toxin in food and feed (Hell and Mutegi, 2011; Farombi, 2006). In the present study maximum AFB1 concentration in corn samples was higher than the results reported by Scudamore *et al.* (1997) in the United Kingdom (41 μg/kg), and Oruç *et al.* (2006) in Turkey (32.30 μg/kg as total aflatoxin), but lower than Shetty and Bhat (1997) in India (109 μg /kg), and Dawlatana *et al.* (2002) in Bangladesh (245 μg/kg). According to Kaaya and Kyamuhangire (2006), maize samples stocked for more than six months were observed to have detectable levels of aflatoxin AFB1 more than 20 μg /kg. In this study, the highest level corn (58 μg/kg) was seen in a sample of corn in more than half the farms which were included in the study (53, 33 % positive samples). This is due to for long period and bad storage of corn in all majority of the farm. Mean level of AFB_1 with Standard Deviation (SD) for corn (24.7±15.5) were higher than the USA and Lebanese legal limit (20 μg/kg), and higher than the European Union (5 μg/kg), but showed no statistical significance when compared to this limit. According to Kabak *et al.* (2006), moisture, poor storage conditions, and poor management all contributed to the elevated levels of aflatoxin, observed in competitive cow's feed, feed additives, and maize. In another study conducted in Iraq, AFB1 was found in 12 out of 24 samples of maize, with levels ranging from 2.30 to 30 ppb detectable through thin layer chromatography (TLC) and 270 to 500 ppb by means of Enzyme–Linked Immunosorbent Assay (ELISA) (Hassan *et al.*, 2014). It is well known that a variety of conditions, including temperature, humidity, insect damage, handling during harvest, and storage, affect the establishment of *Aspergillus spp*. and the subsequent formation of aflatoxins in maize (Hell *et al.*, 2003). Concerning the cotton seeds, in this study 60% of the sample was contaminated and exceeded the limit (20 μg/kg). The mean value and SD of AFB1 concentration was (25.1±15.6 μg/kg), and it was higher more the USA (20 μg/kg) and European Union (5 μg/kg) permissible limit, but no significant difference was detected among means. In comparison with another study from Iran, cotton seeds had highest levels of contamination, even higher than the maximum tolerated levels. All the cotton seed samples (100%) were contaminated by AFB1, which detected by High Performance Liquid Chromatography (HPLC) (Sadegh *et al.*, 2013). According to Azizi *et al.* (2012), cotton seeds show a contaminated level of AFB1 compared to concentrated feed and beet pulp. In a study by Hashemi, (2015), the Cotton seeds had an AFB1 level (2.13±0.31 μg /kg) lesser than the present study (25.1±4.03 µg /kg). In the present study, wheat bran samples showed 40% contamination, and they reached a maximum level of AFB_1 at 32.5 µg/kg. The mean ± SD of AFB1 concentration was 16.4±9.10 µg /kg , but no statistically significant difference was detected in the found values. In comparison and According to some authors (Aydin *et al.* (2008); Joubrane *et al.* (2011), and Almeida–Ferreira *et al.*, (2013)) have high levels of contamination of wheat and their derivatives with AFB1. In an Indian study, Aflatoxin B1 (AFB1) levels in 1646 samples of wheat grains were examined. 40.3% of the samples had AFB1 levels below 5 µg/ kg, while 16% had concentrations over 30 µg /kg, allowed regulatory limit for India (Toteja *et al.*, 2006). Even though in wheat had a high prevalence of AFB1 (44.8%), wheat samples had the lowest maximum limits of 6.0 µg /kg (Elbashir and Ali, 2014). In a study by Hashemi, (2015), Wheat bran had an aflatoxin AFB1 level (2.94±1.38 µg /kg) lesser than our study (16.4±2.35 µg /kg). In this study, mean levels of AFB1 in Soybean is (20.5±15.3 µg /kg), and were higher than the US and Lebanese legal limit (20 µg/kg), and higher than EU limit (5 µg/kg), but showed no statistical significance when compared to this limit concentrations. According to Abdullah Murshed *et al.* (2019), 72% of soybean samples were contaminated with aflatoxins, and 27.6% of them exceed the European Standard, and in only 6.2% of the soybean samples taken, the values of total aflatoxins were above the maximum limit by the FDA standards in Yemen (20 µg/kg). In the present study, the highest level of AFB1 was found in the samples of Soybean (46.5 µg/kg), which was higher than that in United Kingdom (4 µg/kg) reported by Scudamore *et al.* (1997), and slightly higher than that in Turkey (46.3 µg/kg) reported by Oruç *et al.* (2007), and higher than the level from Iran (11.46 µg/kg) reported by Hashemi, (2015) . Regarding barley bran, 33.33% of the contaminated samples by AFB1 exceeded the USA and Lebanese permissible limit (20 µg/kg). Those results were higher compared with the result of Sadegh *et al.* (2013), who established 0% contamination in barley samples, and higher than the result reported by Hashemi (2016), where barley samples show contamination with AFB1 less than Iran regulations and European Union limitations (5 µg/kg) (1.31±0.33 µg/kg). Regarding Aflatoxin B1 detection in the hay, in our study all samples were found contaminated, and 60% of them were with mean value 27.0 µg/kg, and exceeded the USA and Lebanese permissible limit of 20 µg/Kkg. Our results are higher than those reported by Ceniti *et al.* (2021), which they found in all hay samples levels of AFB1 contamination ranging from 2 to 7.7 µg/kg, which are within the limitations set by the European Union (20 µg/kg). In a study done by Karademir *et al.* (2003), fresh Hay shows average values less than the allowed Turkey limits (50 µg/kg). Old Hays' average values were below the limit of Turkey (50 µg/kg), but they were above the maximum limits (20 µg/kg). In Northern Italy, Decastelli *et al.* (2007), reported that 8.1% of the feed samples examined were positive for AFB1. In Iran the concentration of AFB1 in the Hay was higher than the limit of the European Union with 10 % (Bahrami *et al.*, 2016). Contrarily, some investigations have shown a sizable amount of contaminated samples, rarely reaching the set legal limit. 42% of the samples from China included AFB1 levels between 0.05 and 3.53 µg /kg were below the legal limit in Chinese and European standards (10 µg/kg) (Han *et al.*, 2013). In a 10–year investigation in Portugal, Martins *et al.* (2007) discovered 37.4% positive samples with contamination, ranging from 1 to 74 µg/kg, and only 6.2% of these samples surpassed the legal limit of Portuguese (5 µg/kg). Hay samples contamination with mycotoxin in our study, especially with AFB1, is a result of bad storage conditions of the Hay in the farms, e.g., the high percentage of moisture that has not been studied.

All the samples in our study, collected during this period (between the end of June and the start of July), were contaminated with AFB1.

Most of the farms from which were collected the samples were small–scale farms from the three regions (R1, R2, R3). The collection of the samples was from the farm, not the original source. In correlation with the observations that we have made about feed management in the different farms, especially storage, all those are in agreement with the results obtained from this study, after testing feed that shows contamination with AFB1 during this period. According to Richards *et al.* (2009), feed storage management and storage conditions facilitated mold development and, as a result, they are the cause of aflatoxin production.

Conclusion and recommendations

In this study, the levels of AFB1 in different feedstuffs are slightly higher than the acceptable limit concentration for cattle consumption and correlate with the occurrence of AFM1 in raw milk that has been studied before during this period. However, the toxin concentration on additional types of feedstuffs in different geographic locations, more different scale farms and management, conditions for storage, and more seasons must be studied in the future. To avoid a rise in AFB1 in animal feeds, and the occurrence of AFM1 in raw milk, it should support the small dairy farms by giving technical assistance and training to farmers, particularly those in remote regions. Periodic visits and inspections must be carried out, and animal feed and raw milk samples must be collected and examined on a regular basis to ensure their safety. For the storage sites: should enable safe storage for feed by assuring their safety before storage, installing humidity and temperature sensors, and implementing good storage practices. Authorities must also give guidance, and ongoing inspections of various storage facilities must be performed.

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