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Occurrence of Aflatoxin B_1 and T-2 Toxin in Feed and Raw Ingredients Used For Animal Feeding Stuffs

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Summary: Examination of 93 samples of animal feed and feed ingredients for the presence of aflatoxin B_1 (AFB_1) and T-2 toxin has been carried out. These samples were collected from four feed mills and 17 farmers of milk producer in Bursa region, Turkey, and analyzed by competitive enzyme-linked immunoabsorbent assay (ELISA) technique. AFB_1 and T-2 toxin were determined in all samples of dairy cattle feed (except one of them for T-2 toxin), fattening feed, fawn feed, broiler feed, rice bran, rice grain, barley-wheat grain, maize and dry distiller grain soluble (DDGS), soya meal and sunflower meal. The highest levels detected for AFB_1 and T-2 toxin were 431.90 µg/kg in a sample of maize and 131.00 µg/kg in a sample of rice bran, respectively. The mean levels of AFB_1 have shown that the feeds and raw ingredients of samples may lead to some toxic effects for beef cattle, broiler, fawn, dairy cattle, and human because of the conversion of AFB_1 in dairy cattle feed and feed-stuffs to aflatoxin M_1, and aflatoxin M_1 secrete into milk.

Key Words: Animal feed; Feedstuffs; Aflatoxin B_1; T-2 toxin.

Bazı Yem ve Yem Hammaddelerinde Aflatoksin B_1 ve T-2 Toksin Düzeyleri

Özet: Bu çalışmada, hayvan yemi ve yem hammaddesi olarak kullanılan 93 örnek aflatoksin B_1 (AFB_1) ve T-2 toksin yönünden incelendi. Bu örnekler, Bursa bölgesindeki dört yem fabrikası ve 17 süt üreticisi çiftçiden toplandı ve ELISA tekniği ile analiz edildi. AFB_1 ve T-2 toxin, süt sığırı yemleri (bir numunede T-2 toksin tespit edilemedi), besi yemleri, buzağı yemleri, broiler yemleri, pirinç kepeği, tane pirinç, tane arpa-buğday, tane mısır ve dry distiller grain soluble (DDGS) (mısırdan elde edilen bir çeşit yem hammaddesi), soya küspesi ve ayçiçeği küspesi numunelerinin tamamında tespit edilmiştir. AFB_1 ve T-2 toksin için tespit edilen en yüksek miktar sırayla 431.90 µg/kg (tane mısır) ve 131.00 µg/kg'dır (pirinç kepeği). Yem ve yem hammaddelerindeki AFB_1, besi sığırları, etlik piliçler, buzağılar ve süt sığırları üzerinde toksik etkiye neden olabilecek düzeylerdedir. Süt sığırı yem ve hammaddelerindeki AFB_1'in aflatoksin M_1'e dönüşerek sütle atılmasıyla insanlarda toksik etkilere neden olabilecektir.

Anahtar Kelimeler: Yem; Yem hammaddeleri; Aflatoksin B_1; T-2 toksin.

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Introduction

Mycotoxins are toxic metabolites produced by certain types of fungi genera such as *Aspergillus, Penicillium* and *Fusarium,* which invade crops in field and may grow on foods and feedstuffs during storage under favorable conditions of temperature and humidity. Aflatoxin contamination is common in Latin America, Africa, Asia, and Australia[12]. Aflatoxins are toxic compounds which are produced as secondary metabolites by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, known to be carcinogenic, mutagenic, teratogenic and immunosuppressive[2,9]. In the aflatoxin group, approximately 16 compounds are known, but only aflatoxin B_1, B_2, G_1, G_2 and M_1 are routinely monitored. Aflatoxin B_1 (AFB_1) is the prevalent compound in the samples and the most toxic member of the group.

The European Union (EU) and Turkey have established maximum limits of AFB_1 in all feed materials. In EU, the maximum limit of AFB_1 was set as 20 μg/kg in all feed materials, complete and complementary feedingstuffs for cattle, sheep, goats, pigs and poultry (except for young animals); 5 μg/kg in complete feedingstuffs for dairy animals; 10 μg/kg complete feedingstuffs for calves and lambs and, other complete feedingstuffs[10]. In Turkey, the limits were set as 50 μg/kg in all feed materials, complete feedingstuffs for cattle, sheep, goats (except for young animals); 20 μg/kg in complete feedingstuffs for poultry (except for young animals) and 10 μg/kg in complete feedingstuffs for young animals, and worldwide limits for AFB_1 in feed for dairy cattle may change between 5 and 25 μg/kg.[8]

Trichothecenes, zearalenone and fumonisins are the major *Fusarium* mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages. T-2 toxin is type-A trichothecene mycotoxin produced by different *Fusarium* species, including

F. sporotrichioides, F. poae and *F. acuminatum*, which may develop on a variety of cereal grains especially in cold climate regions or during wet storage condition.[3] The immune system is primary target of T-2 toxin, and the effects include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation,[5] oral lesions in poultry. T-2 toxin has also been implicated in reproductive disorders in farm livestock.[6]

T-2 toxin has not been regulated in EU and Turkey, but the toxic levels of T-2 toxin have been reported as 100 μg/kg[12] and 500 μg/kg for growing pigs[17]; the limits set as

25 μg/kg and 100 μg/kg for animal feeds in Iran and Russian Federation, respectively[8]. In EU, control programmes have been coordinated due to lack of reliable data on T-2 and HT-2 toxins.[4]

AFB_1 is more important than T-2 toxin for animal and human health because of the prevalent compound in the samples, and due to aflatoxin M_1 (AFM_1). When AFB_1 contaminates the feed of lactating animals, milks from these animals will contain aflatoxin M_1 (AFM_1) the principal metabolite arising from biotransformation of AFB_1, which is also a toxic compound. Milk and milk products are very important for human consumption, especially babies and children need milk and dairy products**.** In Turkey, high AFM_1 (higher than 50 ng/L and 250 ng/kg for milk and cheese, respectively) concentration could be find in milk and cheese samples as reported by previous studies.[1,11,13,14,16,19,21,26]

The present work was performed to collect data on AFB_1 concentrations in dairy cattle feed and feedstuffs firstly due to the previous studies reporting higher concentrations of AFM_1 than EU and Turkish legal limits in the same region, and then to collect data on AFB_1 levels in other feeds. In addition, we could not find any data on T-2 toxin concentrations in animal feed and feedstuffs (except poultry feeds) in Turkey, so we also aimed to determine the toxin levels in the same samples.

Materials and Methods

The research materials were 93 samples consisting of 1-2 kilos of dairy cattle feed (n = 34), fattening feed (n = 5), fawn feed (n = 5), broiler feed (n = 10), rice bran (n = 10), rice grain (n = 5), barley-wheat grain (n = 5), maize grain (n = 5) and maize products (dry distiller grain soluble/DDGS) (n = 5), soya meal (n = 6) and sunflower meal (n = 3). The samples were collected from four feed mills and 17 farmers of milk producer in Bursa region (province of Bursa, Balıkesir and Eskisehir), Turkey. The dairy cattle feeds obtained from four feed mills (n = 17) and 17 farmers of milk producer (n = 17). All samples were collected and analyzed between September and December of 2006. Analyses were carried out after arriving the samples, if necessary the samples were stored at +4 °C for a week. AFB_1 and T-2 toxin concentrations were measured by competitive ELISA (EL 312e Biotek, biokinetics reader, USA) according to the procedure described by the manufacturer[18]

by using RIDASCREEN® Aflatoxin B_1 30/15 and T-2 Toxin-Test kits (Art. No. R 1211 and Art. No. R 3801, respectively). Detection limit of the methods for AFB_1 and T-2 toxin used was 1 µg/kg and 3.5 µg/kg for cereals and feed, respectively. All statistical analyses were performed using SPSS 10.0 Program. Differences between feed samples obtained from feed mills and dairy farmers were calculated by T-test. For statistical analysis of among the feedstuffs multiple comparisons of variance (ANOVA) was used.

Results

The determined levels of AFB_1 and T-2 toxin in the samples were variable particularly for AFB_1 and these mycotoxins were found in all samples (except one of samples for T-2 toxin). Minimum, maximum and mean values of AFB_1 and T-2 toxin have been shown in Table I and Table II, respectively.

Discussion

AFB_1 concentrations in feed and feed ingredients are very important for animal health, and human health due to the presence of AFM_1 in dairy animals that consume these dairy cattle feeds contaminated with AFB_1. T-2 Toxin levels in feed and feed ingredients are also important for animal health. The highest level was detected in a sample of maize (431.90 µg/kg of AFB_1) and in a sample of rice bran (131.00 µg/kg of T-2 toxin) (Table 1 and Table 2, respectively). All samples (except one for T-2 toxin) were contaminated with both AFB_1 and T-2 toxin. The mean total AFB_1 concentration of the dairy cattle feed samples (26.36±2.27 µg/kg) was lower than the Turkish legal limit[25] (50 µg/kg), but higher than the levels set by EU[10] (10 µg/kg) and many countries where the legal limits are between 5 µg/kg and 25 µg/kg [8]. The AFB_1 level in 1 of 34 (%3) dairy cattle feed samples exceeded the legal limit of Turkish (50 µg/kg) and in 30 of 34 (%88) dairy cattle feed samples exceeded the legal limit of EU (10 µg/kg). The mean AFB_1 concentration of the feed mill group (27.30±2.14 µg/kg) was slightly higher than dairy producer feed group (25.46±4.01 µg/kg), however this difference was not significant (P>0.05). The mean AFB_1 level of the fattening feed samples (39.10±3.86 µg/kg) was lower than the Turkish legal limit [25] (50 µg/kg), but higher than the levels set by EU[10] (20 µg/kg). The mean AFB_1 level of the fawn feed samples (44.50±6.76 µg/kg) was higher than the Turkish and EU legal limits (10 µg/kg). Similarly, the mean AFB_1 concentration of the broiler feed samples (27.60±3.96 µg/kg) was higher than the Turkish and EU legal limits (10 µg/kg). While Thirumala-Devi et al.[24] reported that AFB_1 levels in 18 of 30 (60%) poultry feed samples were higher than 10 µg/kg in India, in our study 9 of 10 (90%) broiler feeds had higher levels than 10 µg/kg. The maximum AFB_1 level of the broiler feeds (49.80 µg/kg) was lower than the value in poultry feeds (160 µg/kg) reported by Dawlatana et al.[7] in Bangladesh.

Table I. AFB_1 levels (µg/kg) in different feed and feedstuffs.

Tablo I. Farklı yem ve yem hammaddelerindeki AFB_1 düzeyleri (µg/kg)

Feed and feedstuffs	*n*	Mean	S.E.M.	Minimum	Maximum
Dairy cattle (total)	34	26.36	2.27	8.10	72.80
Dairy cattle (feed mill)	17	27.30	2.14	19.50	46.30
Dairy cattle (dairy producer)	17	25.46	4.01	8.10	72.80
Fattening feed	5	39.10	3.86	30.00	51.00
Fawn feed	5	44.50	6.76	30.20	70.00
Broiler feed	10	27.60	3.96	8.40	49.80
Rice bran	10	21.87	6.52	9.20	78.80
Rice grain	5	11.80	2.23	6.90	19.30
Barley-wheat grain	5	10.20	0.55	8.90	11.70
Maize grain	5	133.00	77.30	17.20	431.90
DDGS	5	58.40	8.15	38.00	73.60
Soya meal	6	18.50	6.15	5.20	46.30
Sunflower meal	3	21.00	1.67	18.10	23.90

Table II. T-2 Toxin levels (µg/kg) in different feed and feedstuffs.

Tablo II. Farklı yem ve yem hammaddelerindeki T-2 toksin düzeyleri (µg/kg)

Feed and feedstuffs	*n*	Mean	S.E.M.	Minimum	Maximum
Dairy cattle (total)	34	22.16	0.94	-*	29.50
Dairy cattle (feed mill)	17	23.50	1.10	12.90	29.50
Dairy cattle (dairy producer)	17	20.80	1.50	-*	27.70
Fattening feed	5	17.60	2.51	7.80	21.80
Fawn feed	5	21.80	1.68	18.60	28.00
Broiler feed	10	14.60	0.83	11.60	21.30
Rice bran	10	37.70	10.39	23.00	131.00
Rice grain	5	16.50	0.40	15.50	17.80
Barley-wheat grain	5	15.60	1.97	9.80	20.70
Maize grain	5	16.60	3.20	12.10	29.10
DDGS	5	19.20	1.10	16.50	21.60
Soya meal	6	14.00	0.70	11.90	16.50
Sunflower meal	3	12.10	0.80	10.50	13.00

* non-detectable

The mean AFB_1 concentration of the all dairy feedstuff groups was lower than the Turkish legal limit[25] (50 µg/kg), but higher than the levels set by EU[10] (5 µg/kg). Among the feedstuff materials, the mean AFB_1 level of the rice bran samples (21.87±6.52 µg/kg), rice grain samples (11.80±2.23 µg/kg), barley-wheat grain samples (10.20±0.55 µg/kg), maize grain samples (133.00±77.30 µg/kg), DDGS samples (58.40±8.15 µg/kg), soya meal samples (18.50±6.15 µg/kg) and sunflower meal samples (21.00±1.67 µg/kg) were higher than the Turkish and EU legal limits (10 µg/kg). In our study, the highest levels belonging to rice bran, soya and sunflower meal samples (78.80 µg/kg, 46.30 µg/kg and 23.90 µg/kg, respectively) were higher than the results belonging to rice bran, soya and sunflower samples (13 µg/kg, 4 µg/kg and 15 µg/kg), respectively, reported by Scudamore et al.[20] in United Kingdom. The maximum AFB_1 concentration of the maize samples (431.90 µg/kg) was higher than the results reported by Scudamore et al.[20] in United Kingdom (41 µg/kg), Shetty and Bhat[22] in India (109 µg/kg), Dawlatana et al.[7] in Bangladesh (245 µg/kg) and Oruc et al.[15] in Turkey (32.30 µg/kg as total aflatoxin). The highest level detected in maize sample (431.90 µg/kg) was collected from a dairy farm in Bursa, where the dairy feed was prepared by the farmer from a maize grain that had been bought four months previously. The maize grain samples and DDGS, a kind of maize sample imported from USA, had excess AFB_1 levels. Differences among the feedstuffs were calculated. Statistically important difference ($p< 0.05$) were observed between maize grain samples and each of other feedstuff groups for AFB_1 including soya meal soya meal, rice bran, rice grain, sunflower meal and barley-wheat grain.

The mean T-2 toxin level of the all samples groups were between 12.10±0.80 µg/kg (sunflower meal) and 37.70±10.39 µg/kg (rice bran) (Table 2). Although T-2 toxin level is not yet regulated in EU and Turkey, these concentrations were lower than the toxic levels of T-2 toxin have been reported as 100 µg/kg for animals by Mabbett,[12] and 500 µg/kg for growing pigs by Rafai et al.,[17] but only the mean levels of rice bran samples (37.70±10.39 µg/kg) was higher than the limits set as 25 µg/kg in animal feeds in Iran [8]. One of rice bran samples contained 131.00 µg/kg of T-2 toxin, which is higher than 100 µg/kg. The mean level of broiler feeds (14.60 µg/kg) was lower than the result in poultry feeds reported by Sonal and Oruc [23] (58.59 µg/kg) in Turkey.

AFB_1 levels in dairy feed and feedstuffs were especially important for human health since the presence of AFM_1 in milk and dairy products. When lactating mammals, such as cows, sheep, goats are fed with feed and feedstuffs containing AFB_1, this metabolite can be converted to AFM_1, which is cytotoxic, genotoxic and carcinogenic. Approximately 1-2% of AFB_1 in animal feed is transformed to AFM_1 in milk with variations from one animal to another.[27] Therefore, when the AFB_1 concentration in samples is higher than 5 µg/kg, especially 10 µg/kg, the milk can contain higher AFM_1 level than the legal limit of 50 ng/L in Turkey and EU, and when the AFB_1 concentration in samples is higher than 50 µg/kg, the milk can contain higher AFM_1 level than the limit of Codex Alimentarius (500 ng/L). These results were supported by some previous studies done in the same region and other regions of Turkey [1, 11, 13, 14, 16, 19, 21, 26] reporting that the milk samples contained higher than 50 ng/L AFM_1.

The results of this study indicate that the incidence of AFB_1 and T-2 toxin contamination in feed and feedstuffs were very high (100% and 99%, respectively), and the feed and feedstuff samples contained high levels of AFB_1 according to legal limits to EU. The AFB_1 concentrations are at the risk levels for animal health, and human health due to AFM_1, is secreted into milk from the mammary glands as the metabolite of AFB_1. The AFB_1 results supported the previous studies reporting higher AFM_1 concentrations than EU and Turkish legal limits in the same region (Bursa region, Turkey). T-2 toxin levels were generally lower than the toxic levels of T-2 toxin that have been reported previously. Relatively high AFB_1 concentrations in feed and feedstuffs, and limited data on factual contamination of feed and feedstuffs with mycotoxins imply that more emphasis should be given to the routine AFB_1 and AFM_1 inspection of feed-feedstuffs and milk, respectively, in Bursa region as well as in other parts of Turkey. In addition, to protect the animal and human health against mycotoxins, governmental agencies need to inform both farmers and dairy companies about the importance of mycotoxins. Factors influencing mycotoxin production in feedstuffs have to be clearly defined and preventive measures should be taken to decrease the risk of mycotoxins, especially AFB_1 and AFM_1, and the consequences of AFM_1 in their products.

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