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RESEARCH ARTICLE

Determination of Various Mycotoxin Concentrations in the Feedstuffs and Feed Produced by A Feed Manufacturer in Turkey

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Summary

The aim of this study was to determine aflatoxin B_1 (AFB_1), T-2 toxin, fumonisin, deoxynivalenol (DON) and zearalenone (ZEN) levels in various feedstuffs (local and imported feed materials) and feed produced in a feed manufacturer. In addition, aflatoxin M_1 (AFM_1) levels were determined in raw cow milks (n = 24) collected from 10 certain dairy cattle farms. The samples consisted of dairy cattle (n = 26), fattening (n = 4), and calf starter (n = 4) feed, as well as feedstuffs (n = 40). All of the samples were analysed by ELISA. In the analysed samples, the incidence of AFB_1, AFM_1 and T-2 toxins, fumonisin, DON and ZEN were 100%, 80%, 75% and 33%, respectively. The results of this study in feedstuffs and feed revealed that the mycotoxin levels were lower than the mycotoxin limits of the European Union (EU) and Turkey. In previous studies, high concentrations of mycotoxins, especially AFB_1 and AFM_1, were identified in feedstuffs, feeds and milk in Turkey. In contrast, the mycotoxin levels in the current study were lower than that from the previous studies. The mycotoxin concentrations in imported feed materials (except fumonisin) were lower than those in feed materials obtained from Turkey. The results of this study indicate that the mean mycotoxin levels in feedstuff and feeds do not cause toxic effects in animals, and humans for AFM_1.

Keywords: *Feedstuffs, Feed, Feed manufacturer, Mycotoxin levels, Multiple mycotoxin*

Bir Yem Fabrikası Tarafından Türkiye'de Üretilen Yem ve Yem Hammaddelerinde Çeşitli Mikotoksinlerin Miktarlarının Belirlenmesi

Özet

Bu çalışmanın amacı bir yem fabrikası tarafından üretilen yemlerde ve çeşitli yem hammaddelerinde (yerli ve ithal) aflatoksin B_1 (AFB_1), T-2 toksin, fumonisin, deoksinivalenol (DON) ve zearalenone (ZEN) düzeylerinin belirlenmesidir. Ayrıca, seçilen 10 farklı süt işletmesinden toplanan inek sütlerinde (n = 24) aflatoksin M_1 (AFM_1) miktarlarının belirlenmesi amaçlanmıştır. Numuneler, süt yemi (n = 26), besi yemi (n = 4) ve buzağı başlangıç yemi (n = 4), ile yem hammaddelerinden (n = 40) oluşmaktadır. Mikotoksin miktarları ELISA ile analiz edildi. Mikotoksinlerin tespit edilme oranı AFB_1, AFM_1 ve T-2 toksin, fumonisin, DON ve ZEN için sırasıyla %100, %80, %75 ve %33'tür. Yem ve yem hammaddelerinde bulduğumuz mikotoksin miktarlarının Türkiye ve Avrupa Birliği'nde uygulanan limitlerin altında olduğu görüldü. Türkiye'de süt, yem ve yem hammaddelerinde daha önce yapılan çalışmalarda özellikle AFB_1 ve AFM_1 limitlerin üzerinde tespit edilmiştir. Bu çalışmanın sonuçları benzer olan önceki çalışmaların sonuçlarının aksine daha düşüktür. İthal yem hammaddeleri Türkiye'den sağlanan yem hammaddelerinden (fumonisin hariç) daha düşük miktarlarda mikotoksin içermektedir. Bu çalışmanın sonuçlarına göre, yem ve hammaddelerindeki ortalama mikotoksin düzeylerinin hayvan sağlığı; sütlerdeki AFM_1 miktarlarının da insan sağlığı açısından toksik bir etkiye neden olamayacağı görüldü.

Anahtar sözcükler: *Yem hammaddesi, Yem, Yem fabrikası, Mikotoksin düzeyleri, Multiple mikotoksin*

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INTRODUCTION

Mycotoxins are toxic metabolites that are produced by certain types of fungi genera such as *Aspergillus, Penicillium* and *Fusarium,* which invade crops in fields and may grow on foods and feedstuffs during storage under favourable conditions of temperature and humidity. The contamination of feed materials and feed with mycotoxins is a major problem of animal health concerns. Toxicological, environmental and epidemiological studies have addressed the health and economical problems of individual mycotoxins; however, more than one mycotoxin is usually found in the same contaminated commodities.

The best-known mycotoxins are aflatoxins, trichothecenes, ochratoxin, zearalenone and fumonisin. Aflatoxin contamination in feedstuffs and feed is common in Latin America, Africa, Asia and Australia [1]. Aflatoxins are toxic compounds that are produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These fungi are known to be carcinogenic, mutagenic, teratogenic and immunosuppressive [2,3]. In the aflatoxin group, approximately 16 compounds are known; however, only aflatoxin B_1, B_2, G_1, G_2 and M_1 are routinely monitored. Aflatoxin B_1 (AFB_1) is the most prevalent and most toxic member of the group. When AFB_1 contaminates the feed of lactating animals, milk from these animals will contain aflatoxin M_1 (AFM_1), which is the principal metabolite that arises from the biotransformation of AFB_1 and is also a toxic compound.

T-2 toxin, fumonisins (B_1 and B_2), deoxynivalenol (DON) and zearalenone (ZEN), as well as toxins such as nivalenol and diacetoxyscirpenol, are trichothecenes. These toxins are produced by *Fusarium* moulds, which may develop in a variety of cereal grains, especially in cold climate regions or during wet storage conditions [4]. Trichothecenes cause vomiting and refusal to feed. The immune system is the primary target of the toxins and inhibits protein synthesis in animals depending on the species [5]. The effects of T-2 toxin include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation and oral lesions in poultry [6]. T-2 toxin has also been implicated in reproductive disorders in livestock. ZEN causes hyperoestrogenism, severe reproductive and infertility problems [7]. Fumonisins are known to be the cause of equine leucoencephalomalacia and porcine pulmonary oedema syndrome, which are both associated with the consumption of corn-based feed [8].

Mycotoxins are found in cereal crops, such as maize, wheat, barley, oats, rye and mixed feeds. Mycotoxins have a significant impact on the industry because they result in livestock loss and cause problems in rearing and breeding of animals [5]. AFM_1 concentrations in milk and milk products are very important for human health, especially for babies and children that need milk and dairy products. Therefore, worldwide regulations were established for mycotoxins in food and feed [9]. Although several countries routinely monitor certain mycotoxins depending on their climate conditions and imported feed materials, there is not enough monitoring of feedstuffs and feed in Turkey. In previous studies, high AFB_1 [10,11] and AFM_1 [12-15] concentrations were identified in feedstuffs, feed and milk in Turkey. In addition, to the best of our knowledge, this is the first report to evaluate the ZEN and DON levels in feed materials in Turkey. The current study was performed to collect data on certain mycotoxins including AFB_1, T-2 toxin, fumonisin, ZEN and DON levels in local and imported feedstuffs and feed produced by a feed manufacturer. We also aimed to determine the AFM_1 concentrations in the certain raw milks of which the AFB_1 levels in the ration of cows were already determined by us.

MATERIAL and METHODS

The research materials consisted of dairy cattle, fattening and calf starter feed, and feed materials including wheat bran, rice bran, rice, barley, wheat, wheat middling, maize, maize gluten feed, maize gluten meal, sunflower meal, soybean meal, full fat soy, canola meal, dried distiller grains soluble (DDGS), beet pulp and biscuit samples. The samples were collected from a feed manufacturer, which obtained the samples from different regions of Turkey *(n* = 29) and from imported *(n* = 11) feed materials in Bursa province, which produced feed for all of Turkey. Imported materials were obtained from Ukraine *(n* = 6), USA *(n* = 2), Bulgaria *(n* = 2) and Russia *(n* = 1). Raw milk samples (n = 24) were collected from 10 certain dairy cattle farms. The analyses were usually performed after the samples arrived in laboratory. If necessary, feed and feedstuff samples were stored at 4°C, and raw milk was stored at -20°C. The samples involved the collection of approximately 2 kilograms for feed materials and feed and 500-1000 ml for raw milk and were analysed between 2 August and 1 September 2010, the summer season in Turkey. The temperature was between 20°C and 32°C, and the humidity was between 50% and 75% in feedstuff storage facility for 24 h during sample collection. The number of research materials is presented in *Table 1*.

AFB_1 (Aflatoxin B_1 30/15, Art. No. R1211) in all of the samples, T-2 toxin (Fast T-2 toxin, Art. No. R5302), fumonisin (Fast Fumonisin, Art. No. R5602), DON (Fast DON, Art. No. R5902) and ZEN (Fast Zearalenon, Art. No. R5502) in all feed materials, and AFM_1 (Art. No. R1111) concentrations in milk were measured by competitive ELISA (EL 312e Biotek, Bio-Kinetics reader, USA) using Ridascreen® test kits. The manufacturer procedures [16] were used to detect mycotoxins. Aflatoxin Clean-up Columns (R-Biopharm, P25) were used for dark coloured filtrates of samples.

All of the statistical analyses were performed using the Minitab Statistical Program [17]. AFB_1, AFM_1, T-2 toxin, fumonisin, DON and ZEN in local and imported feed materials were compared using the t-test. The differences were considered significant at *P*<0.05. Descriptive data were provided for AFB_1, T-2 toxin, fumonisin, DON and ZEN in all feed materials.

RESULTS

The determined levels of AFB_1 and AFM_1 as well as the determined levels of T-2 toxin, fumonisin, DON and ZEN in the samples are shown in *Table 1* and *Table 2*, respectively.

The incidence of each mycotoxin and multiple mycotoxins in feed materials is presented in *Table 3*. The incidence of AFB_1 in all samples (40 feedstuffs + 34 feed = 74) was 100%, and the incidence of AFM_1 in raw milk samples was also 100%.

DISCUSSION

Mycotoxin concentrations in feed and feed ingredients are very important for animal health and also imported for

Table 1. *AFB_1 (µg/kg) and AFM_1 (ng/kg) levels in feed, feedstuffs and raw milk, respectively*
Tablo 1. *Yem ve yem hammaddelerinde AFB_1 (µg/kg) ve çiğ sütlerde AFM_1 (ng/kg) miktarları*

Feed and Feedstuffs	*n*	Mean	SEM	Min	Max
Dairy cattle	26	3.37	0.90	0.80	25.00
Fattening	4	2.82	0.82	0.65	4.52
Calf starter	4	3.47	0.57	2.30	4.68
Wheat bran	7	1.47	0.37	0.30	2.80
Wheat grain	1	1.40	-	-	-
Wheat middling	1	1.60	-	-	-
Rice bran	2	2.39	0.78	1.62	3.17
Rice grain	1	3.20	-	-	-
Maize grain	7	16.84	7.40	1.86	58.00
Maize gluten feed	3	11.59	7.71	3.64	27.00
Maize gluten meal	2	7.52	4.08	3.44	11.60
DDGS	1	70.00	-	-	-
Sunflower meal	4	1.77	0.77	0.60	3.21
Barley grain	3	4.42	1.87	2.55	6.29
Soybean meal	2	2.44	0.76	1.68	3.20
Full fat soy	2	3.73	0.38	3.35	4.10
Canola meal	2	3.33	0.33	3.00	3.66
Beet pulp	1	11.00	-	-	-
Biscuit	1	3.11	-	-	-
Feedstuffs (total)	40	8.26	2.19	0.30	70.00
Feedstuffs (local)	29	10.09	2.90	0.60	70.00
Feedstuffs (imported)	11	3.08	0.95	0.30	11.00
Raw milk (AFM_1)	24	7.50	0.86	2.48	18.93

Table 2. *T-2 Toxin, fumonisin, DON and ZEN levels in feedstuffs*
Tablo 2. *Yem hammaddelerinde T-2 Toksin, fumonisin, DON ve ZEN düzeyleri*

Feed and feedstuffs	*n*	*Mean±SEM			
		T-2 Toxin (µg/kg)	Fumonisin (mg/kg)	DON (mg/kg)	ZEN (µg/kg)
Feedstuffs (total)	40	30.40±1.87	0.51±0.24	0.72±0.18	55.40±15.20
Feedstuffs (local)	29	30.76±2.37	0.32±0.16	0.89±0.25	65.80±18.20
Feedstuffs (imported)	11	29.87±3.16	0.85±0.60	0.38±0.11	29.50±24.50

* *Mean levels were calculated according to number of positive samples*

***Table 3.** Incidence of analysed samples for each and multi-mycotoxins in feedstuffs (%)*
***Tablo 3.** Yem hammaddelerinde mikotoksinlerin bireysel ve birlikte bulunma oranları (%)*

Feedstuffs	n	$^{*}B_1$, T-2	$^{**}F$	DON	ZEN	B_1+T-2
Feedstuffs (T)	40	100	80	75	33	100
Feedstuffs (L)	29	100	87	86	15	100
Feedstuffs (I)	11	100	73	64	18	100
Feedstuffs	*n*	B_1+T-2+F	B_1+T-2+DON	B_1+T-2+F+DON	B_1+T-2+DON+ZEN	B_1+T-2+F+DON+ZEN
Feedstuffs (T)	40	80	75	50	20	15

** B_1: AFB_1, ** F: Fumonisin, T: Total, L: Local, I: Imported*

human health because the presence of some mycotoxins such as AFM_1 in dairy animal milk.

In the current study, the incidence of both AFB_1 and T-2 toxin was 100%, which is a very high ratio. The incidence of fumonisin (80%), DON (75%) and ZEN (33%) *(Table 3)* decreased, and the incidence of ZEN in feedstuffs was the lowest. The analysis of multi- mycotoxins in feed materials revealed that the incidences were high for AFB_1 + T-2 toxin (100%) and AFB_1 + T-2 toxin + fumonisin (80%), average for AFB_1 + T-2 toxin + DON (75%) and AFB_1 + T-2 toxin + fumonisin + DON (50%), and low for AFB_1 + T-2 toxin + DON + ZEN (20%), AFB_1 + T-2 toxin + fumonisin + ZEN (15%) and AFB_1 + T-2 toxin + fumonisin + DON + ZEN (15%). The results show that AFB_1, T-2 toxin and fumonisin incidence was high and ZEN and the multiple mycotoxin contamination with ZEN were low in the same feed materials. The speculation on the interaction between mycotoxins on the cellular level raises many questions, which remain unanswered. It is now understood that in humans and animals, toxicokinetic [18] and toxicodynamic interactions result from a simultaneous influence of the amount and type of mycotoxins [19]. The incidence of AFB_1 and T-2 toxin in our current study were similar to the study in feed and feedstuffs that was reported by Oruc et al.[11]. Although the incidence of AFB_1 in this study was higher than that reported by Sonal and Oruc [20] in poultry feed, AFB_1, T-2 toxin, fumonisin and ZEN incidences were lower than those reported in poultry feed by Sonal and Oruc [20] in Turkey.

In the current study, the highest level of AFB_1, T-2 toxin, fumonisin and DON were usually found in maize and maize-based feed materials between 1.86 and 70.00 µg/kg *(Table 1)*. The highest level of AFB_1 was detected in a sample of DDGS (70.00 µg/kg). The highest T-2 toxin concentration was found in maize gluten feed (102.35 µg/kg). The highest level of fumonisin was identified in a maize grain (5.06 mg/kg). The highest DON concentration was found in maize gluten meal (2.50 mg/kg), and the highest ZEN concentration was detected in wheat middlings (110.00 µg/kg).

The mean AFB_1 concentration (8.26±2.19 µg/kg) in all of the feed materials was lower than the limits established by the European Union (EU) (20 µg/kg) [21] and by Turkey (50 µg/kg) [22]. However, a maize grain (58 µg/kg) and a DDGS (70 µg/kg) sample levels were higher than the Turkish and EU legal limit. The mean AFB_1 results do not support the previous study [11], which reported higher AFB_1 concentrations in the same region (Bursa region, Turkey). The AFB_1 levels were 21.87±6.52 µg/kg in rice bran, 21.00±1.67 µg/kg in sunflower meal, 58.40±8.15 µg/kg in DDGS and 133.00±77.30 µg/kg in maize grain. Nevertheless, the mean AFB_1 level in the current study was similar to that in the maize samples (10.94±2.32 µg/kg, total aflatoxin) reported by Oruc et al.[10].

The mean AFB_1 concentration in the dairy cattle feed samples (3.37±0.90 µg/kg) was lower than the EU limit and the Turkish limit of 5 µg/kg. The legal limits fluctuate between 5 and 25 µg/kg in many countries [9]. A dairy cattle feed sample value (25 µg/kg) was higher than the level of 5 µg/kg *(Table 1)*. The mean AFB_1 level of the calf starter (3.47±0.57 µg/kg) and fattening (2.82±0.82 µg/kg) feed samples were lower than the Turkish and EU legal limits (10 µg/kg).

The mean T-2 toxin level in all of the feed materials groups was between 29.87±3.16 and 30.76±2.37 µg/kg *(Table 2)*. Although the T-2 toxin level has not been regulated in EU and Turkey, these concentrations were lower than the toxic levels of T-2 toxin that have been reported as 100 µg/kg for animals by Mabbett [1] and 500µg/kg for growing pigs by Rafai et al.[23]. One of maize gluten feed samples contained 102.35 µg/kg of T-2 toxin, which is higher than 100 µg/kg. The mean levels of the samples were lower than those identified in poultry feeds (58.59 µg/kg), which was reported by Sonal and Oruc [20], and similar (between 12.10±0.80 and 37.70±10.39 µg/kg in feed materials) to those in Turkey, which was previously reported by Oruc et al.[11].

The mean fumonisin ($B_1 + B_2$) levels in all of the samples groups were between 0.32±0.16 and 0.85±0.60 mg/kg *(Table 2)*. Fumonisin limits have not been regulated by the EU and Turkey. However, we determined that fumonisin concentrations were lower than that provided by the EU Commission Recommendation [24]. The EU Commission Recommendation for the products that are intended for animal feeding ranges from 5 to 60 mg/kg depending on feed and feedstuffs. Although the mean levels of the samples were higher than those in poultry feeds (0.18 mg/kg), which was reported by Sonal and Oruc [20], and considerably lower than those in local (88.24 mg/kg) and imported (74.15 mg/kg) maize samples in Turkey, which was reported by Oruc et al.[10].

The mean DON concentrations were between 0.38±0.11 and 0.89±0.25 mg/kg *(Table 2)*. The DON limits have not been regulated by the EU and Turkey. However, DON levels were lower than that provided by the EU Commission Recommendation [24]. The EU Commission Recommendation for the products that are intended for animal feeding ranges from 0.9 to 12 mg/kg depending on feed and feedstuffs. To the best of our knowledge, DON levels were studied for first time in feed materials in Turkey.

The mean ZEN concentrations were between 29.50±24.50 µg/kg and 65.80±18.20 µg/kg *(Table 2)*. The ZEN limit has not been regulated in EU and Turkey; however the ZEN levels were lower than that provided by the EU Commission Recommendation [24]. The EU Commission Recommendation for the products that are intended for animal feeding ranges from 0.1 to 3 mg/kg depending on feed and feedstuffs. Although ZEN levels were studied in poultry feeds [20], corn silage [25] and fresh grass silage [26], ZEN was studied for first time in all feed materials in Turkey.

The AFM_1 levels ranged from 2.48 to 18.93 ng/kg in raw milk samples that were obtained from dairy cattle farms (mean = 7.50±0.86) *(Table 1)*. The mean AFM_1 concentration was lower than the Turkish [27] and EU limit of 0.050 µg/kg [28], and the Codex Alimentarius [29] limit of 0.50 µg/kg. The AFB_1 levels in dairy cattle feed were usually low (between 0.87 and 6.25 µg/kg), which the cows that produced the raw milks fed with these feeds in their rations. Therefore, AFM_1 level were low in the milk samples. These results did not support several previous studies that were performed in the same region or other regions of Turkey [12-15], which reported that the milk samples contained more than 50 ng/kg of AFM_1.

In the current study, the mean value of the samples from Turkey was usually (except fumonisin) higher than those of the imported samples (*Table 1* and *2*). A statistically significant difference ($P<0.05$) was observed between the local and imported feedstuff samples for AFB_1 (P=0.027). A statistically significant difference ($P>0.05$) was not observed between the local and imported feedstuff samples for T-2 toxin, fumonisin, DON and ZEN.

The mean mycotoxin levels in feed, feed material and raw milk samples were lower than the legal limits. Because of the low levels, there is no risk to animal and human health from AFM_1. Mycotoxin levels in feed, feed material and raw milk samples, especially AFB_1 and AFM_1 in raw milks, were usually lower than those reported in previous studies in Turkey. For multiple mycotoxin, the incidences of AFB_1, T-2 toxin and fumonisin in feed materials were high, while the incidence of ZEN was low. The mycotoxin levels (except for fumonisin) in imported feed materials were lower than those in the local feed materials.

During the current study and in our routine analyses, we observed that the technical staffs of feed manufacturing and dairy cattle companies were usually well-informed about the factors influencing mycotoxin production in feed stuffs. Nevertheless, preventive measures should be taken to decrease the risk of mycotoxins, especially AFB_1 in feed materials such as maize and maize-based materials and AFM_1 in milk and dairy products.

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