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Research Article

Regional Distribution of Aflatoxin and Ochratoxin A Contaminated Beef and Dairy Cattle Feeds in Turkey

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ABSTRACT

The objectives of this study were to determine the aflatoxin (AF) and ochratoxin (OTA) contents and their correlations with chemical constituents of beef and dairy cattle feeds. Eighty-two commercial feed samples were collected from five different geographical regions of Turkey during fall season of 2006. The AF and OTA analyses were performed in HPLC while chemical constituents were determined with wet-chemistry. The highest and the lowest total AF contents were observed in Mediterranean (7.51 µg/kg) and Aegean (0.34 µg/kg) regions, respectively (P< 0.01). In addition, the OTA content was found to be the highest in Marmara (4.04 µg/kg) but lowest in Aegean (0.76 µg/kg) regions (P<0.01). Among the type of feeds, AFB1 content was higher in beef cattle feeds only in Mediterranean region (9.40 vs. 3.74 µg/kg) while it was higher in dairy cattle feeds in Eastern Anatolia (1.90 vs. 4.25 µg/kg), Aegean (0.27 vs. 0.38 µg/kg), Southeastern Anatolia (4.94 vs. 6.87 µg/kg) and Marmara (0.25 vs. 0.64 µg/kg) regions (P< 0.05). Overall, AF and OTA contents were not affected by the feed type and averaged 3.85 and 3.64 µg/kg for AF and 2.27 and 1.81 µg/kg for OTA, respectively. The moisture content among those feeds was the highest in Mediterranean (9.0%) and Southeastern Anatolia (8.8%) regions but the lowest in Marmara (7.6%) region. Correlation coefficients indicated that AFB1 (r= 0.74), AFG1 (r= 0.32), and AFB2 (r= 0.61) were highly correlated with moisture content of the feeds (P< 0.01). In addition, AFB1 was negatively correlated with OM (r= -0.26) and CP (r= -0.29; P< 0.05). However the OTA content was not correlated with either moisture or any other chemical constituents. The results indicated that, the levels of AF and OTA contaminated feeds were below the indicated safe limits of Food and Drug Administration (FDA) in U.S.A. (20 µg/kg of AF for dairy; 300 µg/kg of AF for beef cattle feeds; while no limit for OTA). However these levels were above the safe limits for AF in dairy cattle feeds in Southeastern Anatolia region and close to safe limits in Mediterranean and Eastern Anatolia regions according to European Food Safety Authority (EFSA) and Turkish Ministry of Agriculture (5 µg/kg for dairy cattle feeds). The OTA levels across the regions were below the EFSA and Turkish Ministry of Agriculture limits (250 µg/kg). The results also indicated that AF contents were highly correlated with moisture, OM, and CP contents of the feeds. However there was no correlation found between OTA and chemical constituents of the feeds.

Keywords: Aflatoxin, Beef cattle feed, Dairy cattle feed, Ochratoxin A

Aflatoksin ve Okratoksin A ile Kontamine Olmuş Besi ve Süt Sığırı Yemlerinin Türkiye'deki Bölgesel Dağılımı

ÖZET

Bu çalışmanın amacı, besi ve süt sığırı yemlerinde aflatoksin (AF) ve okratoksin A (OTA) içerikleri ve bunların kimyasal kompozisyonları ile aralarındaki korelasyonu belirlemedir. Bunun için, 82 ticari yem örneği 2006 yılının sonbahar döneminde Türkiye'nin 5 farklı coğrafik bölgesinden toplanmıştır. Yemlerdeki AF ve OTA analizleri HPLC, besin madde içerikleri ise kimyasal yöntemlerle yapılmıştır. En yüksek ve en düşük AF içerikleri sırasıyla Akdeniz (7.51 µg/kg) ve Ege (0.34 µg/kg) bölgelerinde gözlemlenmiştir (P< 0.01). Ayrıca yemlerdeki OTA içeriği Marmara bölgesinde en yüksek (4.04 µg/kg), Ege bölgesinde ise en düşük (0.76 µg/kg) düzeyde bulunmuştur (P< 0.01). Yem çeşitleri arasında AFB1 içeriği, en yüksek olarak besi sığırı yemlerinde sadece Akdeniz bölgesinde (9.40 vs. 3.74 µg/kg) tespit edilmiştir. Ancak, Doğu Anadolu (1.90 vs. 4.25 µg/kg), Ege (0.27 vs. 0.38 µg/kg), Güneydoğu Anadolu (4.94 vs. 6.87 µg/kg) ve Marmara (0.25 vs. 0.64 µg/kg) bölgelerinde AFB1 içeriği süt sığırı yemlerinde daha yüksek bulunmuştur (P< 0.05). Genel olarak, AF ve OTA içerikleri yem çeşitlerinden etkilenmemiş ve sırasıyla ortalama olarak AF yönünden 3.85 ve 3.64 µg/kg, OTA yönünden ise 2.27 ve 1.81 µg/kg olarak bulunmuştur. Yemlerdeki nem içeriği yönünden en yüksek değerler Akdeniz (%9.0) ve Güneydoğu Anadolu (%8.8) bölgelerinde gözlemlenirken, en düşük değer Marmara bölgesinde (%7.6) gözlenmiştir. Korelasyon katsayıları, yemlerdeki AFB1 (r= 0.74), AFG1 (r= 0.32) ve AFB2 (r= 0.61) içerikleri ile nem içeriği arasında yüksek ilişki olduğunu göstermiştir (P< 0.01). Ayrıca, yemlerdeki AFB1 içeriğinin OM (r= -0.26) ve HP (r= -0.29) içerikleriyle negatif yönde bir korelasyona sahip olduğu bulunmuştur. Ancak, OTA içeriğiyle yemlerdeki nem ve diğer besin madde içerikleri arasında bir korelasyon tespit edilememiştir. Sonuçlar, mevcut çalışmadaki AF ve OTA ile kontamine olmuş yemlerdeki toksin düzeylerinin Amerikan FDA güven limitlerinin (süt sığırı yemleri için 20 µg/kg AF; besi sığırı yemleri için 300 µg/kg AF; OTA için limit yok) altında olduğunu göstermiştir. Ancak, mevcut çalışmada Güneydoğu Anadolu bölgesinden alınan süt sığırı yemlerinde bulunan AF düzeyleri Avrupa Gıda Güvenliği Otoritesi (EFSA) ve T.C. Tarım Bakanlığı güvenlik limitlerinin (süt sığırı yemlerinde 5 µg/kg) üzerinde, Akdeniz ve Doğu Anadolu bölgelerinden alınan yemler de ise güvenlik limitine çok yakın bulunmuştur. Bütün bölgelerden alınan yemlerdeki OTA düzeyleri ise EFSA ve T.C. Tarım Bakanlığı limitlerinin (250 µg/kg) altında tespit edilmiştir. Sonuçlar, yemlerdeki AF içeriği ile nem, OM ve HP içerikleri arasında yüksek korelasyon olduğunu göstermiştir. Ancak, yemlerdeki OTA içeriği ile kimyasal kompozisyonlar arasında bir korelasyon tespit edilememiştir.

Anahtar Kelimeler: Aflatoksin, Besi sığırı yemi, Okratoksin A, Süt sığırı yemi

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Introduction

The mycotoxins are produced by actively growing molds. The mycotoxin formation in feeds and feed ingredients may occur from various reasons, such as using high moisture feed ingredients, wet storage conditions, and cross-contamination with other agricultural products (Benneth and Klich, 2003). In general, mycotoxin contamination of feeds or feed ingredients results in first poor feed efficiency, second animal health problems. Although ruminants are capable of detoxifying (degrading) those mycotoxins in their rumen by ruminal microorganisms, they are still prone to liver damage, contaminated milk (aflatoxin M1), depressed immune system function, and impaired reproductive performance. Aflatoxins (AF) have been shown to be the most potent carcinogen in animals (Squire, 1981). In addition, ochratoxin A (OTA) has been isolated from a commercial corn sample in the U.S.A., and recognized as a potent nephrotoxin (Shotwell et al., 1969). Besides to its nephrotoxic effect, OTA was shown to be a liver toxin, an immune suppressant, a teratogen, and a carcinogen in animals (Kuiper-Goodman and Scott, 1989). Similar to AF, OTA has also the potential for human indirect exposure through the animal derived foods consumed, such as meat (Guillamont et al., 2005) and milk (Skaug, 1999). Most livestock feeds contain mold spores on their surface. These spores counting of less than 10,000 cfu/g are commonly found on feed grains (DiCostanzo et al., 1995). When environmental conditions are optimal for mold colonization on grains or processed and mixed feeds, their first effect is the utilization of nutrients then reduce the nutritional value of feeds. The final outcomes of this mold colonization are hazardous mycotoxin production for animal health and maleficent feed. Tindall (1983) indicated that energy, crude protein, crude fat values of mycotoxin contaminated corn were decreased 5, 7, and 63% respectively. Although OTA causes several production disturbances in poultry, such as reductions in feed intake, weight gain and egg yield as well as transferring to egg and meat (Dwivedi and Burns, 1986), OTA toxicity in ruminants might be less potent due to microbial activity in foregut (Höhler et al., 1999). However cows in the transition period particularly in late pregnancy, parturition and during the early lactation are more susceptible to OTA (Fink-Gremmels, 2008). Duarte et al. (2011) also indicated that cows may also turn out to be more susceptible to OTA when a drastic change is occurred in feed composition and a high percentage of protein-rich concentrates is present in the diet.

Our objective in this study was to show the AF and OTA contents of commercial beef and dairy cattle concentrate feeds sampled from various geographical regions of Turkey along with their correlations with chemical constituents.

Materials and Methods

Beef and dairy cattle concentrate feeds (n= 82) from representing at least four different commercial feed processing companies were collected from five different geographical regions of Turkey. The feed samples were collected from feed manufacturing plant's processing lines. The AF and OTA standards for HPLC were certified (SUPELCO®) and in a liquid form of 2.6 and 50 µg/ml, respectively. The AF analysis was performed based on the modified method of AOAC 999.07 (Stroka et al., 2000). The recoveries for AF were 91, 87, 85, and 55% for AFB1, AFB2, AFG1, and AFG2, respectively. In addition, detection limits were 0.06, 0.04, 0.06, and 0.08 ppb for AFB1, AFB2, AFG1, and AFG2, respectively. The OTA analysis was performed based on the modified method of AOAC 2000.3. The recovery rate and detection limits for OTA were 80% and 0.09 ppb, respectively. The immuno-affinity column principle (VICAM, Afla Test-P) was performed to extract the AF and OTA. The HPLC conditions for AF and OTA were Dionex P680 HPLC pump, Dionex ASI-100 automated sample injector, Dionex RF 2000 Fluorescence detector, Dionex thermostatted column compartment TCC-100, and ODS-2 (C18-250 mm-5µm-4.6 mm) column.

The chemical constituent analyses (moisture, OM, Ash, CP, and EE) were performed based on AOAC (1990) methods. The data were analyzed by PROC GLM procedure of SAS® (1999). Treatment mean differences were tested using the LSD method after a significant F-test (P< 0.05). Correlation coefficients were determined between mycotoxins (AF and OTA) and chemical constituents of treatment feeds.

Table 1. Aflatoxin (AF), ochratoxin A (OTA), and chemical constituents of beef and dairy cattle feeds sampled from different geographical regions.
Tablo 1. Farklı coğrafik bölgelerden örneklenen besi ve süt sığırı yemlerinin aflatoksin (AF), okratoksin A (OTA) ve kimyasal içerikleri.

	Region													
	Mediterranean		Eastern Anatolia		Aegean		Southeastern Anatolia		Marmara					
	Feed (n= 82)													
Parameter[1]	Beef (n= 10)	Dairy (n= 6)	Beef (n= 6)	Dairy (n= 6)	Beef (n= 6)	Dairy (n= 6)	Beef (n= 14)	Dairy (n= 16)	Beef (n= 6)	Dairy (n= 6)	SEM[3]	Effects[2]		
												Region	Feed	Region*Feed
µg/kg														
AFB1	9.40	3.74	1.90	4.25	0.27	0.38	4.94	6.87	0.25	0.64	0.51	**	NS	*
AFG1	0	0	0	0	0	0	0.14	0.14	0	0.05	0.02	**	NS	NS
AFB2	1.24	0.64	0.29	0.59	0	0.04	0.58	0.65	0.05	0.09	0.05	**	NS	**
AFG2	0	0	0	0	0	0	0.01	0.01	0.15	0.11	0.01	**	NS	NS
Total AF	10.64	4.38	2.20	4.84	0.27	0.41	5.67	7.67	0.45	0.89	0.57	**	NS	*
OTA	1.68	0.65	2.67	3.40	1.03	0.49	0.75	1.66	5.23	2.85	0.27	**	NS	NS
%														
Moisture	9.3	8.8	8.1	7.9	7.7	8.0	9.0	8.7	7.7	7.5	0.1	**	NS	NS
OM	83.4	84.1	85.2	82.8	84.3	84.7	83.4	83.5	83.9	85.7	0.2	NS	NS	*
Ash	7.3	7.1	6.7	9.4	8.1	7.3	7.7	7.7	8.4	6.8	0.2	NS	NS	*
CP	15.7	18.6	16.1	17.8	17.7	19.7	15.6	17.9	16.5	19.4	0.2	**	**	NS
EE	2.6	3.3	2.2	2.4	3.3	3.8	2.9	3.4	3.0	4.4	0.1	**	**	NS

[1]AFB1: Aflatoxin B1, AFG1: Aflatoxin G1, AFB2: Aflatoxin B2, AFG2: Aflatoxin G2, OTA: Ochratoxin A, OM: Organic matter, CP: Crude protein, EE: Ether extract
[2]*P< 0.05, **P< 0.01, NS: non-significant
[3]SEM: standard error of mean

Results

The AF and OTA concentrations of treatment beef and dairy concentrate feeds are presented in Table 1. The highest and the lowest AFB1 concentrations of beef cattle feeds were found in Mediterranean (9.40 µg/kg) and Marmara (0.25 µg/kg) regions, respectively (P< 0.01). In addition, dairy cattle feeds contaminated with AFB1 were highest and lowest in concentration from Southeastern Anatolia (6.87 µg/kg) and Aegean (0.38 µg/kg) regions, respectively. The similar trend was also observed for AFB2 concentration for beef and dairy cattle feeds (region*-feed interaction). In general, feeds from Mediterranean and Southeastern Anatolia regions had a higher total AF concentrations for beef (10.64 µg/kg) and dairy cattle (7.67 µg/kg) feeds, respectively. The highest OTA concentration was detected in Marmara region for both beef and dairy cattle feeds (5.23 and 2.85 µg/kg; P< 0.01). The Aegean region had the lowest AF and OTA concentrations for both beef and dairy cattle feeds averaging 0.34 and 0.76 µg/kg, respectively (refer to Figure 1; P< 0.01).

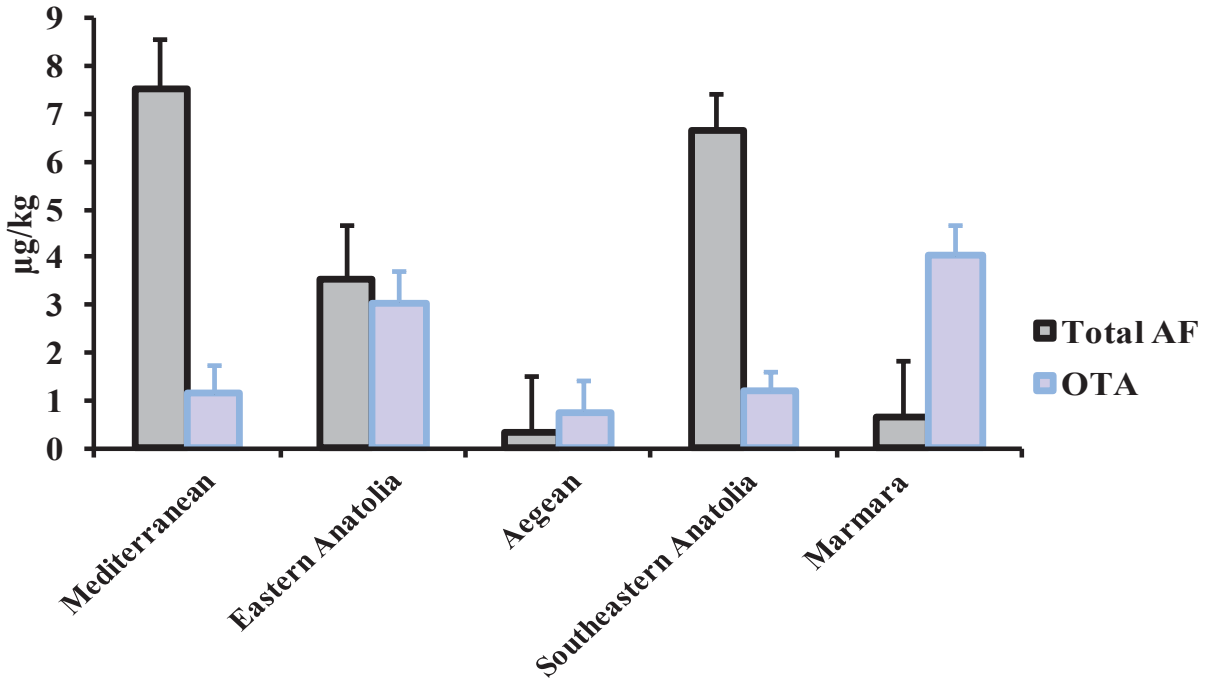

Figure 1. Regional distribution of beef and dairy cattle concentrate feeds contaminated with total aflatoxin (AF) and ochratoxin A (OTA).
Şekil 1. Aflatoksin (AF) ve okratoksin A (OTA) ile kontamine olmuş besi ve süt sığırı yemlerinin bölgesel dağılımı.

The moisture contents of the feeds varied between 7.5 to 9.3%. The feeds sampled from Mediterranean region showed the highest moisture level averaging 9.1%, while the Aegean region had the lowest moisture level averaging 7.8%. The averaged CP contents for beef and dairy cattle feeds were 16.3 and 18.7%, respectively. The highest and the lowest averaged CP contents were detected on feeds sampled from Aegean (18.0%) and Eastern Anatolia (16.7%) regions (P< 0.01).

The correlation coefficients between tested mycotoxins and chemical compositions of feeds are presented in Table 2. The significant correlation coefficients were found between individual AFs in such AFB1 was highly correlated with AFG1 (r= 0.60; P< 0.01). In addition, AFB1 (r= 0.75), AFG1 (r= 0.32), and AFB2 (r= 0.61) were positively correlated with moisture contents of the feeds (P< 0.01). In general, total AF content was also positively correlated with moisture contents of the feeds (r= 0.72; P< 0.01). However, there was no significant correlation between OTA and moisture contents of the feeds. In spite of the moisture content, the CP content of the feeds was negatively correlated with AFB1 (r= -0.29), AFB2 (r= -0.38), and total AF (r= -0.30; P< 0.05). Similar to moisture content, CP content of the feeds were also not correlated with OTA.

Discussion

The FDA reports indicate that levels of AF greater than 20 µg/kg in the commercial feeds and species of food producing animals may not result in aflatoxicosis (Price et al., 1993). Based on this information, the AF contaminated beef and dairy cattle feeds across the regions in the present study were below this limit, averaging 3.74 µg/kg. Price et al. (1993) also indicated that the domestic surveillance of feedstuffs for AFB1 by the FDA between 1989 and 1992 showed that out of 133 feedstuffs (corn, mixed feeds, cottonseed, grain byproducts, soybeans etc.) 57% had no, 30% had 1-20 µg/kg, 9% had 21-100 µg/kg, and 2% had 101-300 µg/kg AFB1. However based on the EFSA and Turkish Ministry of Agriculture limits for AF (5 µg/kg) in livestock feeds our study showed 49% of the beef and dairy cattle feeds were above this limit. These feeds were for beef cattle from Mediterranean (n= 10) and Southeastern Anatolia (n= 14), and dairy cattle from Southeastern Anatolia (n= 16) region. Most of the commercial feed companies in Mediterranean and Southeastern Anatolia regions use corn and cottonseed as the major feed ingredients in beef and dairy cattle concentrate feeds. Cheeke and Shull (1985) indicated that these two feedstuffs are the most important sources of AF in animal feeds. *Aspergillus flavus* and *A. parasiticus* molds may rarely colonize on small cereal grains (wheat and barley) and cause low to moderate levels of aflatoxicosis (Pier, 1992). Basalan et al. (2004) also studied to evaluate fungi growth and aflatoxin B1 contamination in horse and dog feeds. Among 41 feed sam-

Table 2. Correlation coefficients and their significance between mycotoxins (aflatoxin and ochratoxin A) and chemical constituents of beef and dairy cattle concentrate feeds sampled from different geographical regions.
Tablo 2. Farklı coğrafik bölgelerden örneklenen besi ve süt sığırı yemlerinin mikotoksin (aflatoksin ve okratoksin A) ve kimyasal içerikleri arasındaki korelasyon katsayıları ve bunların istatistiksel anlamlı farklılıkları.

Variables[1]	AFG1	AFB2	AFG2	Total AF	OTA	Moisture	OM	Ash	CP	EE
AFB1	0.60**	0.88**	-0.12	1.00**	-0.09	0.74**	-0.26*	-0.13	-0.29*	0.09
AFG1		0.35**	0.16	0.60**	-0.10	0.32**	-0.02	-0.16	-0.09	0.39**
AFB2			-0.23*	0.89**	-0.06	0.61**	-0.22	-0.10	-0.38**	-0.14
AFG2				-0.11	0.28*	-0.28*	0.14	0.00	0.13	0.34**
Total AF					-0.09	0.72**	-0.25*	-0.14	-0.30*	0.08
OTA						-0.23	0.01	0.12	-0.14	0.05
Moisture							-0.39**	-0.13	-0.35**	0.03
OM								-0.86**	0.15	0.01
Ash									0.03	-0.02
CP										0.28*

[1]AFB1: Aflatoxin B1, AFG1: Aflatoxin G1, AFB2: Aflatoxin B2, AFG2: Aflatoxin G2, OTA: Ochratoxin A, OM: Organic matter, CP: Crude protein, EE: ether extract
*P< 0.05
**P< 0.01

ples examined both feeds had AFB1 content in allowable limits (10 µg/kg). The moisture content of the feed ingredients or storage environment may promote the growth of the toxigenic molds. Feed processing stages (sieving, cracking, grinding) may also increase the severity of the aflatoxicosis where these stages remove the natural barrier to infestation by destroying the kernels or seeds. In general, the AF production on feed ingredients might happen if the moisture content of the feed is ≥15% (Pier, 1992). Although moisture contents of the sampled beef and dairy cattle feeds were below 10% in the present study, we did not know the moisture contents of the individual ingredients before the feeds were processed. In recent years, binding agents fed with AF may reduce the availability of the toxins and thereby reduce their effects in livestock animals (Akkaya and Bal, 2012).

The OTA concentrations of sampled beef and dairy cattle feed across the regions averaging 2.0 µg/kg were below the EFSA and Turkish Ministry of Agriculture limits (250 µg/kg). Several researches showed that either individual raw feed ingredients or finished livestock feeds were contaminated with OTA in some extend. Rafai et al. (2000) surveyed various feed ingredients contaminated with OTA within 7-years period in Hungary and found corn (320 µg/kg), wheat (207.8 µg/kg), barley (76.7 µg/kg), and sunflower (160 µg/kg) had an incidence of 9.3, 2.5, 2.7, and 18.2% of OTA contamination, respectively. Similarly, Beg et al. (2006) showed that poultry feed ingredients (wheat bran, yellow maize) also contaminated with OTA had an incidence of 7.4 and 96.8% with 12.1 and 14.5 µg/kg concentration. In addition, Rosa et al. (2008) surveyed both raw ingredients and finished bovine feeds contaminated with OTA. They found that corn (132 µg/kg), brewer's grains (439 µg/kg), barley (637 µg/kg), and finished feed (324 µg/kg) had an incidence of 31, 45, 21.6, and 25% of OTA contamination. Similar to our findings, the recent study from Turkey (Yıldız, 2009) showed that the OTA contamination was more pronounced in feeds from Marmara region (52.9% of the feeds having the OTA concentration greater than 20 µg/kg) than the feeds obtained from all regions of Turkey. The author also concluded that 80.7% (1.1-975.0 µg/kg) of the ruminant feeds were contaminated with the OTA across the country.

Dietary manipulations are also important in ruminants for detoxifying the OTA in the rumen. Xiao et al. (1991a, b) reported that The OTA can be hydrolyzed (detoxified) much faster in the rumen of sheep fed hay than grain and concluded that the OTA bioavailability of grain was 4.3 times greater. A research indicated that sometimes the OTA biodegradation by ruminal and intestinal microorganisms may not be complete and it can be found in the feces and urine in significant concentrations (Höhler et al., 1999). This indicates that the OTA can escape from the fermentation of rumen and hindgut. Gibson et al. (1989) proposed that protein concentration in the diet of growing chicks could ameliorate the toxicity of OTA. They showed that consumption of high-protein diets (26%) compared to a lower protein (14%) decreased the toxicity produced by 4 mg/kg of OTA as indicated by rate of growth and mortality. Similarly, the highest CP concentration for beef (17.7%) and dairy cattle (19.7%) feeds in Aegean region compared to other regions reflected to the lowest OTA concentration (0.76 µg/kg).

Although there seems to be critical limits for AF and OTA concentrations were somewhat at the borderline of EFSA and Turkish Ministry of Agriculture, feed producers and farmers should pay more attention for producing and using mycotoxin contaminated feeds or commodities.

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