Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Detection of Aflatoxin, Zearalenone and Deoxynivalenol in Some Feed and Feedstuffs in Turkey

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ARTICLE HISTORY (13-271)

Received: July 13, 2013
Revised: January 31, 2014
Accepted: April 20, 2014

Key words:
Aflatoxin
Deoxynivalenol
Feed
Feedstuff
Zearalenone

ABSTRACT

The aim of this study was to determine aflatoxin, zearalenone (ZON) and deoxynivalenol (DON) in some feed and feedstuff samples obtained from several farms and animal feed manufacturers in Turkey. A total of 106 samples (76 feedstuffs and 30 feeds) were analyzed by HPLC method. In samples of feedstuffs, ZON occurred at a high incident rate, however, AFG1 and G2 had a lower incident rate. In feed samples, although AFB2 and G2 occurred at a low incident rate, ZON had a higher incident rate. The data obtained in this study showed that toxin levels in feed and feedstuff samples were lower than maximal allowed levels.

To Cite This Article: Bilal T, DH Aksakal, S Sünnetci, O Keser and H Eseceli, 2014. Detection of aflatoxin, zearalenone and deoxynivalenol in some feed and feedstuffs in Turkey. Pak Vet J, 34(4): 459-463.

INTRODUCTION

In animal production, feed costs comprise about 70% of total cost of production. Besides potential hazards for human and animal health, molds may cause important economic losses by their toxic metabolites. Mycotoxins produced molds are found in food and feed supply chain and the infection of cereal plants with fungi triggers the risk of grain contamination with secondary metabolites, *i.e.*, mycotoxins, and their subsequent transfer to food and feed (Gahukar, 2014; Waśkiewicz *et al*., 2014).

Aflatoxins (AF) are produced by three species of *Aspergillus* (*A. flavus, A. parasiticus and A. nominus*) which contaminate plants and plant products. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins (Creppy, 2002; Jubeen *et al*., 2012). All cereals, especially wheat and its by-products, and feeds are considered as the most hazardous materials with regard to AF. In a comparative study on the production of AF in wheat, corn, rice and groundnuts reported the highest level of AF in wheat (Demet *et al.,* 1995). Zearalenone is a fusariotoxin produced by some species of genus *Fusarium* and leads to a number of diseases in animals resulting in considerable losses of production and a high rate of mortality (Valcheva and Valchev, 2007). Zearalenone is a non-steroidal estrogen and its major metabolites α-zearalenol and β-zearalenol elicit significant estrogenic activity in animals, corresponding to their binding affinities for hepatic, uterine, mammary and hypothalamic estrogen receptors (Fink-Gremmels and Malekinejad, 2007). Deoxynivalenol (DON) is one of the toxins mainly produced by *Fusarium graminearum*. This water-soluble toxin, also known as vomitoxin, responsible for emesis and feed refusal in non-ruminant animals (Forsyth *et al.,* 1997). DON appears predominantly in wheat, corn, rye, rice and barley. Studies in the USA, Germany, the Netherlands, Bulgaria, Hungary, Russia, China, Korea and Argentina have shown that 60-100% of samples tested had DON contamination (Cahill *et al.,* 1999). Previous studies on the occurrence of mycotoxins in feeds and feedstuffs shown that the concentration of AF, ZON and DON varied. Sonal and Oruç (2000) reported that average total AF, AFB1 and ZON were 6.94, 0.86, 78.64 µg/kg and incidence were 100, 65.38 and 100% respectively in 27 poultry feeds that were collected from chicken farms during June 2000 in Bursa province of Turkey. In a study carried out by Yildiz (2009) in Turkey, 145 roughage samples were examined for AF and 70 samples for ZON contamination. Ninety samples were AF-positive (62.07%, 2.00-214.80 µg/kg) and 21 samples were ZON-positive (30%, 50.00-442.60 µg/kg). It was also reported that AF contamination in Aegean and Mediterranean and ZON contamination in Mediterranean and Black Sea region was at higher level than the other regions in Turkey. Chilaka *et al.* (2012) analyzed 40 maize samples from Kwazulu Natal in South Africa and reported

that contamination level of mycotoxins, as determined by an HPLC method, ranged from 0-762 ppb for AFB and 0-135 ppb for ZON. Data obtained by TLC (thin layer chromatography) method showed that the prevalence were 33% for AFB and 30% for ZON. Binder *et al.* (2007) analyzed 1507 samples from European and Mediterranean markets and 1291 samples from Asian-Pacific region to investigate the occurrence of mycotoxins in commodities, feeds and feed ingredients. They found that more than half of materials sampled in Europe were contaminated by AF, ZON and DON at levels above the limit and Asian-Pacific sourced samples were also positive. However, Aydin and Oğuz (2012) reported that AFB_1 and ZON contamination were not detected in any of the sample in 260 corn silage samples which were the crops of the year 2007 collected from dairy cattle and sheep enterprises in several provinces of Anatolia- Turkey.

Although there are many methods for the determination of mycotoxin contamination, high-performance liquid chromatography (HPLC) is most useful in terms of specificity and sensitivity, especially with an immunoaffinity column clean-up step to concentrate and purify mycotoxins (Pirestan *et al.,* 2011; Khan *et al*., 2013). The aim of this study was to investigate the occurrence of AF (B_1, B_2, G_1, G_2), ZON and DON in some feed and feedstuffs obtained from different farm and enterprises in Turkey by HPLC.

MATERIALS AND METHODS

Samples: A total of 106 samples (76 feedstuffs and 30 feeds) of feeds and feedstuffs were obtained between April and June 2011 from several farms and animal feed manufacturers in Marmara, Aegean and Mediterranean Region in Turkey. As mentioned by Sokolovic and Simpraga (2006), primary large samples of approximately 10 kg were composed of several samples collected from different part of storage lots. The primary samples were homogenized and quartered to obtain a 1 kg of laboratory sample. All samples were stored at 4°C for further analysis.

Standards, chemicals and instrument: Aflatoxin, ZON and deoxynivalenol standards were purchased from R-Biopharm AG (Darmstadt, Germany). High performance liquid chromatography (HPLC) solvents and other chemicals were purchased from Merck (Darmstadt, Germany). HPLC system (model 1100, Agilent, USA) equipped with ODS-2 (5 µm, 4.6 mm x 250 mm) and ODS-EP (5 µm, 4.6 mm x 150 mm) columns (Hicrom Ltd., UK) and FLD-Fluorescent detector (Thermo Fisher Scientific Inc., Waltham, MA, USA) and DAD-Diode array detector (model 1100, Agilent, USA) was used for measurements. Immunoaffinity columns (EASI-EXTRACT® AFLATOXIN, EASI-EXTRACT® ZEARALENONE, DONPREP) were purchased from R-Biopharm AG (Darmstadt, Germany).

Preparation of calibrant solutions for HPLC and calibration: For preparation of aflatoxin mix stock solution, 1 ml of main stock solution was diluted with 10 ml toluene/acetonitrile (98:2 v/v) and grade II stock standard was prepared. AF B1-G1 and AF B2-G2 concentrations of grade II stock standard were 1000 and 200 ng/ml, respectively. Grade II stock standard was pipetted into a volumetric flask and completed to 10 ml with toluene/acetonitrile (98:2 v/v) and shaked again. After shaking, the concentrations of this mix (grade III stock standard) were 0.1 ng/ml for AF B_1-G_1 and 0.02 ng/ml for AF B_2-G_2. For post-column derivatization, 10, 30, 50, 70 and 90 µl of grade III stock standard solutions were pipetted into vials, then toluene/acetonitrile solution was evaporated just to dryness under a stream of nitrogen at room temperature. Then, 1 ml of HPLC-grade methanol was added to each vial and shaked to dissolve aflatoxins, and mixes were completed up to 2.5 ml with ultra pure water. These prepared standards were analyzed six times in HPLC for calibration.

For zearalenone, main stock solution was diluted with acetonitrile and concentration of grade II stock solution was 5 µg/ml. After pipetting of 20, 40, 80, 200, 400, 800 and 2000 µl of grade II solution into vials, acetonitrile solutions were evaporated under a stream of nitrogen at room temperature. Then, 2 ml acetonitrile/water (3:7 v/v) was added to each vial and shaked. To prepare a calibration table, first three and last four solutions were injected to HPLC five and four times respectively.

For DON, main stock solution was diluted with acetonitrile and concentration of grade II stock solution was 20 µg/ml. 500 µl of grade II solution was pipetted into a vial and evaporated under a stream of nitrogen at room temperature. Grade III calibrant solution (2 µg/ml) was prepared by dilution of residue in the vial with 5 ml of mobile phase. After pipetting of 62.5, 125, 250, 500, 1000 and 2000 µl of grade III solution into vials, each vial was completed up to 2 ml with ultra-pure water/acetonitrile/methanol (94:3:3 v/v/v) and shaked. Each of these calibrants was injected to HPLC five times for calibration table. Values of calibration accuracy at the point of 0.999 from each treatment were accepted.

Extraction procedures and HPLC method for detection of mycotoxins: For extraction of aflatoxin (Anonymous, 2005), 25 g of each sample, 5 g NaCI and 125 ml of methanol/water (70:30 v/v) were added into a blender. The mixture was homogenized for 2 min in 22000 rpm and then filtered through common filter paper. After that, 15 ml of filtrate was pipetted into a beaker and mixed with 30 ml of pure water. This dilution was completely filtered through glass microfiber filter paper (Watman, pore size 1.6 µm). After filtration, 15 ml of filtrate was passed through EASI-EXTRACT® AFLATOXIN immuno-affinity column at a flow rate of 1-2 drops/sec. The column was then washed with 10 ml of ultra pure water. After washing, 1 ml HPLC-grade methanol was passed (1 drop/sec.) through column to elute bounded aflatoxins into a vial and diluted with 1 ml ultra pure water. The mobile phase, water/acetonitrile/methanol (6:2:3 v/v/v) was run at a flow rate of 1 ml/min. Detection of aflatoxins were done at excitation and emission wavelengths of 360 and 430 nm, respectively, on a FLD-fluorescence detector coupled with a coring cell (COBrA cell) for derivatization.

For extraction of zearalenone (Fazekas and Tar, 2001), 50 g of each sample, 2 g NaCI and 100 ml of methanol/water (8:2 v/v) were added into a flask and shaked for 60 min. in 22000 rpm. Extract was filtered and 25 ml of filtrate was mixed with 75 ml of ultra pure water.

Diluted extract was filtered through glass microfiber filter paper. 50 ml of filtrate was passed through EASI-EXTRACT® ZEARALENONE immunoaffinity column at a flow rate of 1-2 drops/sec. After that, the column was washed with 10 ml of ultra pure water at a flow rate of 1-2 drops/sec. Bounded zearalenone eluted slowly with 2 ml HPLC-grade acetonitrile at a flow rate of 1-2 drop/sec into a vial. The mobile phase, water/acetonitrile (50:50 v/v) was run at a flow rate of 1 ml/min. Detection of zearalenone was done at excitation and emission wavelengths of 232 and 440 nm, respectively, on a FLD-fluorescence detector.

For extraction of DON (R-Biopharm Ref no: A4.P50.V1, 2003), 25 g of each sample homogenized with 200 ml ultra pure water for 120 min. in 22000 rpm. Extract was filtered through filter paper (Watman no. 4). 2 ml of filtrate was passed through DONPREP immuno-affinity column at a flow rate of 1-2 drops/sec. and then the column was washed with 5 ml of ultra pure water. After washing, bounded DON eluted with 1.5 ml HPLC-grade methanol into a vial at a flow rate of 1-2 drops/sec. The eluate was evaporated to dryness at 60˚C with a stream of nitrogen gas and vortexed with 1 ml of mobile phase. The mobile phase, water/acetonitrile/ methanol (94:3:3 v/v/v) was run at a flow rate of 1 ml/min. Detection of DON was done at wavelengths of 218 nm on a DAD-Diode array detector.

RESULTS

A total of 76 feedstuffs and 30 feeds were analyzed for mycotoxin and data obtained on HPLC analysis demonstrated the occurrence of AFB1, AFB2, AFG1, AFG2, ZON and DON as summarized in Tables 1 and 2. In feedstuff samples, results showed that incident rates were 26.32, 7.89, 5.26, 5.26, 31.58 and 18.42 percent for AFB1, AFB2, AFG1, AFG2, ZON and DON, respectively. ZON occurred at a high incident rate with a range of 0-96.61 ppb. However, AFG1 and G2 had a lower incident rate ranged 0-1.90 and 0-0.04 ppb, respectively. According to the data from the analysis of beef and dairy cattle, calf, sheep, lamb and broiler feeds, incident rates were 56.66, 3.33, 30.00, 3.33, 73.33 and 43.33 percent for AFB1, AFB2, AFG1, AFG2, ZON DON, respectively. Although ZON had a higher incident rate with a range of 0-37.72 ppb rather than the other toxins, AFB2 and G2 occurred at a low incident rate ranged 0-0.31 and 0-0.67 ppb, respectively. Limits of quantification and recoveries levels have been presented in Tables 3 and 4, respectively.

DISCUSSION

The present study revealed that AF levels of feedstuffs and feeds were low and none of samples exceeded the maximum permitted level by EU and Turkey. According to a recent study carried out by Oruc *et al.* (2012), the incidence of AFB_1, DON and ZON in feed and feedstuff samples was 100, 75 and 33%, respectively, and these levels were lower than the mycotoxin limits of the EU and Turkey. Oguz *et al.* (2011) reported that none of 210 wheat flour samples were found positive for AF contamination and AF levels in 150 mixed feed samples were found below the maximum permissible level as determined by Ministry of Agricultural and Rural Affairs of Turkey. Sonal and Oruc (2000) carried out an study on mycotoxin levels in 27 mixed feed samples taken from poultry farms in Bursa Province of Turkey and the mean total AF and AFB1 concentrations were 6.94 and 0.86 ppb whereas the incidence of total AF and AFB1 were 100 and 65.38%, respectively. They also reported that these levels of AF could not be considered a risk to poultry health and productivity. Binder *et al.* (2007) reported that there were no evidence of aflatoxin contamination in the 98 wheat samples tested and his result agreed with data obtained from 33 wheat samples in present study. In comparison with the previous studies the AF concentrations in feed

Table 1: Aflatoxin contamination and incidence in feedstuff and feed samples

	Feedstuffs											
	AF											
	AFB1, ppb			AFB2, ppb			AFG1, ppb			AFG2, ppb		
	Occurrence	Content	Mean	Occurrence	Content	Mean	Occurrence	Content	Mean	Occurrence	Content	Mean
Maize	1/4	0-1.58	0.39	0/4	ND	0	0/4	ND	0	0/4	ND	0
Maize gluten	3/3	0.32-5.55	2.85	1/3	0-0.27	0.09	0/3	ND	0	0/3	ND	0
Maize bran	3/3	0.14-2.70	0.15	2/3	0-0.27	0.15	1/3	0-0.27	0.09	0/3	ND	0
Maize DDGS	3/4	0-0.32	0.15	1/4	0-0.07	0.01	0/4	ND	0	0/4	ND	0
Wheat	0/33	ND	0	0/33	ND	0	0/33	ND	0	0/33	ND	0
Wheat bran	0/5	ND	0	0/5	ND	0	0/5	ND	0	0/5	ND	0
Barley	1/5	0-0.10	0.02	0/5	ND	0	0/5	ND	0	0/5	ND	0
Full fat soybean	1/3	0-0.12	0.04	0/3	ND	0	0/3	ND	0	0/3	ND	0
Soybean meal	0/5	ND	0	0/5	ND	0	0/5	ND	0	0/5	ND	0
Sunflower meal	5/8	0-0.76	0.23	0/8	ND	0	2/8	0-1.90	0.27	4/8	0-0.40	0.09
Cottonseed meal	3/3	0.92-11.37	7.36	2/3	0-1.76	1.15	1/3	0-0.36	0.12	0/3	ND	0
Total	20/76	0-11.37	1.02	6/76	0-1.76	0.13	4/76	0-1.90	0.04	4/76	0-0.04	0.01
Incident rate, %		26.32			7.89			5.26			5.26	
	Feeds											
	AF											
	AFB1, ppb			AFB2, ppb			AFG1, ppb			AFG2, ppb		
	Occurrence	Content	Mean	Occurrence	Content	Mean	Occurrence	Content	Mean	Occurrence	Content	Mean
Beef cattle	5/7	0-0.51	0.21	0/7	ND	0	2/7	0-0.99	0.22	0/7	ND	0
Dairy cattle	6/9	0-3.31	0.91	1/9	0-0.31	0.03	2/9	0-0.35	0.07	0/9	ND	0
Calf	2/3	0-0.22	0.12	0/3	ND	0	1/3	0-0.47	0.16	0/3	ND	0
Sheep	0/1	ND	0	0/1	ND	0	1/1	1.1	1.1	1/1	0.67	0.67
Lamb	1/3	0-0.17	0.06	0/3	ND	0	1/3	0.46	0.15	0/3	ND	0
Broiler	3/7	0-0.99	0.30	0/7	ND	0	2/7	0-0.56	0.12	0/7	ND	0
Total	17/30	0-3.31	0.26	1/30	0-0.31	0.01	9/30	0-1.1	0.30	1/30	0-0.67	0.11
Incident rate, %		56.66			3.33			30.00			3.33	

Table 2: Zearalenone and deoxynivalenol contamination and incidence in feedstuff and feed samples

	Feedstuffs					
	ZEA, ppb			DON, ppb		
	Occurrence	Content	Mean	Occurrence	Content	Mean
Maize	1/4	0-25.01	6.25	3/4	0-283.66	122.34
Maize gluten	2/3	10.48-27.64	12.70	1/4	0-4769.63	1589.87
Maize bran	3/3	16.81-42.39	25.51	2/3	0-1335.31	641.23
Maize DDGS	2/4	0-96.61	35.85	4/4	115.05-3484.41	1816.47
Wheat	6/33	0-38.32	4.31	1/33	0-1226.76	37.17
Wheat bran	1/5	0-14.16	2.83	0/5	ND	0
Barley	0/5	ND	0	1/5	0-424.58	84.91
Full fat soybean	2/3	0-12.78	7.68	0/3	ND	0
Soybean meal	2/5	0-10.52	4.15	0/5	ND	0
Sunflower meal	2/8	0-11.83	2.77	2/8	0-2108.04	362.88
Cottonseed meal	3/3	9.95-11.37	10.71	0/3	ND	0
Total	24/76	0-96.61	7.34	14/76	0-4769.63	423.17
Incident rate, %		31.58			18.42	
	Feeds					
	Occurrence	Content	Mean	Occurrence	Content	Mean
Beef cattle	4/7	0-37.41	12.36	4/7	0-88.92	33.57
Dairy cattle	6/9	0-37.72	12.12	6/9	0-486.20	130.94
Calf	3/3	13.40-27.54	20.00	1/3	0-168.16	56.05
Sheep	1/1	16.97	16.97	1/1	67.66	67.66
Lamb	2/3	0-12.11	7.83	1/3	0-266.68	88.89
Broiler	6/7	0-20.87	11.15	0/7	ND	0
Total	22/30	0-37.72	13.40	13/30	0-486.20	62.85
Incident rate, %		73.33			43.33	

Table 3: Limits of quantification (ppb) of method applied in feedstuffs and feed samples

	Feedstuffs					
	AFB1	AFB2	AFG1	AFG2	ZEA	DON
Maize	0.2	0.1	0.3	0.5	15	250
Maize gluten	0.2	0.1	0.3	0.5	15	250
Maize bran	0.2	0.1	0.3	0.5	15	250
Maize DDGS	0.2	0.1	0.3	0.5	15	250
Wheat	0.2	0.1	0.3	0.5	15	250
Wheat bran	0.2	0.1	0.3	0.5	15	250
Barley	0.2	0.1	0.3	0.5	15	250
Full fat soybean	0.2	0.1	0.3	0.5	15	250
Soybean meal	0.2	0.1	0.3	0.5	15	250
Sunflower meal	0.2	0.1	0.3	0.5	15	250
Cottonseed meal	0.2	0.1	0.3	0.5	15	250
	Feeds					
Beef cattle	0.6	0.4	0.8	0.8	15	200
Dairy cattle	0.6	0.4	0.8	0.8	15	200
Calf	0.6	0.4	0.8	0.8	15	200
Sheep	0.6	0.4	0.8	0.8	15	200
Lamb	0.6	0.4	0.8	0.8	15	200
Broiler	0.6	0.4	0.8	0.8	15	200

and feedstuff samples were below legal limits as in our study. In comparison of feedstuffs and feeds for AF incidence, it was observed that feeds had higher incidence rate rather than feedstuffs. Similarly, in a study carried out in Poland with 625 mixed feed and 1120 feedstuff samples, it was reported that feeds had more AF contamination than those of feedstuff samples (Juszkiewicz *et al.,* 1992).

The frequent contamination of grain with the Fusarium toxins, DON and ZON is an important issue in animal and human nutrition (Goyarts *et al.,* 2007). The Fusarium fungi are the most prevalent toxin-producing fungi of northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia (Creppy, 2002). It was reported that ZON could play an indicator role in the contamination of other Fusarium toxins (Kutay, 2003). Therefore, it would be meaningful to discuss ZON together with DON. Although many studies have focused on aflatoxicosis in Turkey, few have examined the impact of Fusarium toxins (Ozpinar *et al.,* 2001). In this study, 24 and 14 samples of feedstuffs and 22 and 13 samples of feeds had ZON and DON contamination, respectively. Incidence rates of ZON and DON were 31.58 and 18.42% in feedstuffs and 73.33 and 43.33% in feed samples, respectively. In 40 maize samples, Chilaka *et al.* (2012) reported that the contamination level of ZON as determined by HPLC were 0-135 ppb and occurred at a high incidence rate of 90%. Pleadin *et al.* (2013) investigated ZON and DON levels in 63 maize, 51 wheat, 34 barley and 34 oats samples and percentage of positive samples were 78, 69, 9 and 6 for ZON, 71, 65, 53 and 21 for DON, respectively. Minimum and maximum levels for ZON were 10-611 (mean 187), 7-107 (mean 56), 5-68 (mean 32) and 4-43 ppb (mean 44 ppb), and for DON were 215-2942 (mean 1565), 115-278 (mean 223), 74-228 (mean 342) and 34-201 ppb (mean 145 ppb), respectively. They also reported that maize was the most contaminated cereal and the mean concentrations of ZON and DON found maize were significantly higher than those found in other samples. In contrary, the range of ZON and DON concentration in maize samples were 0-25.01 (mean 6.25) and 0-283.66 ppb (mean 122.34 ppb), respectively in our study. Furthermore, maize products (gluten, bran and DDGS) had higher mean ZON and DON concentrations rather than the other feedstuffs in our study. In a survey study on 1507 samples sourced from European and Mediterranean markets, Binder *et al.* (2007) reported that mean and maximum levels of ZON were 180 and 970 ppb for Northern Europe, 273 and 1392 ppb for Central Europe, 174 and 2348 ppb for Southern Europe + Mediterranean samples. For DON, mean and maximum levels were 559 and 5510 ppb for Northern Europe, 571 and 8020 ppb for Central Europe, 304 and 3036 ppb for Southern Europe + Mediterranean samples. In Croatia, a total of 465 grains and animal feed samples were tested and the overall incidence of DON was 41.2% and the percentage of samples positive for DON varied up to 71.4% (Sokolovic and Simpraga, 2006). Kutay (2003) reported that DON concentrations of 91 feedstuff samples of wheat, maize, barley, oat, soybean meal, sunflower meal and 30 feed samples were analyzed and all samples, except oat and sunflower meal, had DON contamination in range of 200-6200 ppb. In our study,

Table 4: Recoveries (ppb) of method applied in feedstuffs and feed samples

Feedstuffs	AFB1	AFB2	AFG1	AFG2	ZEA	DON
Maize	81	87	91	82	93	90
Maize gluten	80	79	86	76	92	92
Maize bran	83	86	82	78	91	94
Maize DDGS	81	78	79	77	89	92
Wheat	89	90	81	82	87	89
Wheat bran	88	87	86	78	89	85
Barley	92	89	87	78	85	88
Full fat soybean	91	90	85	84	88	85
Soybean meal	92	89	785	79	87	87
Sunflower meal	93	87	85	78	88	86
Cottonseed meal	91	89	84	75	84	86
Feeds						
Beef cattle	99	98	97	96	95	94
Dairy cattle	100	93	91	94	94	90
Calf	98	95	96	78	96	92
Sheep	96	93	82	80	95	91
Lamb	95	92	84	79	93	90
Broiler	94	91	93	78	92	89

DON were not detected in wheat bran, barley, full fat soybean, soybean meal, cottonseed meal and broiler feed samples and these results disagreed with that of Kutay (2003). Yildiz *et al.* (2005) reported that ZON levels were higher than 60 ppb in 29 of 128 feed samples and ZON incidence rates in poultry and ruminant feeds were 28.13 and 31.34 %, respectively. Similarly Sonal and Oruc (2000) reported that mean ZON levels and incidence rate were 78.64 ppb and 100% in 27 poultry feed samples. It was reported that the legal tolerable level of ZON in Germany was 250 ppb for pre-ruminants and beef cattle and 500 ppb for calves and dairy cows (Yildiz, 2009). In present study, none of the samples exceeded these levels and ZON and DON levels of all samples were below from the permissible levels in Turkey and Europe.

Conclusion: The data obtained in this study showed that feedstuffs and feeds available in Turkey were contaminated with varied levels of AF, ZON and DON and that these levels lesser than the tolerable limits. However, it must be considered that mycotoxin contamination and concentrations in feedstuffs and feeds can be vary according to regional climate, annual rainfall regime, harvesting methods, storing conditions etc. The contriving of comprehensive and detailed studies regularly with the greater number of samples in several countries will be effective and beneficial in the detection of mycotoxin profile.

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