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Occurrence of Aflatoxin B1, T-2 Toxin and Zearalenone in Compound Animal Feed

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Abstract: This study was performed to determine mycotoxin (aflatoxin B1 (AFB1), T-2 toxin and Zearalenone (ZEA)) levels in compound ruminant feeds collected from local feed sellers in Samsun province, Turkey. Forty compound feed samples were collected and analyzed by the ELISA method. The mean and standard error of the AFB1 were 6.81±0.81 (range 0.15-32) μg kg^{-1}. T-2 toxin and ZEA levels were 33.87±5.80 (2.24-132.2) μg kg^{-1} and 175.26±43.05 (51.61-1023.25) μg kg^{-1}, respectively. The incidence of Aflatoxin B1 (AFB1), T-2 toxin and Zearalenone (ZEA) in compound feeds was found to be 95, 65 and 87.5%, respectively. Although, most of samples were found to be contaminated with mycotoxins, the levels of contamination for AFB1 and T-2 toxins were found to be relatively lower than that of ZEA levels in the feed samples. The types and levels of mycotoxins present varied. The most common contaminants were the AFB1 and ZEA. Twenty-three of the samples contained three types of mycotoxins (57.5%). Only one sample did not contain any mycotoxin (2.5%). Two samples contained only AFB1 (5%) and the rest (14 samples) contained two types of mycotoxins (35%). The mycotoxin found in highest levels was ZEA and the mycotoxin with the higher frequency of occurrence was AFB1. Correlation between the occurrence of the Fusarium toxins (T-2 toxins and ZEA) was found to be statistically significant ($p<0.05$).

Key words: Mycotoxin, feed, ELISA, Samsun province

INTRODUCTION

Mycotoxins are fungal toxins produced by the mycelial structure of filamentous fungi, commonly called the molds (Hussein and Brasel, 2001). They are mainly produced by fungi belonging to *Aspergillus, Penicillium* and *Fusarium* genera (Wagacha and Muthomi, 2008). Mycotoxins are found to occur widespread in food and feed varieties under environmental conditions that favor their growth such as suitable moisture and temperature (Adanyi *et al*., 2007), sufficient oxygen, physical damage to the commodity and presence of the fungal spores (Sforza *et al*., 2006). Consumption of a mycotoxin-contaminated food or feed may cause acute and long-term chronic effects that can range from teratogenic, carcinogenic, neurotoxic and estrogenic or immune-suppressive effect in humans and/or animals (Kabak *et al*., 2006; Binder *et al*., 2007). The direct consequences of consumption of mycotoxin-contaminated animal feed include decreased feed intake, feed rejection, reduced body weight gain and reproductive capacity, poor feed conversion and increased disease incidence (Morgavi and Riley, 2007; Voss *et al*., 2007).

AFB1 is a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* commonly found to grow in many animal feeds (Battacone *et al*., 2003). Aflatoxins were classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (WHO-IARC, 2002). AFB1 is known to increase the incidence of digestive disorders causing hemorrhage and necrosis and to reduce production efficiency. Toxicity occurs at the cellular level, causing DNA changes, cellular changes and death (Martins *et al*., 2007).

Trichothecenes constitute a large group of mycotoxins produced by various species of moulds, in particular those belonging to genus *Fusarium.* Approximately 170 trichothecene mycotoxins have been identified to date. The most prevalent mycotoxins of these groups are deoxynivalenol (vomitoxin), nivalenol, 3- or 15-acetyl-deoxynivalenol, fusarenon X, T-2 toxin, HT-2 toxin and diacetoxyscirpenol (Binder, 2007). T-2 toxin is a type A trichothecene and may be found in grains such as wheat, barley, oat, rice, rye and other crops (Richard, 2007), occuring mainly in cold areas (Molinelli *et al*., 2008). T-2 toxin has been reported to be toxic to in humans and various farm and laboratory animal species. The chronic toxicity of trichothecenes is

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characterized by anorexia, reduced weight gain, neuroendocrine changes and immunological effects (Rezar *et al*., 2007). The major physiological effect of T-2 toxin and other trichothecenes is that they inhibit protein synthesis, which is followed by a secondary disruption of DNA and RNA synthesis (Richard, 2007; Sokolovic *et al*., 2008).

ZEA is a secondary metabolite produced by several species of *Fusarium* fungi among, which *F. graminearum* and *F. culmorum* are mainly found to grow on several commodities (Sforza *et al*., 2006). ZEA and its metabolites are know to cause reproductive disorders (Richard, 2007; Thieu *et al*., 2008) described as hyperestrogenism (Gremmels, 2008) by mimicking the action of estradiol-17β (Chen *et al*., 2008). ZEA is better classified as a nonsteroidal estrogen or mycoestrogen. It is also, sometimes called a phytoestrogen (Bennett and Klich, 2003). Moreover, ZEA has also been shown to be genotoxic (Wang and Groopman, 1999; Stopper *et al*., 2005) hepatotoxic, hematotoxic and immunotoxic (Zinedine *et al*., 2007). ZEA is classified by IARC under group 3 carcinogens (WHO-IARC, 1993).

Analytical procedures for the determination of mycotoxins have improved. Immunological techniques for mycotoxin determination based on specific monoclonal and polyclonal antibodies produced against several toxins are commercially available and are essentially of 2 types: Immunoaffinity column-based analyses and ELISA tests (Sforza *et al*., 2006). Chromatographic methods have been used widely, including thin-layer chromatograpy, gas chromatography, gas chromatography with mass spectrometry detector and high-performance liquid chromatography (Chu, 1992; Richard *et al*., 1993; Sforza *et al*., 2006).

This study was aimed, at assessing the extent of natural occurrence of AFB1, T-2 toxin and ZAE in compound animal feed samples collected from Samsun province, Turkey.

MATERIALS AND METHODS

Sampling and sample preparation: Forty compound ruminant feed samples were collected from local feed sellers in Samsun province, Turkey. Feed samples were ground using a grinder. Five grams of ground sample was taken in a polypropylene tube and to it was added 25 mL of 70% methanol. The sample was shaken vigorously for 3 min using a shaker and filtered through filter paper (Whatman No.1). The filtrate obtained was diluted (1:1) with ultrapure water (Millipore).

AFB1, T-2 toxin and ZEA analysis: ELISA was performed using a Digital and Analog Systems microplate reader (Digital Analog System, Model A3, Rome, Italy). RIDASCREEN Mycotoxin ELISA kits (R-Biopharm AG, Darmstadt, Germany) were used for determination of AFB1, T-2 toxin and ZEA. The basis of the tests was antigen-antibody reaction. The wells in the microtiter strips were coated with specific antibodies to AFB1, T-2 toxin or ZEA. The measurements were made photometrically at 450 nm, with the absorbance and mycotoxin concentrations in the sample being inversely proportional to each other.

Statistical analysis was performed using SPSS 14.0 version (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The results of the screening of AFB1, T-2 toxin and ZEA in animal feed are given in Table 1. The types and levels of mycotoxins present varied. Although most of samples were found to be contaminated with mycotoxins, the levels of contamination for AFB1 and T-2 toxin were found to be relatively lower than that of ZEA levels for feed samples. The most common contaminants were the AFB1 and ZEA. Twenty-three samples contained 3 types of mycotoxins (57.5%). Only one sample did not show any mycotoxin (2.5%). Two samples contained only AFB1 (5%). The rest (14 samples) contained 2 types of mycotoxins (35%). Statistically, the correlation of the occurrence of Fusarium toxins between the T-2 toxin and ZEA was found to be significant ($p<0.05$) (Table 2).

In Turkey, tolerance limits for T-2 toxin and ZEA in animal feed have not been set until now. Thus, the present study is limited to determining the incidence of AFB1, T-2 toxin and ZAE in compound animal feed samples and could not extend to draw conclusions on the bearing the present results might have with the Turkish regulatory standards. Only in one feed sample, AFB1 level was found to be higher than the maximum acceptable limit (20 μg kg^{-1}) (Ministry of Agriculture of Turkey, 2008). The mean AFB1 concentration (6.81 μg kg^{-1}) in the present study was found to be lower than that previously reported by Thieu *et al*. (2008) in Southern Vietnam (10.88 μg kg^{-1}), Dutta and Das (2000) in India (412 μg kg^{-1}), Binder *et al*. (2007) in North Asia (35 μg kg^{-1}), in South-East Asia (38 μg kg^{-1}), South Asia (52 μg kg^{-1}) and Oceania (34 μg kg^{-1}). Martins *et al*. (2007) carried out a survey over a period of 10 years and reported on the presence of AFB1 (37.4%) in 1001 samples of cattle feed. The average incidence of AFB1 in 616 feed samples was found to be 8.1% in Northern Italy (Decastelli *et al*. 2007). In the current study, incidence of AFB1 (95%) was found to be higher than in the the studies mentioned.

T-2 toxin was present in 10% of the 1506 corn samples used as animal feed in Brazil (Salay and Mercadante,

Table 1: Occurrence of AFB1, T-2 toxin and ZEA in compound animal feed

	AFB1		T-2 Toxin		Zearalenone	
Mycotoxin level (μg kg^{-1})	n	(%)	n	(%)	n	(%)
Not detected	2	5.00	14	35.00	5	12.50
Detected samples	38	95.00	26	65.00	35	87.50
<1	4	10.00	0	0.00	0	0.00
1-5	5	12.50	2	5.00	0	0.00
5.1-10	27	67.50	1	2.50	0	0.00
11-50	2	5.00	20	50.00	0	0.00
51-100	0	0.00	1	2.50	27	67.50
101-250	0	0.00	2	5.00	3	7.50
>250	0	0.00	0	0.00	5	12.50
Total (n)	40	100.00	40	100.00	40	100.00
X±Sx	6.81±0.81 μg kg^{-1}		33.87±5.80 μg kg^{-1}		175.26±43.05 μg kg^{-1}	
Legal limit (Turkey) (μg kg^{-1})	20		-		-	

Table 2: Correlation coefficients between mycotoxins occurrence

	Zearalenone	T-2 toxin
AFB1	-0.05924	-0.18190
T-2 toxin	0.31709*	-

*p<0.05

2002). Rafai *et al.* (2000) found considerably more contamination with T-2 toxin in animal feeds (50-980 μg kg^{-1}) in contrast to the present study (2.24-132.2 μg kg^{-1}). The mean concentrations of the T-2 toxin in feed samples in Northern and Central Europe were found to be 137 and 190 μg kg^{-1}, respectively and were higher than that reported in the present study. But mean concentration of feed samples in Southern Europe and Mediterranean (30 μg kg^{-1}) was found to be less than that reported in the present study (Binder *et al*., 2007).

In the present study, the contamination of feed samples with ZEA (87.5%) was higher than that reported by Rafai *et al.* (2000) feeds such as maize (18.8%), wheat (58.6%), rye (57.1%) and bran (62.4%). However, mean ZEA concentration (175.27 μg kg^{-1}) in our feed samples was less than that for maize, wheat, rye and bran samples, 228.9, 210.3, 231.3, 308.5 μg kg^{-1}, respectively (Rafai *et al.*, 2000). Similarly, the mean concentration of ZEA from different regions in Asia and Oceania was found to be higher than our findings (Binder *et al*., 2007).

Ruminants such as cattle, sheep, goats and deer are less known for their sensitivity to the negative effects of mycotoxins than are non-ruminants. However, production of milk, beef, or wool, reproduction and growth can be altered when ruminants consume mycotoxin-contaminated feed for extended periods of time (Hussein and Brasel, 2001). The presence of T-2 toxin, a cytotoxin and one of the prominent type A trichothecenes in feed for cattle, can result in severe irritation of the upper digestive tract and cause a hemorrhagic ruminitis (Gremmels, 2008). T-2 toxin is also believed to induce immunosuppression in cattle by decreasing serum concentrations of IgM, IgG and IgA neutrophil functions and lymphocyte blastogenensis and effect the response of lymphocytes to phytohemagglutinin (Hussein and Brasel, 2001). Aflatoxins affect the quality of milk produced by dairy cows and result in carry-over of AFM1 from AF-contaminated feed (D'Mello and Macdonald, 1997; Hussein and Brasel, 2001). Lambs fed daily on feed containing AF at concentrations of 2.5 mg kg^{-1} of feed for 21 days showed symptoms of clinical aflatoxicosis including hepatic and nephritic lesions, altered mineral metabolism and increased size and weight of the liver and kidney (Fernandez *et al*., 1997). In cows, infertility, reduced milk production and hyperestrogenism have been linked with ZEA and with *Fusarium* species producing this mycotoxin (D'Mello and Macdonald, 1997).

Since, mycotoxin affects animal health and the contaminated food can be a serious public health threat, need for permanent monitoring of aflatoxins, trichothecenes, ZEA and other mycotoxins, not only in animal feed but also in food for human consumption is mandatory.

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