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Determination of Ochratoxin A Levels in Mixed Feed and Feed Stuffs Used in Some Laying Hens and Ruminant Enterprises of Sivas City

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ABSTRACT

Mycotoxins, which are produced by some fungi under improper storage conditions before or after harvesting in plant products, cause acute or chronic toxicities. Ochratoxin A (OA) which is also one of the harmful mycotoxins pose a threat to animal and human health. This study was carried out in order to determine OA levels in mixed feed and feedstuffs materials used in livestock enterprises throughout Sivas province. The 59 mixed feeds and 30 feedstuffs materials collected from different enterprises was analysed. Ochratoxin A concentrations were quantified using immunoassay (ELISA). In result, OA was found to be positive in 64 (71.91%) of the 89 samples analysed, whereas OA was not found in 25 samples (28.09%).

INTRODUCTION

Mycotoxins produced by fungi are highly toxic and cause acute or chronic mycotoxicosis in animals consuming feeds contaminated with such compounds (Pfohl-Leszkowicz & Manderville, 2007). It was found that plant-derived feedstuffs may be contaminated with mycotoxins before and after harvesting, and mycotoxins have been found in animal products and in egg, milk and dairy products from animals fed with rations containing such feedstuffs (Hussein & Brasel, 2001; Meucci *et al.,* 2010). It has been reported that OA contamination in plant-based feedstuffs vary during the year due to climate, water content and storage (Jørgensen *et al.,* 1996).

Ochratoxins are mycotoxins, which are produced by *Aspergillus* and *Penicillium* fungi and have three derivatives as A, B and C (Girgin, 2001; Milićević *et al.,* 2010). Ochratoxin A and Ochratoxin B, which is less toxic, were first described by South African chemists in 1965 (Bayman *et al.,* 2002; Castella *et al.,* 2002). Ochratoxin A (OA), one of the mycotoxins commonly found in feedstuffs, is eliminated from the body through urine (Li *et al.,* 1997), feces (Madhyastha *et al.,* 1992) and milk (Breitholz-Emanuelsson *et al.,* 1993). The threshold value for OA in our country was determined as 0.25 ppm in cereal and cereal products; 0.1 ppm in complete and complementary feeds of poultry and 0.05 ppm in compete and complementary feeds of pig (Official Gazette, 2014). In a study conducted, OA was shown to reduce feed consumption, weight gain, and egg yield in birds (Hamilton *et al.,* 1982; Gibson*et al.,* 1989). It has also been reported that 0.34 and 1.68 mg OA/kg body weight in rat's feed (Dortant *et al.,* 2001), 0.5-2 mg / kg in chicken's feed (Singh *et al.*, 1990) and 2.5 mg / kg in pig's feed (Harvey 1992) pressurizes the immune system. In ruminants, OA toxicity is lower than the ones with a single stomach due to microbial activity in the anterior stomach (Höhler *et al.,* 1999). In fact, it was reported that the dose of 14 µg/kg body weight OA does not show any clinical symptoms and toxicities in sheep

(Blank *et al.,* 2004). Furthermore, OA was found to be carcinogenic, immunotoxic and nephrotoxic in many different species (Pfohl-Leszkowicz & Manderville, 2007; El Khoury & Atoui, 2010).

This study was carried out to determine the contamination of Ochratoxin A in various mixed feed and feedstuffs materials used in livestock enterprises throughout Sivas city.

MATERIAL AND METHODS

Collection of Feed and Feedstuffs Samples

Due to its geographical location, the hottest times of the year are during the summer season in Sivas province. For this reason, samples (59 mixed feeds and 30 feedstuffs materials) collected from the randomly selected livestock enterprises in the province in July and August 2016 were gathered according to a method determined by Ergun *et al.* (2007). Mixed feed samples consisted of calf growth (n=10), cattle fattening (n=23), dairy cow (n=20) and laying hen (n=6) feeds, while feedstuffs material samples consisted of cottonseed meal (n=12), sunflower meal (n=10), soybean meal (n=3) and wheat bran (n=5). The samples taken from storage at the enterprises were kept at +4°C in the refrigerator and analysed within one week when the sample collection work was finished (samples were kept in the refrigerator for a maximum of 60 days).

Biochemical Analyses

Levels of Ochratoxin A in feed samples were determined by ELISA device (Multiskan, Thermo Fisher Scientific, USA) using the commercial kit (RIDASCREEN, Art No: R1311) via the competitive inhibition enzyme immunoassay method based on the manufacturer's procedure. Limit of detection for feed 2.5ppb as the described method according to Elisa kit procedure(R-Biopharm, 2016).

For preliminary processing of feed samples; 5 grams were weighed and taken from each sample. Each sample with an addition of 100 ml of 0.13M sodium hydrogen carbonate buffer was vortexed and mixed for 15 minutes. It was centrifuged at room temperature (NF 800 R, Nüve, Ankara, Turkey) for 15 minutes at 3500g. 50µl taken from each pre-processed sample was added to the wells. They were incubated for 30 minutes at room temperature and in a dark place by adding 50 µl of conjugate enzyme. After the laundering process, substrate was added and then absorbance was detected at 450nm wavelength by incubating for 15 minutes under the same conditions.

Statistical Analysis

SPSS 20.00 statistical package program was used to determine mean, minimum and maximum values of obtained data (SPSSChicago, 2011).

RESULTS

Of the 89 samples analysed, 64 of them (71.91%) were found to have OA, while 25 (28.09%) of them were not found to have OA (Table 1). Throughout the 59 samples of mixed feed analysed for Ochratoxin A; OA contamination was found higher than 5-19 ppb in 26 samples (44.07%), 20-40 ppb in 12 samples (20.34%) and >40 ppb (10.17%) in 6 samples. The samples of mixed feed in 15 (25.42%) did not detect ochratoxin A (Table1). During the analysis of the feedstuffs materials, OA contamination was found 5-19 ppb (43.33%) in 13 samples, 20-40 ppb in 4 samples (%13.33) and >40 ppb in 3 of the samples (10.00%). The samples of feedstuffs in 10 (33.33%)

Table 1 - The ochratoxin a levels in mixed feeds and feedstuffs,%

Feeds	n	Undetected		5-19 ppb		20-40 ppb		>40 ppb	
		n	%	n	%	n	%	n	%
Mixed Feeds									
Calf growth feed	10	3	30.00	6	60.00	1	10.00	0	-
Cattle fattening feed	23	6	26.09	9	39.13	5	21.74	3	13.04
Dairy cow feed	20	4	20.00	10	50.00	4	20.00	2	10.00
Laying hen feed	6	2	33.33	1	16.67	2	33.33	1	16.67
Feedstuffs									
Cottonseed meal	12	5	41.67	4	33.33	1	8.33	2	16.67
Sunflower meal	10	2	20.00	5	50.00	2	20.00	1	10.00
Soybean meal	3	1	33.33	1	33.33	1	33.33	0	-
Wheat bran	5	2	40.00	3	60.00	0	-	0	-
Mixed Feeds	59	15	25.42	26	44.07	12	20.34	6	10.17
Feedstuffs	30	10	33.33	13	43.33	4	13.33	3	10
Total	89	25	28.09	39	43.82	16	17.98	9	10.11

did not detect OchratoxinA (Table1). The mean, minimum and maximum values of mixed feed and feedstuffs materials are given in Table 2.

Table 2 - The levels of average, minimum and maximum ochratoxin in mixed feed and feedstuff (ppb).

Feeds	n	X±Sx	Minimum	Maximum
Mixed Feeds				
Calf growth feed	10	8.09±2.20	6.20	18.90
Cattle fattening feed	23	28.36±10.22	5.60	222.40
Dairy cow feed	20	35.46±15.92	7.80	305.60
Laying hen feed	6	27.28±14.96	8.40	96.30
Feedstuffs				
Cottonseed meal	12	28.36±14.15	9.50	157.20
Sunflowermeal	10	20.58±8.28	7.20	89.10
Soybean meal	3	14.00±10.43	7.60	34.40
Wheat bran	5	5.82±2.72	6.80	14.50

X±Sx: mean ± Standard error

DISCUSSION

Ochratoxin A is a very important toxin due to its frequent presence in nature and the pathological conditions it causes. When unprocessed agricultural products contaminated with Ochratoxin A are used as feed, they do not cause problems in ruminant adult animals; it may contaminate the meat products of non-ruminant animals such as pigs and poultry (Walker & Christian Larsen, 2005). In ruminants, protozoa and bacterial enzymes in the anterior stomach hydrolyze OA, resulting in phenylalanine and non-toxic OTα formation (Battacone *et al.*, 2010; Mobashar *et al.*, 2012). However, enzymes involved in the metabolism of phenylalanine indirectly have adverse effects on lipid peroxidation and mitochondrial respiration (Steyn & Stander, 1999).

According to the communiqué published in our country on April 19, 2014, 0.25 ppm in cereal and cereal products, 0.1 ppm in poultry complete and supplementary feeds and 0.05 ppm in pigs complete and supplementary feeds were determined, although the values of ochratoxin in the feeds vary according to the countries (Official Gazette, 2014). In our study, the ratio of OA found was 74.58% in mixed feeds and 66.67% in feedstuffs materials (Table 1). While these results are lower than the limit values that OA is allowed to be found in feeds, it may lead to a loss of yield and health problems in animals after such consumption of feeds for a long time (Moura *et al.,* 2004). In a study conducted in our country, the ratio of OA found was 66.67% in mixed feeds, while it was 47.83% in feedstuffs materials (Yıldız, 2009). In another study, it was found that 18.2% of the 302 mixed feeds, feedstuffs and food samples taken from 25 different feed plants in 7 different geographical regions of Turkey contained OA and 21.4% of these samples had 10-20 ppb of OA, 23.4% of them had 20-40 ppb of OA and 55.2% of them had higher than 40 ppb (Ozkazanc *et al.,* 1992). In a study by Araguás *et al.* (2005), 72 different cereal crops were analysed and although 79% of the samples were found to have OA contamination only 2 samples were above the allowed limit value. In a similar study, 91 grain products were analysed and it was determined that only 1 sample had OA above the allowed value (Vega *et al.,* 2009). In a study by Altintas *et al.* (2011), it was found that only 2 out of 56 samples had OA above the allowed limit.

Although cattle were shown to be more resistant to mycotoxin-induced toxicity than other animal species due to their stomach structure, chronic toxicities were observed in some dairy cattle (Whitlow & Hagler, 2004). It was reported that such toxicities did not have a specific symptom but there may be a decrease in milk yield or susceptibility to diseases and that inflammation and oedema were found especially in the intestines in the pathological examination performed (Whitlow & Hagler, 2004; Whitlow & Hagler, 2005). In our study, the samples taken from calf growth, cattle fattening and dairy cow feedstuffs, respectively contained 70.0%, 73.91% and 80.00% of OA above 5 ppb levels (Table 1). In a survey of feed samples collected from different regions in Turkey, it was reported that OA level was above 5 ppb in 66.66% of calf growth feed, 55.17% of cattle fattening feed and 60.00% of dairy cattle feed (Yıldız, 2009). Rosa *et al.* (2008) determined that OA positive of 25% for finished cow´s feed, 31% for maize and 22% for barley rootlets. When compared to the results of this study, it is seen that there are more samples containing OA in terms of percentage in our study. Accordingly, it can be said that the contamination in the feeds continues at a low level but still has a high rate.

In mixed feed samples (n=27) taken from poultry farms in and around Bursa province, the coincidence rate of OA was reported to be 100% (4.36±0.46 ppb) (Sonal & Oruc, 2000). Yıldız (2009) stated that OA level was 18.43±3.19 ppb in 75% of the laying hen feeds. In our study, 66.67% of laying hen mixed feed samples (n =6) contained OA above 5 ppb, but the quantities found were below 0.1 ppm, the limit value allowed to be found in poultry feeds according to the communiqué issued on 19 April 2014 (Official Gazette, 2014). However, it is known that the likelihood of

a single mycotoxin being present in a feed is very low, and it is generally known that the same fungus species can synthesize many mycotoxins and fungi that produce different mycotoxins (Hussein & Brasel, 2001) can easily infest this feed. For these reasons, the likelihood of other toxins existence in poultry feed is considered when the OA existence is high; there is a high possibility of seeing cases such as decrease in performance, weakening of immunity system and susceptibility to diseases in animals fed with these feeds.

In our research, OA was higher than 5 ppb levels respectively in 58.33%; 80.00%; 66.66% and 60.00% of the feedstuffs materials such as cottonseed meal, sunflower meal, soybean meal and wheat bran (Table 1). In a similar study, it was determined that the OA level was above 5 ppb in 50.00% of the cottonseed meal samples, 56.25% of the sunflower meal samples, 33.33% of the soybean meal samples and 16.67% of the bran samples (Yıldız, 2009). In a study in Sudan, in feeds like crushed sorghum (0.33–1.58 µg/kg), sunflower cake (1.59 µg/kg), wheat bran (0–0.43 µg/kg) and groundnut cake (0–0.31 µg/kg) OA contamination was found (Elzupir *et al.*, 2009). Also in a study in Romania, it was found that 34.6 out of 52 samples had OA positive (avarage 6.39 mg/kg) (Alexa *et al.,* 2013). In a study by Kaya *et al.* (1990) (n=20), soybean (n=9), cottonseed meal (n=11) and sunflower meal (n=11) were used as feedstuffs in the research they carried out. As a result of the study, OA was determined in 1 corn and 6 sunflower meal samples, and the amount of OA was 260 ppb in corn and 200-800 ppb in sunflower meal and 438 ppb on average. The results of the study by Kaya *et al.* (1990) are quite high compared to our results, which are thought to be due to factors such as regional differences, climate, relative air humidity and mechanical injury (Jørgensen *et al.,* 1996; Erzurum, 2001).

CONCLUSIONS

In the feed samples analysed, it was found that the quantities of Ochratoxin A were below the allowed value for feeds. Microbial contamination in feedstuffs can be avoided by hygiene and sanitation measures during crop harvesting and subsequent processes and by ensuring adequate environmental conditions (humidity, temperature, etc.) during storage. Nevertheless, it is thought that it would be useful to carry out routine analyses of coarse and concentrated feeds given to animals in terms of mycotoxins.

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