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HÜSAMETTİN EKİCİ

EBRU YILDIRIM

ENDER YARSAN

TÜBİTAK

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The effect of seasonal variations on the occurrence of certain mycotoxins in concentrate feeds for cattle collected from some provinces in Turkey

Hüsamettin EKİCİ[1,*], Ebru YILDIRIM[1], Ender YARSAN[2]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

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Abstract: The aim of this study was to determine the effects of seasonal variations on the occurrence of total aflatoxin (AFTotal), aflatoxin B1 (AFB1), ochratoxin A (OTA), and total fumonisin (FTotal) in the commercially mixed ruminant feed obtained from feed mills located in Ankara, Kırıkkale, Çankırı, Çorum, and Kırşehir provinces using the enzyme-linked immunoassay (ELISA) method. To represent the annual production, 22 samples were collected every quarter over 1 year (a total of 88 samples) starting in September 2012. AFTotal and AFB1 were detected in 72 samples (81.81%), OTA in 84 samples (95.45%), and FTotal in 83 samples (94.31%). When seasonal changes were taken into consideration, the highest levels of AFTotal, AFB1, and FTotal (13.57 ± 8.78 ppb, 8.54 ± 6.02 ppb, and 0.70 ± 0.88 ppm, respectively) were found in the summer, while the highest OTA level (57.69 ± 14.59 ppb) was observed in the spring. The results of high-performance liquid chromatography methods confirmed the results of the ELISA method. Consequently, although most of the feed samples were contaminated with mycotoxins, all the amounts were within the limits allowed for feedstuffs in Turkey.

Key words: Enzyme-linked immunoassay, high-performance liquid chromatography, mycotoxins, ruminant feed, seasonal variations, Turkey

1. Introduction

Mycotoxins are the toxic metabolites of fungi; they are associated with significant damage to organ systems. The harmful effects of mycotoxins include suppression of immunity, hepatotoxicity, carcinogenicity, nephrotoxicity, and neurotoxicity (1). Major mycotoxins threatening the public health are aflatoxins (AFs), ochratoxins (OTs), and fumonisins (2). AFs are considered to be the most toxic and carcinogenic mycotoxins that pose a risk to both animals and humans (3). AFs are produced by fungi of the genera *Aspergillus* and *Penicillium* (such as *A. flavus*, *A. parasiticus*, and *P. puberulum*). Some major members of AFs include AFB1 (the most potent member), AFB2, AFG1, and AFG2. Depending on the sensitivity of the animal and the amount of the received toxins, AFs can lead to acute, subacute, and chronic poisoning. A small amount of toxins in the animal feed can reduce the growth rate and the feed consumption ratio, thus decreasing the number of animal products and carcass quality, and causing immune system suppression in animals (4). OTs are a group of mycotoxins produced by *A. ochraceus* (also known as *A. alutaceus*) and *Penicillium viridicatum*. The most important members of this group are OTA, OTB, OTC, the methyl ester of OTA, and the ethyl ester of OTB. Of these, OTA is the most common pollutant found in feed and feed ingredients. OTA is very resistant to heat and affects protein, DNA, and RNA synthesis in the body (2–4). Fumonisins, which are synthesized by fungi of the genus *Fusarium*, have been reported to have negative effects on lipid metabolism (5). Fumonisins also have hazardous effects on the liver, cardiovascular system, kidneys, and embryos, as well as teratogenic effects. The ingestion of fumonisin-contaminated corn may even lead to cancer in humans (6).

Seasonal changes and climate variations can have an impact on food safety hazards at various stages of the food chain, from primary production to consumption. Most scientists accept the influence of weather on AF contamination. Appropriate temperature and water activity are critical for the production of mycotoxins and mycotoxin producing fungi. In general, if the temperature increases in cool or temperate climates, AF contamination becomes more problematic. In areas where storage facilities are controlled, it is possible to prevent postharvest contamination problems; however, this increases the cost and causes economic loss (5,7).

* Correspondence: hekici@kku.edu.tr

To our knowledge, no surveys have been undertaken to screen mycotoxin contamination in Ankara, Kırıkkale, Çankırı, Çorum, and Kırşehir provinces in terms of seasonal changes. Therefore, this study investigated the occurrence of mycotoxins in ruminant mixed feed obtained from the region and evaluated the effect of seasonal variations on the level of contamination. The enzyme-linked immunoassay (ELISA) method was used to determine the mycotoxins in feed samples since it provides many advantages to researchers such as feasibility, accuracy, high sensitivity, and time-efficiency (4).

2. Materials and methods

2.1. Samples

Ruminant mixed feed samples were randomly collected from feed mills located in Ankara, Kırıkkale, Çankırı, Çorum, and Kırşehir provinces to determine the occurrence of mycotoxin contamination and the effect of seasonal variation on contamination. To represent the annual production, 22 samples were collected every quarter over 1 year starting in September 2012. The feed samples were transported and stored at 4 °C until analysis.

2.2. Mycotoxin analysis

The quantitative analysis of total AF (AFTotal), AFB1, OTA, and total fumonisin (FTotal) in the samples was carried out using an ELISA commercial kit (HELICA Biosystems, Inc., HELICA for total aflatoxin-981AFL01LM-96, HELICA for aflatoxin B1-981BAFL01LM-96, HELICA for ochratoxin-941OCH01M-96, and HELICA for fumonisin-951FUM01C-96). Mycotoxin extraction and all tests were performed according to the manufacturer's instructions.

High-performance liquid chromatography (HPLC) was used to confirm the results of the samples obtained from the ELISA method. Ten samples with the highest results underwent HPLC. The HPLC apparatus (Shimadzu LC-20A, HPLC, Shimadzu, Tokyo, Japan) had a photo diode-array detector and fluorescence detector. The ten samples that had the highest levels, which were detected in the summer, were used for HPLC analysis of mycotoxins. The AFTotal was determined with the method of Ghali et al. (8), AFB1 was determined with the method of Stroka et al. (9), OTA was determined with the method of Teixeira et al. (10), and the FTotal was determined with the method of Ndube et al. (11). The methods were validated by the parameters of accuracy, recovery (AFTotal: 99.90%, AFB1: 99.50%, OTA: 99.90%, and FTotal: 95.99%), specificity, limit of detection (AFTotal: 0.03 ppb, AFB1: 0.01 ppb, OTA: 0.01 ppb, and FTotal: 0.002 ppm), and limit of quantitation (AFTotal: 0.08 ppb, AFB1: 0.03 ppb, OTA: 0.04 ppb, and FTotal: 0.007 ppm).

2.3. Statistical analysis

The values were assessed using the software provided by the relevant company. The quantitative evaluation was performed according to the standard curve obtained from the software. The calculated values were then evaluated in terms of their compliance with the tolerated limits declared in the announcement on undesirable substances in animal feed issued by the Ministry of Food, Agriculture, and Livestock of the Republic of Turkey (12). Statistical analysis of the data was carried out using SPSS 15.0 for Windows. The data were expressed as arithmetic means ± standard deviation, and the minimum and maximum values were recorded. One-way ANOVA was used to determine the seasonal variations among the groups. When the F value was significant, Duncan's multiple range test was performed. The results obtained from ELISA and HPLC were evaluated using Student's t-test. P values less than 0.05 were considered significant for all statistical calculations.

3. Results

AFTotal and AFB1 were detected in 72 samples (81.81%), OTA in 84 samples (95.45%), and FTotal in 83 samples (94.31%). All the analyzed feed samples were found to be below the values permitted by the Republic of Turkey's Ministry of Food, Agriculture, and Livestock Announcement No: 2014/11 on undesirable substances in animal feed (Tables 1–4). The arithmetic means ± standard deviation, the minimum and maximum values of the feed samples, and the comparison among the seasons are given in Table 5.

Table 1. The level of AFTotal in the analyzed feed samples.

Seasons	n	0–5 ppb	5–10 ppb	10–20 ppb	20 ppb and above
Winter	22	22	-	-	-
Spring	22	21	1	-	-
Summer	22	4	1	12	5
Autumn	22	6	16	-	-

Table 2. The compliance of AFB1 levels in the analyzed feed samples with relevant legislation*.

Seasons	n	0–5 ppb	5–10 ppb	10–20 ppb	20 ppb and above	Compliance
Winter	22	22	-	-	-	100%
Spring	22	22	-	-	-	100%
Summer	22	4	2	9	-	100%
Autumn	22	20	2	-	-	100%

*Republic of Turkey's Ministry of Food, Agriculture and Livestock Announcement No: 2014/11.

Table 3. The compliance of FTotal levels in the analyzed feed samples with the relevant legislation*.

Seasons	n	0–1 ppm	1–50 ppm	50 ppm and above	Compliance
Winter	22	22	-	-	100%
Spring	22	22	-	-	100%
Summer	22	19	3	-	100%
Autumn	22	19	3	-	100%

*Republic of Turkey's Ministry of Food, Agriculture and Livestock Announcement No: 2014/11.

Table 4. The compliance of OTA levels in the analyzed feed samples with the relevant legislation*.

Seasons	n	0–50 ppb	50–100 ppb	100–250 ppb	250 ppb and above	Compliance
Winter	22	22	-	-	-	100%
Spring	22	1	21	-	-	100%
Summer	22	18	4	-	-	100%
Autumn	22	22	-	-	-	100%

*Republic of Turkey's Ministry of Food, Agriculture and Livestock Announcement No: 2014/11.

Table 5. The arithmetic means ± standard deviation, the minimum and maximum values of AFTotal, AFB1, OTA, and FTotal levels in analyzed feed samples and comparison among seasons.

Seasons	Autumn	Winter	Spring	Summer
Mycotoxins	Mean ± SD Min.–Max.	Mean ± SD Min.–Max.	Mean ± SD Min.–Max.	Mean ± SD Min.–Max.
AFTotal (ppb)	4.90 ± 2.62^{a} (0–9.10)	0.58 ± 0.48^{b} (0–1.85)	1.84 ± 1.28^{a} (0–5.55)	13.57 ± 8.78^{c} (0–33.90)
AFB1 (ppb)	1.96 ± 1.67^{a} (0–5.65)	0.25 ± 0.17^{a} (0–0.60)	0.66 ± 0.57^{a} (0–2.36)	8.54 ± 6.02^{b} (0–19.24)
OTA (ppb)	19.25 ± 12.01^{a} (0–36.90)	1.04 ± 1.05^{b} (0–3.4)	57.69 ± 14.59^{c} (0–79.10)	43.80 ± 10.53^{d} (0–52.50)
FTotal (ppm)	0.37 ± 0.42^{a} (0–1.60)	0.04 ± 0.02^{b} (0–0.09)	0.12 ± 0.03^{ab} (0–0.18)	0.70 ± 0.88^{c} (0–3.9)

[a,b,c]The superscript letters within the same row indicate significant differences between the groups ($P < 0.05$). SD: standard deviation.

In terms of the AFTotal levels, there were similarities between the samples collected in the autumn and those collected in the spring; however, a significant decrease ($P < 0.05$) was observed in samples collected in the winter as compared with the other seasons. On the other hand, a significant increase ($P < 0.05$) was seen in samples collected in the summer as compared with the other seasons.

In terms of AFB1, similar results were obtained in the autumn, winter, and spring; however, an increase ($P < 0.05$) was observed in the samples collected in the summer as compared with those collected in the other seasons. The level of OTA showed significant differences ($P < 0.05$) among all seasons. The occurrence of OTA from the lowest to the highest level was detected in the winter, autumn, summer, and spring, respectively. The highest level of FTotal was observed in the summer while the lowest was detected in the winter ($P < 0.05$). No difference was found in terms of FTotal levels in the feed samples collected in the spring and autumn.

4. Discussion

Mycotoxin-forming fungi are commonly found all over the world. Field conditions and collation, storage, handling, and preparation stages are potential factors for the fungal growth and mycotoxin contamination in animal feed and food products. Consuming mycotoxin contaminated food may result in clinical and systemic disorders characterized by liver and kidney failure, skin, blood, and nervous system disorders, and hormonal imbalances as seen in domestic animals with acute or chronic mycotoxin toxicity (13). AFs, OTA, and FTotal are types of mycotoxins found in feeds and feedstuff, and in case of ingestion, they can pose a significant health risk for both humans and animals (14). Therefore, investigating the occurrence of mycotoxins in feed and feed ingredients on a regular basis is extremely important for public health and the economy.

According to relevant legislation in Turkey (Republic of Turkey's Ministry of Food, Agriculture, and Livestock Announcement No: 2014/11 on undesirable substances in animal feed), the maximum tolerable limits of AFB1 are: 0.02 ppm for animal feed and feed products; 0.005 ppm for mixed feed of dairy cattle and calves, dairy sheep and lambs, dairy goats and kids, piglets, and young poultry; 0.02 ppm for the mixed feed of cattle (dairy cows and calves excluded), sheep (dairy sheep and lambs excluded), goats (milk goats and kids excluded), pigs (piglets excluded), and birds (young birds excluded); and 0.01 ppm for supplementary feeds. The maximum tolerable limit for OTA in cereals and cereal products has been determined as 0.25 ppm. In terms of FTotal, the maximum tolerable limit is 60 ppm for corn and corn products used as feed ingredients; 20 ppm for complete and complementary feed of poultry, sheep, goats, and small calves older than 4 months; and 50 ppm for feedstuff of adult ruminants and mink. These values vary from one country to another and should be updated over time (12). None of the analyzed feed samples exceeded the legal limits.

The results of HPLC methods confirmed the results of the ELISA method. These results are presented in Table 6. There was no difference between the ELISA and HPLC results ($P > 0.05$). These results illustrate the reliability of the results obtained from commercial ELISA kits. Pirestani et al. (15) compared HPLC and ELISA methods to determine the concentration of AFs in milk and feed. They found no significant difference between the values obtained from the two procedures. Another study showed

Table 6. The results of ELISA and HPLC for mycotoxins (n = 10)*.

Samples	AF Total (ppb)		AFB1 (ppb)		OTA (ppb)		F Total (ppm)	
	ELISA	HPLC	ELISA	HPLC	ELISA	HPLC	ELISA	HPLC
1	15.1	14.98	9.35	9.3	45.9	45.84	0.503	0.496
2	15.1	14.99	10.49	10.29	47.7	47.56	0.6	0.586
3	15.39	15.12	11.57	11.5	47.7	47.68	0.792	0.79
4	17.59	17.28	12.91	12.86	48.2	48.03	0.792	0.786
5	17.88	17.35	12.35	12.01	49.4	49.26	0.277	0.273
6	21.5	21.15	18.1	18.02	49.4	49.28	0.28	0.279
7	23.1	22.95	13.7	13.5	50.2	50.12	0.287	0.281
8	23.1	22.96	13.7	13.6	50.7	50.59	1.9	1.885
9	24.26	24.05	17.88	17.82	52	51.86	2.2	2.181
10	33.9	33.6	19.24	19.22	52.5	52.44	3.9	3.884
Total (mean ± SD)	20.69 ± 5.84	20.44 ± 5.83	13.93 ± 3.39	13.81 ± 3.42	49.37 ± 2.06	49.27 ± 2.05	1.15 ± 1.18	1.14 ± 1.17

There was no difference between the ELISA and HPLC results ($P > 0.05$). SD: standard deviation.

that the AF levels in peanuts obtained by using HPLC and ELISA methods were similar; therefore, both HPLC and ELISA methods give accurate and reproducible results (16). Colak et al. (17) also stated that the results obtained from ELISA and HPLC methods are compatible. The results of our study are in accordance with these studies (15–17).

Many screening studies have been conducted to determine mycotoxin residues (4,18–20). Oğuz et al. (21) collected 150 feed samples from Konya and Mersin, and found that only 4 samples were contaminated with AFB1 (0.5–1 ppb). In another study, no AFB1 contamination was detected in corn silage samples collected from 13 different provinces of Turkey (22). Akkaya and Bal (23) analyzed 82 commercial feed samples collected from five different geographical regions in Turkey during the fall season and found that AFB1 levels were above 10 ppb for total AF in dairy cattle feeds in the southeastern Anatolia region. However, the OTA levels in other regions were within the acceptable levels. Basalan et al. (24) suggested that neither horse feeds nor dog foods exceeded the legal limit. Altıntaş et al. (4) found AFTotal and AFB1 contamination in 138 of 150 feed samples (90.2%) and OTA contamination in 51 of 56 feed samples (91.07%), and noted that the contamination level was above the maximum tolerable limit in 7 (5.07%) of the positive samples for AFB1 and 2 (3.92%) of the positive samples for OTA. Similarly, in the current study, different levels of OTA, AFTotal, and AFB1 were found in the feed samples, but these were all within the acceptable levels.

In a study conducted by Vega et al. (18), only one of the 91 cereal products was above the tolerable values in terms of OTA. Arslan and Essiz (25) also reported that AFB1 and AFTotal levels in silage were above the acceptable limits. In another study, the rate of mycotoxin contamination was 100% and 80% for AFB1 and FTotal, respectively, in feed obtained from the feed mills (26). Kocasari et al. (20) collected 180 feed samples from Burdur Province in Turkey and found 108 positive samples for AF (3.82–116.83 μg/kg), 84 for OTA (1.01–15.85 μg/kg), and 19 for FTotal (2.69–4.965 μg/kg).

Climate variations affect the presence of mycotoxins in foods. Hot weather, heat waves, heavy precipitation, and droughts are considered conducive conditions for the growth of mycotoxins. The effect of climate on the formation of mycotoxin contamination is considered important in various regions of the world such as Africa, Europe, Asia, Latin America, and North America. The increase in UV radiation can induce mutation in fungi on plants and generate different kinds of mycotoxins (6,13,14). In a study conducted by Pleadin et al. (27), the average value for AFB1 was found to be 81 μg/kg under extremely hot (>98%) and dry (<2%) weather conditions during the growth and harvesting period (May–September 2012).

Researchers have suggested that climate changes should be taken into consideration for the use of preharvest models to predict the risk of mycotoxin contamination in products such as wheat and maize (13). In addition, while environmental conditions such as temperature and CO_2 have little effect on the growth of AF, they were found to have a considerable effect on AF biosynthetic gene expression, thus inducing AFB1 production (28). Iqbal et al. (29) found that the level of AFTotal in 26 of 156 pepper samples was above the maximum tolerable limit by the European Union, and noted that if high levels of mycotoxins occurred due to climatic change, it is important to know the minimum tolerable level.

After analyzing three different kinds of feed (pig feed, wheat, and corn), Monbaliu et al. (19) found that 67 of the 82 samples were contaminated with mycotoxins; they noted that the most frequently occurring mycotoxin types were B-trichothecenes and FTotal. In a study conducted in Argentina, south of Buenos Aires Province, Palacios et al. (30) showed that the FB1 and FB2 range was 10.5–1245 ng/g in the 2007 harvest season, while the levels were lower in the 2008 harvest season. In the current study, the highest occurrence of mycotoxins (AFTotal, AFB1, and FTotal) was in the summer and the highest OTA levels were in the spring. These increases in the levels of mycotoxins can be attributed to an increase in molds or fungi formed in the previous season when the humidity and temperature were favorable for fungal growth.

This study investigated the effect of seasonal changes on mycotoxin contamination of feedstuff collected from different provinces in Turkey. The summer and spring were the critical seasons for contamination by the AFB1, OTA, and FTotal mycotoxins. However, all the levels were within the acceptable limits, which is a favorable result for the consumers. In order to develop strategies to prevent mycotoxin contamination, further screening studies should be undertaken using different types of mycotoxins (other than AF, FTotal, and OTA). Moreover, those studies should be conducted in certain seasons, particularly in the winter and autumn. In addition, we suggest that mycotoxin screening be carried out on a regular basis to provide updated information for producers and animal owners in order to help prevent their feedstuff from being contaminated with mycotoxins.

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