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The Effects of Production, Transportation and Storage Conditions on Mold Growth in Compound Feeds

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***Abstract*—**The objective of the present study is to determine the critical control points during the production, transportation and storage conditions of compound feeds to be used in the Hazard Analysis Critical Control Point (HACCP) feed safety management system. A total of 40 feed samples were taken after 20 and 40 days of storage periods from the 10 dairy and 10 beef cattle farms following the transportation of the compound feeds from the factory. In addition, before transporting the feeds from factory immediately after production of dairy and beef cattle compound feeds, 10 from each total 20 samples were taken as 0 day. In all feed samples, chemical composition and total aflatoxin levels were determined. The aflatoxin levels in all feed samples with the exception of 2 dairy cattle feeds were below the maximum acceptable level. With the increase in storage period in dairy feeds, the aflatoxin levels were increased to 4.96 ppb only in a BS8 dairy farm. This value is below the maximum permissible level (10 ppb) in beef cattle feed. The aflatoxin levels of dairy feed samples taken after production varied between 0.44 and 2.01 ppb. Aflatoxin levels were found to be between 0.89 and 3.01 ppb in dairy cattle feeds taken on the 20th day of storage at 10 dairy cattle farm. On the 40th day, feed aflatoxin levels in the same dairy cattle farm were found between 1.12 and 7.83 ppb. The aflatoxin levels were increased to 7.83 and 6.31 ppb in 2 dairy farms, after a storage period of 40 days. These obtained aflatoxin values are above the maximum permissible level in dairy cattle feeds. The 40 days storage in pellet form in the HACCP feed safety management system can be considered as a critical control point.

***Keywords*—**Aflatoxin, beef cattle feed, compound feed, dairy cattle feed, HACCP.

I. Introduction

MYCOTOXINS are ubiquitous, mold produced toxins that contaminate a wide variety of foods and feeds. Approximately 25% of crop production around the world is affected by mycotoxins [1]. Ingestion of mycotoxins causes a range of toxic responses, from acute toxicity to chronic health disorders. Some mycotoxins have caused outbreaks of human toxicoses, and at least one mycotoxin, aflatoxin B1, is a presumed human hepatocarcinogen [2]. The economic costs of contamination of feeds with mycotoxins are impossible to accurately determine, however, the mean economic annual costs of crop losses from the mycotoxins, aflatoxins, fumonisins, and deoxynivalenol only in USA, are estimated to be $932 million [3]. The severity of mycotoxin contamination of agricultural commodities varies yearly. Humidity, moisture during harvest and storage, temperature extremes, harvesting methods, and insect infestation are major environmental factors that determine the severity of mycotoxin contamination [2], [4]. Mycotoxin contamination reduces growth efficiency, lowers feed conversion and reproductive rates, impairs resistance to infectious diseases, reduces vaccination efficacy, and induces pathologic damage to the liver and other organs [2]. Molds generally grow in feeds if the humidity of medium is higher than 12-15% [36].

Aspergillus flavus produces mycotoxin and infest stored products. *Aspergillus flavus* and the closely related subspecies parasiticus have long been recognized as major contaminants of organic and nonorganic items. *Aspergillus flavus* can infest a wide range of agricultural products. Some *Aspergillus flavus* varieties produce aflatoxins, which are carcinogenic toxins that induce liver cancer in laboratory animals. *Aspergillus flavus* var. flavus, *Aspergillus flavus* subsp. *parasiticus*, and *Aspergillus nomius* share the ability to produce aflatoxins. The main types of naturally occurring aflatoxin are distinguished. These include aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1 and aflatoxin M2 [5]. The most frequent toxigenic fungi in Europe are *Aspergillus*, *Penicillium* and *Fusarium* species. They produce aflatoxin B1 transformed into aflatoxin M1 found in the milk as well as Ochratoxins and Zearalenone, Fumonisin B1, T-2 toxin, HT-2 toxin and deoxynivalenol (vomitoxin), which are of increasing concern in human health. These mycotoxins are under continuous survey in Europe. They are found in foodstuffs and are not destroyed by normal industrial processing or cooking since they are heat-stable. The toxicity of liver and kidney, immune toxicity, reproduction toxicity, fetal toxicity, teratogenicity and carcinogenicity are generally reported in experimental models [6]. Aflatoxins can cause acute toxicity at high doses. Clinical findings of acute aflatoxicosis are observed in most animals. They are namely loss of appetite, weight loss, neurological abnormalities, hepatitis in mucous membranes, contraction, and eventually death. The coloration of the liver or completely decoloration and fat accumulation are significant. In the body cavities, fluid accumulation in the kidney and intestines may also occur [7]. According to the FDA limits, aflatoxin should be below 20 ppb in the feed of animals in the lactation period and 5 ppb in the milk. The main rule is that the aflatoxin concentration in the milk is around 1.7% (0.8 - 2.0) of the afalatoxin concentration in the dry matter of the total ration. More than 5 ppb aflatoxin residues were found in cows consuming 30 ppb aflatoxin. Aflatoxin occurs quickly in milk

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every 3-4 days [8], [9]. Acute aflatoxicosis is characterized by symptoms such as anorexia, lethargy, ataxia, rough hair cover, and fatty liver. Chronic aflatoxicosis is also associated with decreased feed consumption, reduced milk yield, and signs of hepatitis. Aflatoxicosis prevents acquired immunity and reduces resistance to diseases. When fattening cattle were fed with feeds containing 700 ppb of aflatoxin, an increase in CA increase was observed, but an increase in KC weight at 100 ppb could be considered as a criterion for toxicity. The presence of aflatoxin above 100 ppb in rations may affect production and health in dairy farms. It was reported that milk production increased by more than 25% when milk production in lactation consumed 120 ppb of aflatoxin in pasture conditions [10]. Applebaum et al. [11] showed that milk production decreased when the aflatoxin consumed by the cow was reduced, but it did not have a significant effect on milk production if an equal amount of pure aflatoxin was consumed.

Mycotoxins in feed can be analyzed by high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) or biochemical (immunoassays) techniques [12]. Aflatoxins occurring naturally in foods and feeds may be reduced by a variety of procedures. Improved farm management practices, more rapid drying and controlled storage are now defined within GAP (Good Agricultural Practice) or HACCP [13]. By segregation of contaminated lots after aflatoxin analyses and by sorting out contaminated nuts or grains by electronic sorters, contaminated lots of peanuts or maize can be cleaned up to produce food-grade products. Decontamination by ammoniation or other chemical procedures can be used for rendering highly contaminated commodities suitable as animal feeds [14]. The Food Safety Modernization Act (FSMA) has been published in September 2015 by the US Food and Drug Administration (FDA), which includes Good Manufacturing Practice, Hazard Analysis and Risk-Based Protective Controls for Animal Feed [15]. According to the Veterinary Services, Plant Health, Food and Feed Law No. 5996 [37] which entered into force in 2010 in Turkey, unsafe feeds cannot be put on the market and cannot be used in feeding animals. Therefore, production according to hazard analyzes and critical control point principles is a legal requirement for all feed processors. The highest risk for animal food safety comes from chemical pollutants. The most common of these are mycotoxins which are above acceptable levels in feed and feed materials. Official control plans of feed safety system are applied at different parts of feed chain such as in feed processing enterprises, at farms, in feed storage, at sales areas of prohibited substances, for plant and animal originated feeds, feed additives and premixes [16]. Although the details of the official control plan change according to the years, the mycotoxins in the list of unwanted substances that are screened in feeds in 2015 take priority in all feed materials. According to the Regulation on Feed Hygiene [38], the feed processors are obliged to establish and implement a feed safety system based on the principles of hazard analysis and critical control points, excluding primary production. The feed operator must review the implementation of the system based on the principles of hazard analysis and critical control points in case of changes in the product or production, processing and distribution stages, make necessary changes to the system and record these changes. Feed operators are responsible from the safety of feed production [17]. In the HACCP feed safety management system, identification of critical control points is required to control feed processors. In order to prevent, eliminate or reduce identified hazards, critical control points must be determined in feed production enterprises in Turkey. Defined critical control points in the production process should be used in documentation system of HACCP management. Processed feeds should be kept separate from unprocessed feeds and feed additives to prevent cross contamination. The processed feeds should also be transported and stored without contact with contaminants [18].

The aim of this study was to determine the critical control points to be used in HACCP feed safety management system due to mold grow during the production, transportation and storage conditions of compound feeds.

II. Materials and Methods

A. Feed Material

Beef cattle compound feed (BCF) and dairy cattle compound feed (DCF) were prepared in pellet form from the ingredients of wheat bran, sunflower seed meal, distillers dried grains with solubles, tapioca, wheat, corn, barley, sugar beet molasses, calcium carbonate, soybeans, and sodium chloride in feed processing factory (FPF). During production, vitamins and trace minerals mixture were added into compound feeds. After then, vanilla flavor and *Saccharomyces cerevisiae* (live yeast) were added into both BCF and DCF.

B. Collection of Feed Samples

10 BCF and 10 DCF feed samples were collected from FPF just after production as day 0. A total of 40 feed samples were collected from 10 dairy and 10 beef cattle farms after 20 and 40 days of storage of feeds in farms during July, August and September 2017. The temperature were recorded between 17-30 °C in July, 21-31 °C in August and 15-31 °C in September. Recorded humidity levels in July, August and September were 68, 69 and 72% respectively. Collected feed samples were kept in refrigerator at 2-8 °C until analysis.

C. Chemical Composition Analysis

Collected feed samples were milled for passing through a 1 mm sieve. Dry matter (DM), organic matter (OM), crude ash (CA), crude protein (CP) and ether extract (EE) of all samples were analyzed according to AOAC methods [19]. Crude fiber (CF) content of all samples was determined by the method described by Van Soest et al. [20]. Feed samples were studied in two replications for all feeds analysis.

D. Analysis of Aflatoxine by Direct Quantitative ELISA Method in Feed Samples

Total aflatoxin levels in all collected feed samples were determined using the Veratox Aflatoxin Quantitative Assay (USDA-GIBSA 2008-011), which was based on the direct competitive ELISA method [21]. In all aflatoxin analyzes, feed

samples were studied with two replications.

III. Results and Discussion

A. Determination of Chemical Composition of Feeds

Chemical composition of BCF and DCF collected from FPF is shown in Table I. The mean CP% ,CF%, CA%, OM% and% DM values of BCF in pellet form were 14.45 ± 1.11; 9.45 ± 1.29; 4.45 ± 1.56; 9.05 ± 1.03; 92,03 ± 3,05 and 88,03 ± 1,05 respectively. The mean CP%, CF%, CA%, OM% and %DM values of DCF in pellet form were 21.13 ± 1.41, 13.00 ± 1.77, 5.28 ± 1.54, 9.54 ± 1.92; 91,46 ± 2,92 and 89,83 ± 0,95 respectively. The average moisture content of BCF in the 10 beef cattle farms was determined as 12% and the average moisture content of DCF in the 10 beef cattle farms was 11%. Molds may grow in feeds containing more than 12-15% moisture. In feeds containing high moisture such as silage, if oxygen is available in medium, mold growth occurs in feeds [22]. In our study the moisture content determined in the storage conditions of the farms where the feeds were collected was found to be lower than the value reported for mold growth.

TABLE I
Chemical Composition of BCF and DCF

Nutrients (%) n=20	BCF X±SE	DCF X±SE
CP	14,45±1,11	21,13±1,41
CF	9,45±1,29	13,00±1,77
EE	4,45±1,56	5,28±1,54
CA	9,05±1,03	9,54±1,92
OM	92,03±3,05	91,46±2,92
DM	88,03±1,05	89,83±0,95

n: number of samples

B. Analysis of Aflatoxin by Direct Quantitative ELISA Method in Feed Samples

Total aflatoxin levels in feed samples taken from 0, 20 and 40 days after storage period in beef cattle farms are shown in Table II. The critical control points to be taken into consideration in HACCP feed safety management system were identified based on the measurements of total aflatoxin levels in collected BCF and DCF feeds in the period from feed production to consumption. In July, August and September when the feed samples were collected, determined air temperatures were between 17-30 °C, 21-31 °C and 15-31 °C respectively. The recorded average humidity rates were 68%, 69% and 72% in July, August and September, respectively

After the storage period of 0 and 20 days in two beef cattle farms, aflatoxine levels were determined as 0.01 ppb at BC1 and 0.81 ppb at BC2 as shown in Table II. Aflatoxin levels of post-production of BCF varied between 1.08 and 3.01 ppb. In 10 beef cattle farms, aflatoxin levels in the BCFs taken on the day 20 of storage were determined between 0.95-4.00 ppb. In the same farms aflatoxin levels were found between 0.81-4.96 ppb on day 40. With an increase in storage period, aflatoxin levels increased to 4.96 ppb in only BC8 farm. However this value is below the maximum acceptable level for beef cattle feeds [18].

Scott [23] reported that aflatoxin B1 was among 10-2000 ppb in 43 out of 126 BCF samples in France. Demirer et al. [24] stated that they found 30 ppb AFB1 in only one of the samples among 106 compound feeds' and feed ingredients' (n = 92) aflatoxin analysis. Kulmanov [25] reported that they found AFB1 in 4-5% of wheat in 160 stored grain products of corn, wheat, barley and rice in Alma-Ata and Dzhambul. Dutton and Westlake [26] reported that the incidence of AFB1 was 27% in the samples of 400 grains, compound feed, straw and corn silage. Altıntas et al. [27] reported that AFB1 residues were found at different levels in all 69 ruminant feed collected from Ankara, Konya and Bolu provinces by using ELISA method.

In addition, it was determined that the levels of AFB1 in the feeds that were analyzed were above the officially allowed levels. Oguz et al. [28] reported that only 4 (2,66%) of 150 compound feed samples collected in different seasons between May 2005 and August 2006 from Konya, Karaman and Mersin provincial centers and districts were found to have AFB1 contaminations and the results were below the permissible level. Dogan [29] reported that AFB1 levels were higher than 10 ppb in all 100 feed samples which were collected from Kars province and districts. They reported that they were below the values that would affect the health and productivity of the animals at the determined levels. Dokuzlu [30] reported that 7 of 50 collected cattle compound feed samples were contaminated with AFB1. AFB1 levels of collected feed samples were determined by thin layer chromatography method [30].

Total aflatoxin levels in feed samples taken from 0, 20 and 40 days after storage period in dairy cattle farms are shown in Table III.

TABLE II
Total Aflatoxin Levels in Feed Samples Taken From 0, 20 and 40 Days after Storage Period in Beef Cattle Farms

Beef Cattle (BC) Farm No	Day 0. (ppb*)	Day 20. (ppb)	Day 40. (ppb)
BC1	1.19	2.51	2.02
BC2	<Min	<Min	0.81
BC3	1.66	2.01	2.52
BC4	<Min	<Min	0.01
BC5	1.68	2.10	2.70
BC6	1.08	0.95	1.00
BC7	1.18	1.25	2.26
BC8	3.01	4.00	4.96
BC9	2.10	3.66	4.01
BC10	2.65	3.80	7.53

*ppb: Parts per billion

Aflatoxin levels were found under minimum levels of the samples collected after 0, 20 and 40 days of storage periods in DC4 and DC10 dairy cattle farms (Table III). Aflatoxin levels were also found to be below the minimum official levels in DCF samples taken after production in DC2 and DC8 dairy farms. Aflatoxin levels of DCF samples after production changed between 0.44-2.01 ppb. Aflatoxin levels were determined between 0.89-3.01 ppb in the DCF samples taken on the 20th day of storage in 10 dairy cattle farms. On the 40th day storage, aflatoxin levels in the same dairy farms were found to be between 1.12-7.83 ppb.

TABLE III
TOTAL AFLATOXIN LEVELS IN FEED SAMPLES TAKEN FROM 0, 20 AND 40 DAYS AFTER STORAGE PERIOD IN DAIRY CATTLE FARMS

Dairy Cattle (DC) Farm No	Day 0 ppb*	Day 20 ppb	Day 40 ppb
DC1	0.92	1.47	2.62
DC2	1.24	1.80	3.01
DC3	<Min	2.62	3.02
DC4	<Min	<Min	<Min
DC5	0.44	0.76	1.12
DC6	2.01	3.01	7.83
DC7	1.80	1.90	2.01
DC8	<Min	0.89	1.79
DC9	1.16	1.21	6.31
DC10	<Min	<Min	<Min

*ppb: Parts per billion

After 40 days of storage, aflatoxin levels increased to 7.83 and 6.31 ppb in farms DC6 and DC9. These values are above the maximum acceptable level in dairy cattle feeds [18]. Sabatini et al. [31] conducted an AFB1 analysis of 45 compound feeds collected from 15 dairy cattle farms from May to October in 2015 and found that all of the feeds were contaminated with AFB1. They reported that the AFB1 concentration ranged between 0.03-7.76 ppb. Bilal et al. [32] reported that 6 of 9 DCF samples were contaminated with AFB1.Determined AFB1 concentrations were changed between 0,3-31 ppb. Martinez and Blasco [33] reported that AFB1 was found in 90% of the 78 collected DCF samples from various dairy cattle breeding companies in 5 different regions in Spain. Seasonal contamination was reported as 0.086, 0.075, 0.030 and 0.017 ppb in spring, winter, summer and autumn seasons respectively. Udom et al. [34] reported that obtained AFB1 levels were over 5 ppb in 92% of analyzed DCF samples in a study conducted in Nigeria. Shreeve and Patterson [35] found 40 ppb aflatoxin B1 in compound feeds for daily cattle produced in the FPF.

As a result, 68-72% moisture density of the BCF in pellet form and a temperature between 15-20 °C were not found to be critical for storage condition for 40 days in the warehouses with good thermal and moisture insulation. On the contrary, in dairy cattle farms, aflatoxin was detected on the maximum acceptable level (5 ppb) of DCF for 40 days of storage. In terms of feed safety, 40 days of storage can be considered as critical control point, although pelleted dairy cattle compound feeds are kept under appropriate storage conditions in HACCP management.

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