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Research Article

Aflatoxin contamination in dairy feed during wet and dry seasons in selected rural areas of Sidama zone in Southern Ethiopia

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Abstract

This study was conducted in the southern region of Ethiopia. The objectives were to assess the occurrence and level of aflatoxin B₁ contamination in dairy cow feeds. The analytical technique used was Enzyme-linked immunosorbent assay (ELISA). Two-way ANOVA was used to analyze the season and location interaction effect. Laboratory analysis showed that 81.2% of the feed samples had a detectable level of AFB₁ within the range of 0.067-29.69 µg/kg and with an average of 7.91±5.83 µg/kg. From the positive samples, 52.5 and 4.58% of the feed sample were above EU (5 µg/kg) and USA (20 µg/kg) limits for dairy cattle feed respectively. Average AFB₁ concentration during the dry season (8.74± 5.6 µg/kg) is significantly higher (P<0.05) than wet season (7.02± 5.86 µg/kg). This result implies that sustainable good practices should be maintained for all feed harvesting, storage, and feeding practices by feed producers and dairy farmers regarding aflatoxin contamination.

Introduction

Mycotoxins are fungal secondary metabolites when ingested causing a variety of adverse effects in both humans and animals [1]. Aflatoxins are a highly toxic group of mycotoxins produced by different species of fungi such as *Aspergillus fumigatus*, *A. flavus*, *A. parasiticus*, *A. niger*, *A. tamari*, and *A. nomius* [2]. These fungal metabolites are primarily found in various plants and their grains, such as nuts and maize. *Aspergillus flavus* and *A. parasiticus* especially represent 93% of strains that produce aflatoxins worldwide [3,4].

Aflatoxin was first discovered in 1960 after contamination of turkeys in Britain when they were identified as toxic compounds of the fungus *Aspergillus flavus*. During that time, 100,000 turkeys died of the so-called "Turkey-X disease" [5]. There are more than 20 known aflatoxins that have been identified. The four main ones are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), and aflatoxin G₂ (AFG₂) [6]. Letter

"B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin-layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively [3]. Aflatoxin B₁ is the most toxic, carcinogenic, teratogenic, and a mutagenic class of aflatoxins [7]. And is listed as a group I carcinogen by the International Agency for Research on Cancer [8-10]. In situations, where animal feeds are derived from AFB₁-contaminated plant materials or their byproducts from the farm or processing industries, once ingested the toxin is converted into a secondary hydroxylated metabolite named aflatoxin M₁ (AFM₁), which is subsequently secreted in milk in the mammary glands of both humans and lactating animals [11,12]. The ratio between ingested and excreted AFM₁ seen in ruminants is usually 1-3%, but can reach 6% in worst case scenarios and high yielders cows [13].

Aflatoxin contamination of key staples compromises the production of the agriculture sector and worsens the food

safety situation in low-income countries [14]. According to the estimate of the Food and Agriculture Organization of the United Nations (FAO), mycotoxicosis, a disease caused by a natural toxin produced by *A. flavus* and *A. parasiticus* affects 25% of world food crops and every year 1 billion metric tons of food products are wasted due to same [15]. In many developing countries Aflatoxin also lowers agricultural product quality and compromises export earnings [16].

Aflatoxins are considered to affect a wide range of food commodities. Cereals, oilseeds, spices, nuts, and dried fruits are particularly vulnerable [17]. Mold growth and toxin production especially occur in prolonged crop storage under high temperature and humid conditions [18].

In Ethiopia, limited evidence is available regarding the extent of aflatoxin contamination in dairy feed used in the rural areas, since the majority of milk is produced at the smallholder level in the rural areas. The available studies in the urban and per urban areas reported the presence of a high concentration of aflatoxin B₁ in dairy feed. A recent study in the greater Addis Ababa milk shed found high levels of AFM₁ in raw cow's milk and AFB₁ in the dairy feed from EU and USA limits [19]. This has scared consumers and has affected the market of both milk producers and processors. Smallholder dairy farmers, whose daily lives depend on the sale of milk and other dairy products, are hard hit by the fall in demand. Dairy farmers harvest feed traditionally and stored it in exposed environments commonly. Which is exposed to cold and hot temperatures sometimes rain, which causes mold growth in feed. The moisture content of the feed, susceptibility of feed, and environmental humidity primarily determine mold growth rate in feed. Various methods to determine AFB₁ have been developed such as high-performance liquid chromatography (HPLC), thin-layer chromatography, gas chromatography, and immunochemical method such as enzyme-linked immunosorbent assay (ELISA). A variety of immunological detection and quantification methods such as ELISA are precise, rapid, and require usually no further sample purification [20]. Doing this research is important for assisting as baseline information for government and stakeholders for policy development. Therefore, the purpose of this study was to determine the level of aflatoxin B₁ contamination in dairy feed at the smallholder farmer's level in the rural areas of Ethiopia.

Materials and methods

Study areas

This study was conducted in the Dale and Hula districts of Sidama zone, Southern Nation Nationalities, and People Regional States (SNNPRS) Ethiopia. These districts are purposely selected to represent a rural area of Sidama zones during the two seasons which are wet (July 2017) and dry (March 2018). Dale districts have a moist to humid, warm subtropical climate with a mean annual precipitation of 1000 to 1800 mm, and a mean annual temperature of 15 to 20°C. On the other hand Hula district has a wet, cool temperate climate and receives an annual rainfall of 1200 to 1800 mm, and has a mean annual temperature of 10 to 15°C [21].

Sample size and sampling approach

The minimum sample size was determined according to [22].

$$n = \frac{z^2 P (1 - P)}{\delta^2}$$

Where n = minimum sample size

z = Standard normal deviate that corresponds to 95% confidence interval (1.96)

P = Estimated prevalence

δ = is the level of significance (5%)

Since there is little information on the occurrence of AFB₁ in dairy feed in the rural area of Ethiopia. A prevalence rate of 80% was used to calculate the sample size. Assuming this prevalence at a 95% confidence interval the minimum estimated sample size was given as 245.8 approximately 240 samples were taken using the formula given below.

$$\text{Therefore; } n = \frac{(1.96)^2 \times 0.8(1-0.8)}{(0.05)^2} = 245.8$$

Initially, the total sample size (n=120) of one season was equally distributed to the two districts (60 per district). Then from each of the districts, two peasant associations having the rural areas characteristic were chosen randomly. From each peasant association 30 subjects that fulfill the inclusion criteria (households owning at least one lactating cow) were selected randomly. Prior to the data collection households that meet the inclusion criteria were listed through rapid enumeration and the list was ultimately used as a sampling frame. A similar sampling procedure was followed during the dry season.

Feed sample collection and preparation

During feed sample collection, 200 g of representative composite feed samples (containing fresh fodder, hay, grain by-products, and crop residue especially maize stover) were collected from each feed ingredient of the ration according to the proportion of feed the farmers use during sampling time from each dairy farms during the dry and wet season. The samples collected were kept in polyethylene bags and transported to the nutrition laboratory of the school of animal and range sciences. The samples were then dried in a dry air-forced oven at 65°C for 48 hr according to AOAC [23]. And were ground to pass a 1 mm mesh size. The ground samples were stored in a sample container at room temperature for further analysis. Then 80% acetonitrile extraction solvent was prepared by adding 40mL of distilled water to 160 mL of acetonitrile for each sample to be tasted. Two hundred mL of 80% acetonitrile solution was transferred to a container and 2 g of the ground sample was added. Then it was mixed by shaking for 10 minutes and centrifuged at 3500rpm for 5 minutes. The supernatant was collected and diluted in 1:10 reconstituted wash buffer. The samples were ready to be tested according to the assay procedure. The final dilution factor was 1:1000.

Analysis of AFB₁ in animal feed

The assay was performed based on the protocol provided by the manufacturer (Helica Biosystems Inc. 1527 W. Alton Ave Santa Ana, CA 92704, USA) using the commercial Enzyme-Linked Immunosorbent Assay (ELISA)-based test kits and reagents. The detection limit was 0.2 ng/ml (parts per billion [ppb]). We use an ST-360 microplate ELISA reader (model Shanghai Kehua). The concentration of AFB₁ was measured according to the procedures (#941BAFL01B1-96) provided by the manufacturer (Helica Biosystems, Inc., 2020b) [24].

Data analysis

The data were analyzed using SPSS 20 software. Aflatoxin contaminations in different samples were described using the appropriate measure of central tendency and dispersion. An independent t-test was conducted to determine differences in AFB₁ concentrations in dairy cow feed samples between wet and dry seasons. A P-value less than 0.05 was considered statistically significant. Concentrations of AFB₁ were expressed as Mean ± SD, minimum and maximum value. Two-way ANOVA was performed for the season-location interaction effect analysis of AFB₁.

Results and discussion

Aflatoxin B₁ in dairy feed in the study area

Under the current study from overall samples collected in dry and wet seasons, 17.08% were below the detection capacity of the ELISA detection method. The positive samples had Aflatoxin B₁ that ranged from 0.067 – 29.69 with an average of 7.91 ± 5.78 µg/kg. From the positive samples, 52.5% had above the EU/FAO/WHO limit of 5µg/kg while 4.58% were above the USA limit of 20 µg/kg for dairy feed. This study showed a lower result than the study by [25]. In the rural area of Kenya which reported a range between 2.31 – 84.41 µg/kg range with an average of 25.94 ± 28.71 µg/kg. Similarly, the result of the current study was much lower than the previous report in Ethiopia, where the contamination level was in the range of 7 – 419 µg/kg [19]. Yohannes et al. [26]. A study from the Gurage zone also reported concentrations ranging from 1.88 – 31.2 µg/kg with an average of 11.42 µg/kg of aflatoxin B₁ in animal feed. The current study, however, has found a higher concentration than the report by [27]. Who reported 0 – 20 µg/kg with an average of 5.63 µg/kg in the central highland of Ethiopia. The difficulty of comparing results between and within the countries is due to different investigative procedures used, farmers/households practice, the number of samples, animals, different detection methods, type of feed, different on-farm feeding practices, climatic situations, and animal feed handling and storage conditions, the sampling time and procedures.

Seasonal pattern of aflatoxin B₁ in the study area: Aflatoxin B₁ in animal feed is season dependent due to available feed type, feed harvesting, and storage practice. The result of the current study showed that aflatoxin B₁ during the dry season (March) was significantly higher (P< 0.05) than wet season (July). Both the lowest and the highest concentrations were recorded during the dry season (Table 1). This might be due

to the availability of feeds from fresh enset (leaf, stem, and root) up to dry hay and crop residue, especially at the Hulla site. During the dry season, there is no availability of rainfall and there are no fresh green fodders expected except for enset which is drought tolerant. The main source of feed during this season is dry and stored feed. A higher percentage of samples (59.1%) from the dry season had aflatoxin levels higher than the EU limit (5 µg/kg) while only 45.8% of samples from the wet season were higher (Table 1). A similar finding was reported by [28]. In Greece higher concentration than the EU limit was reported for the dry season (April – October) than in the wet season (November – February).

The result of this study is in contrast with studies by [29]. And [30]. In Pakistan where they reported higher concentrations of AFB₁ in dairy feeds during the wet season (December to March) than in dry season (April – August). The reason for the low AFB₁ concentration under the current study during the wet season, and the high availability of rainfall, could be due to animals' feed which is mainly pasture grasses, fresh green fodder, and weeds in this season. A report by [25]. Showed that the susceptibility of roughage feeds for AFB₁ (5.14 g/kg) is almost 10 times lower than concentrate feeds (47.84 µg/kg). A similar situation study by [31]. In the rural areas of Pakistan reported that chances of aflatoxin contamination of fresh fodder for dairy animals were low. High concentration during the dry season might be due to the feed type which mainly consists of stored crop residue, dried maize stover, and hay, especially at the Hulla site. The variation of aflatoxin B₁ from country to country or from place to place might be also related to feeding type, storage, feed moisture, environmental humidity, and detection method difference.

AFB₁ concentration in feed at a different location: Aflatoxin B₁ concentration detected at the Hulla site was significantly higher (P<0.05) than at Dale (Table 2). The lowest (0.067 µg/kg) aflatoxin B₁ concentration was recorded from the Dale site while the highest concentrations (29.69 µg/kg) were recorded from the Hulla site. A higher percentage (67.5 %) of samples collected from the Hulla site had a concentration above the EU limit (5µg/kg) while only 46.6% of the samples from the Dale site were above the EU limit (5µg/kg) (Table 2). This might

Table 1: Seasonal pattern of aflatoxin B₁ in the study area.

Season	N	Positive N (µg/kg)	Mean ± SD (µg/kg)	Range (µg/kg)	Above EU limit(5 µg/kg)	P
Wet	120	40.4%	7.02±5.86	0.124-26.87	45.8%	P = 0.036
Dry	120	42.5%	8.74 ±5.6	0.067-29.69	59.1%	
Total	240	82.9%	7.91±5.78	0.067-29.69	52.5%	

N= total sample SD= standard deviation

Table 2: AFB₁ concentration in feed between two districts, Dale and Hulla.

Districts	N	Positive (N)	Mean ± SD	Range	above EU limit (5µg/kg)	P
Dale	120	40.83%	6.72 ±5.56	0.067-26.87	46.6%	P = 0.004
Hulla	120	42.08%	9.07±5.78	0.24-29.69	67.5%	
Total	240	82.9%	7.91±5.78	0.067-29.69	52.5%	

N= total sample SD= standard deviation



be due to the availability of crop residue and hay in the Hula site as a resource of feed and feeding practice. The common crop cultivated in the Hula site is Maize [21]. Which is highly susceptible to the mycotoxin. They use this crop and its residues mainly in addition to storing the available feed in the exposed area for rain and sunlight for about a month where the storage favors the growth of mold. In addition to this majority of the respondents feed their cows around the backyard and they offer feed without removing the previous leftover this encourages mold growth and formation of mycotoxin due to mixing of high moisture leftover with dry feeds. In Dale districts, they use fresh fodder like enset (*Ensete ventricosum*) which can't store and is exposed for mold formation.

Interaction effects of location and season on aflatoxin content of dairy feed in the study area: The two-way ANOVA for the interaction effect of season and location on AFB₁ in dairy cattle feed (Table 3) indicated that there is no significant interaction effect on AFB₁ concentration in the study area. Season ($P= 0.028$) and location ($P= 0.003$) however, had a significant effect on the concentration of aflatoxin B₁ in the study area. This might be due to feeding type availability difference between locations with more crop residue and other dried feed being available at the Hulla site.

The feed resources between wet and dry seasons from the Dale site were almost similar but different at the Hula site which leads to the result in the season being less significant for AFB₁ production when compared with the location.

Conclusion

The laboratory analysis showed that 82.9% of animal feed samples had a detectable level of Aflatoxin where 52.5% and 4.58% were above EU and USA limits respectively which are not safe for dairy cow's feed according to EU and USA limits. Feed samples collected during the dry season were significantly higher in aflatoxin B₁ concentration than in the wet season. According to these Regional agricultural offices, livestock experts and other concerning personals should focus on awareness creation about feed harvesting, storing, and feeding practices regarding aflatoxins and health effects. In addition, sustainable good practices should be maintained for all feeds

Table 3: Interaction effects of location and season on aflatoxin content of dairy feed and milk.

Location	Aflatoxin B ₁ (µg/kg) feed
	Mean ±SD
Dale	6.72 ± 5.56
Hula	9.05 ±5.78
P value	0.003
Season (S)	
Wet	7.03 ±5.86
Dry	8.74 ±5.6
P value	0.029
Interaction	
Location*Season	0.288 ^{NS}

SD= Standard Deviation µg/kg= Microgram per kilogram

harvesting, storage, and feeding practices by feed producers and dairy farmers regarding aflatoxin contamination.

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