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DETERMINATION OF AFLATOXIN IN DAIRY FEEDS AND MILK IN SOME SELECTED AREAS OF ETHIOPIA

Rehrahie MESFIN[1,3], Getnet ASSEFA[2] and Fassil ASSEFA[3]
[1]Holetta Agricultural Research Center, P.O. Box 2003, Addis Ababa, Ethiopia, phone: +251-112-370300; e-mail: rehrahiemesfin@gmail.com
[2]Addis Ababa University (AAU), Science faculty, Department of Microbial, Cellular and Molecular Biology, P.O.Box 1176, Addis Ababa, Ethiopia, phone: +251-111-239472 e-mail: asefafasil2013@gmail.com
[3]Ethiopian Institute of Agricultural Research (EIAR), Livestock Research Directorate Office, P. O. Box 2003, Addis Ababa, Ethiopia, phone +251-116-454432; e-mail: getnetassefa@yahoo.com
*Corresponding author
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Abstract*: Aflatoxin B1 (AFB1) in feeds and aflatoxin M1 (AFM1) in milk were studied in Holetta, Bishoftu and Hawassa, Ethiopia using Enzyme-linked Immunosorbent Assay (ELISA). Concentrate feeds were more contaminated (7.67 ± 0.80 µg/kg) than roughage feeds (0.41 ± 0.14 µg/kg); hay was more contaminated (0.72 ± 0.25 µg/kg) than straw (0.05 ± 0.05 µg/kg) and oilseed cake based concentrate feeds were more contaminated (13.09 ± 1.12 µg/kg) than concentrate feeds without oilseed cake (2.78 ± 0.66 µg/kg). Fifty percent of the feed samples contained 0 µg/kg aflatoxin and 69% and 31% of them fulfilled the permissible limit of the EU (5µg/kg) and the USA (20 µg/kg) for feeds respectively. The average AFB1 of feeds in Bishoftu, Holetta and Hawassa were 9.76µg/kg, 6.33µg/kg and 1.19µg/kg, respectively. The AFB1 of feeds in dairy producers, feed manufacturers and feed retailers were 9.35 ± 1.04µg/kg, 7.50 ± 1.43 µg/kg and 6.91 ± 1.09µg/kg respectively. Twenty nine percent of the milk samples had aflatoxin content of 0 µg/L and; 58% and 42% of them fullfilled the EU (0.05µg/L) and the U.S.A.(0.5 µg/L) permissible limits respectively. Further studies are required by using other techniques such as HPLC, GC and multi-mycotoxin assay using LC-MS-MS. The contribution of too acidic and too alkaline PH on aflaoxin reduction should also be studied. Awareness creation is required to feed processers, feed traders and dairy producers along the feed-milk production and marketing chain. To minimize the incidence of aflatoxin, feed processers, feed traders and dairy producers should employ better feed/grain storage practices.*

Key words: *Safety; Concentrate; Hay; Straw; Holetta; Bishoftu; Hawassa*

1. Introduction

Agriculture in Ethiopia is an important component of rural livelihoods which creates job opportunity for > 85% of the population. It is a major source of food, income, raw materials for domestic industries and foreign exchange [1]. Ethiopia has diverse agro-ecologies suitable for production of different livestock species and contributes to the national economy in terms of food security, employment, draught power and foreign currency [2]. Livestock resources in the country are also the sole source of livelihoods for about 10 million pastoral communities [3]. Ethiopia has good potential for dairy production. With the growing trend of urbanization, tourism industry and rising of incomes, there is an increasing demand for production of safe and better quality milk [4]. However, the quality and safety of milk is influenced by different feed contaminants. Mycotoxins are among the many contaminants that affect the quality of feeds. Aflatoxins are

among the many members of mycotoxin that contaminate food and feedstuffs. Cereals and oilseed grains and their by products are regularly contaminated with fungi occurring as plant pathogens in the field or developing during storage, transportation and during feeding [5]. The aflatoxin group found in feeds consists of B_1, B_2, G_1 and G_2 and Aflatoxin B_1 is the most common, carcinogenic and potent [6]. Aflatoxin M_1 is hydroxylated aflatoxin B1 released in milk when dairy cows consume aflatoxin contaminated feed. Livestock species exposed to aflatoxin contamination experiences higher susceptibility to infectious diseases and reduce their yield of meat, milk and egg [7]. In human beings, aflatoxin is carcinogenic and poses to liver cancer. Epidemiological studies carried out in China, Kenya, Mozambique, Philippines, Swaziland, Thailand and South Africa showed, around 100.000 new cases of hepatocellular carcinoma attributable to aflatoxin exposure [8]. In children it poses to stunted growth and susceptibility to infectious diseases [7]. Children living in high aflatoxin exposure zones in Malawi were found to lose 22% less height than children in low-exposure zones [8]. Aflatoxin is potent and the largest outbreak reported in the world was in 2004 when 317 cases were attributed to 125 deaths due to consumption of aflatoxin contaminated maize in Kenya [9]. In Indonesia, Philippines and Thailand, 5% of the aflatoxin contaminated maize and peanuts were discarded [7]; in 1980s' in Malawi, the share of groundnut exports had collapsed from 64% to 0.2% [8] (Rios *et al*., 2013). Past research efforts in Ethiopia had paid little attention to feed quality particularly to aflatoxins. Since feed is an integral part of the food chain, it needs assesemnt for its quality. The current study therefore was undertaken to detect and quantify the contamination level of aflatoxin in dairy feeds and milk following the production, processing, marketing and utilization of feeds.

2. Materials and methods

Description of study locations

The study was undertaken in the central highlands of Ethiopia particularly in Western Shoa (Holetta), Eastern Shoa (Bishoftu) and Southern Shoa (Hawassa). The study sites were located on the important feed and milk production chain of the country and represent the different temperature and humidity conditions of the central highlands. Holetta is locateed in Western Shoa, representing the cooller (Dega) climatic regions of the country and is situated at 38° 30`E, 9° 3`N and 45 km west of Addis Ababa and lies at an elevation of 2400 m.a.s.l. The annual rainfall is 1066 mm and the average annual minimum and maximum temperatures were 6° and 22°C respectively [10]. Holetta could represent sub humid areas of the country. Within Holetta, there were different sampling sites including Welmera, Ejere (Addis Alem), Dendi (Ginchi), Ambo and Guder. Bishoftu (Debre-zeit) is considered to have relatively warmer (Weynadega) climatic condition and dry in its humidity level. It is located at 8^O50 to 8^O53 latitude and 38^O55 to 38^O59 longitude. It is located 45 km East of Addis Ababa, at an altitude of 1600-2400 m a.s.l. [11]. The mean minimum and maximum temperature are 10.9 and 27.28 °C, respectively. The mean annual rainfall and relative humidity are 56.81mm and 65.92% respectively [12]. Hawassa is located in the Southern Nations, Nationalities and Peoples (SNNP)

and Sidama zone of Southern Ethiopia, 285 km far from Addis Ababa in the Great Rift Valley. It is situated at an altitude of 1708 m, latitude 7°3′N and longitude of 38°28′E. The average annual rainfall was 1100 mm and average low and high temperature of 12.6 Cº and 27.3 Cº respectively [13]. Tabour sub-city and Monopol areas in Hawassa town were considered as sampling sites. Within each study location, dairy feed chain actors representing feed processors, feed retailers and dairy producers were delineated and the study locations are demonstrated in the following maps.

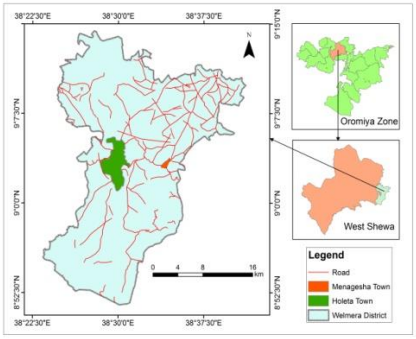

Fig 1. Map of Holetta in Western Shoa

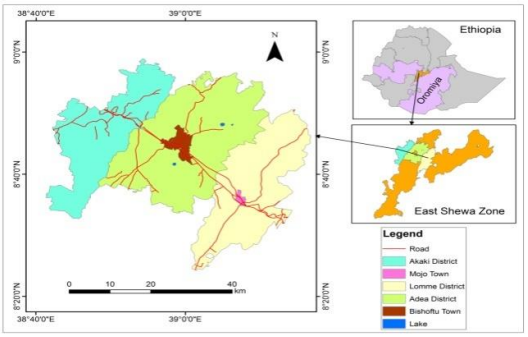

Fig 2. Map of Bishoftu Eastern Shoa

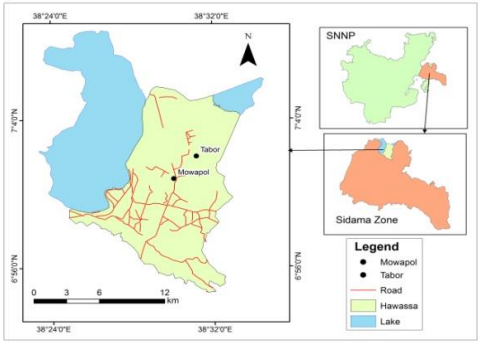

Fig 3. Map of Hawassa

Description and collection of feeds samples

Feed and milk samples were collected based on purposive sampling and a total of 205 samples consisting of 160 feeds (115 concentrate feed and 45 hay) and 45 milk were collected from all study locations. The collected samples from the three study locations include: 73 samples containing 15 hay, 43 concentrate feed and 15 milk (Holetta), 63 samples containing 15 wheat straw, 33 concentrate feed and 15 milk (Bishoftu), 69 samples consisting of 15 hay, 39 concentrate and 15 milk (Hawassa). Hundred ml of fresh milk samples were collected in ice box from each household from the morning milking and stored in a refrigerator. Season of sampling for feeds was in the dry and warmer season of April to June and milk samples were collected from September to November 2014.

The collected samples were dairy feeds commonly utilized for dairy cattle which included roughage (basal feed) and concentrate (supplemental feed). The concentrate feeds included by-products from agro-industry of flour milling by-

products such as wheat bran and wheat middling's, by-products from oil-extracting factories including noug seed cake, cotton seed cake and linseed cake. Majority of the concentrate feed samples were mixture of wheat bran, oilseed meals and byproducts of cereals, pulses which were categorized in to three: oilseed cake based concentrate feed, concentrate feeds without oilseed cakes and pure wheat bran. The oilseed cake based concentrates included wheat bran, wheat middlings and either or mixture of noug seed cake, linseed cake or cotton seed cake. The concentrate mixture feeds without oilseed cakes included wheat bran, wheat middlings and other products of pulses and cereal by products. The roughage feeds samples were categorized in to hay, wheat straw and straw of haricot bean. Majority of market oriented dairy producers in Ethiopia mainly utilize hay as roughage feed and different concentrate feeds as supplemental feeds.

Description of target people

The study has examined aflatoxin situation of dairy feeds and milk along the production, processing, marketing and consumption chain. The target people considered for this study included dairy feed processors, feed retailers and dairy producers. Feed processors are individuals that owe flour milling and oil extracting factories and engaged in production of concentrate feeds. They produce, sell and distribute flour milling by products and oilseed meals as concentrate feeds for dairy cattle feeding. The second target people were feed retailers that buy concentrate feed from feed processors and sell to dairy producers. The third group of people was market oriented urban smallholder milk producers having crossbred dairy cows and buy concentrate feeds from feed retailers to supplement dairy cows and sell their milk to consumers.

Sample preparation and laboratory investigations

Sample preparations including drying, chopping, milling and packing and labeling processes were carried out at Holetta Agricultural Research Center and laboratory investigation for aflatoxin was carried out in Kenya, Naivashsa Dairy Research Coordination Center.

Extraction process of feed samples

Extraction process of feed samples was carried out according of the protocol provided by (Helica Biosystem Inc.). Twenty g of each feed sample was transferred into 250 ml Erlenmeyer flasks containing 100 ml of 70% methanol and allowed to digest for 30 minutes. The particulate matter was filtered with whatman ♯1 filter paper No 541 or 542 and the filtrate was transferred into 100 µl centrifuge tubes for determination of aflatoxin.

Assay process of feed samples

Assay process was performed based on the protocol provided by the manufacturer [14] using the commercial Enzyme-Linked Immunosorbent Assay (ELISA) - based test kits and micro plate ELISA reader of model Biotech USA. Hundred µl of the sample solutions or standards were added in to each dilution wells containing 200 µl of assay diluient and the mixture was mixed using the multi-channel priming micro pipette. Then, hundred µl of the mixture was transferred in to each of the antibody coated micro wells and incubated for 15 minutes at room temperature. After adding 200 µl of horseradishperoxidase (HRP) as a conjugate, incubation

continued further for 30 minutes. Then the liquid was poured out of the wells and wells were washed 5 times using distilled water and the bound substances was stacked to the antibody coated micro wells. Wells were tapped face down on a layer of paper towel to remove the residual wash solution. Next, hundred µl of tetramethylbenzidine (TMB) as enzyme substrate solution was added in to each well and incubated for 5 minutes at room temperature in the dark. Then 100 µl of acidic stop solution was added which changed the chromagen color from blue to yellow. The optical density (OD) of each samples in the micro well was read using ELISA reader at 450 nm and the concentration of AFB1 (µg/kg) was directly measured from the standard curve that was constructed with known concentration of AFB1 reagent grade standards.

Assay process of milk samples

The laboratory investigation for AFM1 was carried out at Naivashsa Dairy Research Coordination Center, Nairobi, Kenya. The standards were presented in homogenized skim milk (milk plasma). An aliquot of unprocessed raw fatty milk was placed at refrigeration temperature overnight to allow the fat globules to rise to the surface in a natural "creaming" effect. The upper fatty layer was removed by aspiration and the lower plasma layer was used for the assay. The aflatoxin standards were: 0.0 pg/ml, 5.0 pg/ml, 10.0 pg/ml, 25.0 pg/ml and 100.0 pg/ml. The reagents were brought from refrigeration temperature to room temperature before starting the assay. Using a new pipette tip for each, 100 µl aliquots of standards and samples were dispensed into the appropriate wells. This was incubated at room temperature for 2 hours. The contents of the wells were discarded into an appropriate receptacle. The micro wells were washed by filling each with PBS-Tween wash buffer and were decanted into an appropriate receptacle. This was repeated for three washings. The wells were tapped face down on a layer of absorbent towel to remove residual wash buffer. Two hundred µl of conjugate was added to each well and incubated at room temperature for 15 minutes. Then hundred µl of enzyme substrate was placed to each micro-well and incubated at room temperature for 5 minutes in the dark. Next, hundred µl of stop solution was added in the same sequence and at the same pace as the substrate was added. The optical density (OD) of the standards and the samples were read on each well with a micro plate reader using a 450nm filter. A standard curve was run for quantification of aflatoxin concentration for each feed sample. The graph plotted was based on the optical density (Y-axis) and the aflatoxin concentration of the standards (X-axis). The concentration of aflatoxin M1 was measured in picogram per milliliter (pg/ml). The manual used for determination of aflatoxin in milk was [15]**.**

Study design and statistical analysis

The design of the study was Completely Randomized Design (CRD) and the statistical model was

$$Yi = \mu + Li + ei$$

where, Yi = level of aflatoxin
μ = the overall mean
Li = effect of location
ei = the error term

The data was subjected to Analysis of variance using the General Linear Model Procedures of Statistical Analysis System [16]. Different statistical analysis including

descriptive statistics and t-test were employed to determine the differences.

3. Results and discussion

Level of aflatoxin in feeds

The detection and level of aflatoxin in this study showed that 81 feed samples (50.6%), did not contain any level of the toxins; whereas 29 of the feed samples (18.1%) and 50 samples (31.3%) contained aflatoxins within the EU permitted level of 5µg/kg, and the U.S.A. permitted level of 20 µg/kg AFB1, respectively (Table 3.2). This result was similar to the AFB1 level of feeds reported in Portugal almost all (98%) of the feed samples fulfilled the permissible limits of the E.U. and the U.S.A [17].

The aflatoxin status and level of feeds in this study was much lower than the previous report in the country where the contaminated level was in a range of 7-419 µg/kg [18]. The results of this study is in contrary with the AFB1 level of feeds reported in India, Kenya and previous authors in Ethiopia in which 17% and 15%, 46% and 45% of the feed samples did not fulfill the U.S.A AFB1 level recommended for feeds (Table 1). The reason for the difference of AFB1 in feeds between Ethiopia and Kenya; as well as Ethiopia and India could be due to the difference in feed type used in Ethiopia (wheat by-products based feeds) and other African countries and India (by-products of maize and groundnut based feeds) which are the most susceptible crops to AFB1 contamination as compared to wheat-based feeds [19].

Table 1.
AFB1 (µg/kg) in feeds in this study & other countries in comparison with EU and USA permissible limits (%)

Country	*N*	*Range (µg/kg)*	*0 level*	*EU standard*	*USA standard*	*> USA Standard*	*Reference*
Ethiopia	160	0-20	50.6	68.7	31.3	0	This study
Portugal	312	5.1-74	0	84	14	2	[18]
India	40	1.8-245	28	13	55	17	[21]
Kenya	144	1-9661	0	27	54	46	[22]
India	356	-	46	NA	39	15	[23]
Ethiopia	156	7-419	0	5	50	45	[19]

N= number of samples, NA=not available

AFB1 mean, range and percent of feed samples belonging to different level of aflatoxin

The AFBI distribution showed variations among the different feed types (Table 2). Accordingly, concentrate feeds contained significantly ($P<0.05$) higher AFB1 (7.67µg/kg) contents than roughage feeds (0.41µg/kg). Among the concentrate feeds, oil seed cake based concentrate feed contained significantly ($P<0.05$) higher AFB1 (13.09µg/kg) than those without oilseed cake and grass hay showed significantly ($P<0.05$) higher AFB1 (0.72 µg/kg) level than the other roughage feed types.

Table 2.

AFB1 mean, range and percent of feed samples belonging to different aflatoxin categories

Type of feed	*N*	*Level of aflatoxin (µg/kg)*			*Range (µg/kg)*	*Mean (µg/kg)*
		0	*0.1 - 5*	*5.1 - 20*		
		Percent				
Roughage feeds	45	21.9	6.3	0.63	0-0.55	0.41 ± 0.14^{f}
Grass Hay	25	33.3	22.2	2.2	0-5.5	0.72 ± 0.25^{e}
Wheat straw	15	31.1	2.2	0	0-1	0.05 ± 0.05^{g}
Harricot bean	5	11.1	0	0	0	0
Concentrate feeds	115	6.3	2.5	22.5	0-20	7.67 ± 0.8^{b}
With oil seed cake	47	8.7	3.5	31.3	0-20	13.09 ± 1.12^{a}
Without oil seed Cake	68	33.9	8.7	14.8	0-20	2.78 ± 0.66^{c}
Wheat bran	44	26.1	6.1	4.4	0-20	2.15^{d}
Mean						5.63

N= number of samples, LS-means with different superscripts among three consecutive feeds in the last columns were significantly different.

The lowest AFBI was recorded from wheat straw (roughage feed) and wheat bran (concentrate feed) with AFBI of 0.05µg/kg and 2.15µg/kg, respectively. Interestingly, no aflatoxin was detected from the haricot roughage feed that may be associated with the limited number of samples tested for AFBI.The pattern of AFB1 contamination in roughage feeds and concentrate feeds was similar to the report of [22]. Who showed that concentrate feeds (47.841 µg/kg), contained almost 10 times more AFBI than the roughage feeds (5.14 g/kg) indicating concentrate feeds are more susceptible to AFB1 contamination than roughage feeds.

This may be due to the fact that concentrate feeds are rich in nutritional composition than roughage feeds which can provide adequate substrate for growth and reproduction of fungi. This is justified by the fact that, compared to grass hay which contains 59 g CP/kg feed, noug and noug based concentrate feeds contained CP contents of 312 g/kg and 251.3g/kg, respectively [23], [24] also reported that food stuffs with high fat content are good substrates for aflatoxin producing fungi and are more susceptible to AFB1contamination as compared to other dairy feeds [25]; [18]. It was reported that the first effect of mold on feeds is utilization of nutrients for their metabolism and propagation which results in decreased nutritional value of feeds. The energy, crude protein and crude fat values of moldy feeds were decreased by 5%, 6% and 63% respectively and dietary fats are affected more extensively than proteins or carbohydrates and they are decreased 37% to 40% after 25 days of storage or 52% to 57% after 50 days of storage [26].

The result showed that higher proportion (22%) of the roughage feed samples contained 0µg/kg of AFB1 with fewer samples (0.63%) with AFB1 level in the range of 5.1-20 µg/kg. On the contrary, higher proportions (22.5%) of the concentrate feed samples contained AFB1 in the range of 5.1-20 µg/kg, and fewer proportions (6.3%) of the concentrate feed samples were free from aflatoxin contamination

Similarly, higher proportions (31.3%) of AFB1 was detected in the range of 5.1-20 µg/kg, from concentrate feeds containing oilseed cake compared to the lower proportions (14.8%) detected from the same concentrate without fortification with

oil seed cake (Table 2). The overall mean and range of AFB1 in feeds in this study was 5.63 µg/kg and 0-20 µg/kg respectively which was lower than the mean and range AFB1 of feeds (25.53 µg/kg and 0.54-204.72 µg/kg) reported in Chennai, India [27].

Level of aflatoxin in different feeds sampled from different study locations

The average AFB1 content of feeds in Bishoftu (9.76µg/kg**)** was significantly ($p<0.05$) higher than the AFB1 content of feeds in Holetta (6.33µg/kg) and this then was significantly higher than that of Hawassa (1.19µg/kg) (table 3) in which case, it had fulfilled the EU standard of 5µg/kg [28].

The AFB1 contamination of feeds in Bishoftu (9.76 µg/kg) was higher than the average AFB1 of Holetta and Hawassa (3.8 µg/kg) which had similar trend with report of [19] in that AFB1 level in Bishoftu (156 µg/kg) was higher than the average AFB1 content of other study locations (Sendafa, Sululta and Addis Ababa) which was 133 µg/kg. The lowest AFB1 content of feeds observed in Hawassa in this study was mainly due to the short storage duration (<1 month) of dairy feeds practiced in that area.

The roughage feeding practice in urban dairying in Hawassa was mainly based on cut and carry system collected from protected areas like universities and prison stations and roughage was not stored not for more than 10 days. In addition, the concentrate feeds belonging to feed manufacturers and feed retailers in Hawassa was mainly (80%) wheat bran with some mixture of linseed cake and other home produced grain by products from barley and pulses which are relatively less susceptible to aflatoxin contamination [29].

Table 3.

Level of aflatoxin (µg/kg) in different feeds by study locations

Type of feed	*Study location*					
	Holetta	***%***	***Bishoftu***	***%***	***Hawassa***	***%***
No of samples	58		48		54	
Roughage feeds						
Grass hay	1.07 ± 1.4^{a}	13.8			0.13 ± 1.7^{a}	1.9
Wheat straw			0.07 ± 1.4^{a}	2.1		
Concentrate feeds						
With oil seed cake	10.29 ± 1.1^{b}	29.3	17.5 ± 1.3^{a}	35.4	8.13 ± 2.8^{b}	7.4
Without oil seed cake	5.24 ± 1.3^{b}	24.1	9.64 ± 1.5^{a}	20.8	2.0 ± 1.6^{b}	11.1
Wheat bran	3.61 ± 2.1^{ab}	10.3	6.43 ± 2.1^{a}	8.3	0.81 ± 1.0^{b}	9.3
Mean AFB1	6.33 ± 1^{b}		9.76 ± 1.3^{a}		1.19 ± 1.1^{c}	
Storage time (month)	3-6	77.5	3-6	66.6	< 1 month	29.7

% - percentage of aflatoxin contamination for each type of feed under each study location
LS - means with different superscripts between columns were significantly different

Though there was no significance difference among roughage feeds across the study locations, AFB1 level of wheat straw in Bishoftu had the lowest concentration (0.07µg/kg) as compared to AFB1 content of hay in Holetta (1.07µg/kg) and Hawassa (0.13µg/kg) (tabe 3). The lowest AFB1 level in wheat straw is related to its poor nutritional composition [30:19-20] which makes it less vulnerable to fungal contamination.

The data also showed that the AFB1 in concentrate feeds fortified with oilseed cakes was significantly ($p<0.05$) higher (17.50 ± 1.3 µg/kg) in Bishoftu compared to the same type of feed in Holetta with AFB1 content 10.29 ± 1.1 µg/kg and AFB1 of 8.13 ± 2.8 µg/kg at Hawassa respectively. Similarly, the highest AFB1 content of concentrate feeds without oilseed cakes was observed in Bishoftu (9.64 ± 1.5µg/kg) as compared to Holetta (5.24 ± 1.3µg/kg) and Hawassa (2.0 ± 1.6µg/kg). Similar pattern of AFB1 contamination was also observed on other feed types in Bishoftu sampling sites (table 3).

In general, the highest AFB1 content in feeds observed in Bishoftu might be due to the relatively higher temperature which could be aggravated by the relatively longer storage duration (temperature 25^0C and storage duration 3-6 month) employed in this area as compared to Holetta (temperature 22^0C and storage duration 3-6 month) and Hawassa (temperature 27^0C and storage duration < 1 month).

Aflatoxin contamination level of feeds across the feed value chain

The AFB1 contamination level of feeds belonging to dairy producers was significantly ($P<0.05$) higher (9.35 ± 1.04µg/kg) than feed retailers (6.91 ± 1.09 µg/kg) and was also higher than the AFB1 content of feeds in feed manufacturers (7.50 ± 1.43 µg/kg) (Table 4).

Regarding the study locations, the AFB1 content of feeds produced by feed manufactures in Bishoftu (12.45 ± 3.25µg/kg) was significantly higher than that of Holetta (7.92 ± 1.84µg/kg) and Hawassa (2.14 ± 2.42 µg/kg), respectively. Similarly, the AFB1 content of feeds handled by feed retailers in Bishoftu (15.40 ± 2.01µg/kg) was significantly higher than that of Holetta (5.33 ± 2.02µg/kg) and Hawassa (0µg/kg), respectively. The trend was also the same for AFB1 content of feeds handled by dairy producers across the study locations (table 4).

Table 4.

AFB1 (µg/kg) contamination of feeds across the feed value chain

Dairy feed chain actors	*N*	*Study location*			*Mean AFB1*
		Holetta	*Bishoftu*	*Hawassa*	
Feed processors	29	7.92 ± 1.84^{ab}	12.45 ± 3.25^{a}	2.14 ± 2.42^{c}	7.50 ± 1.43^{ab}
Feed retailers	41	5.33 ± 2.02^{b}	15.40 ± 2.01^{a}	0.00^{c}	6.91 ± 1.09^{bc}
Dairy producers	45	11.37 ± 1.88^{ab}	15.18 ± 1.88^{a}	1.5 ± 1.30^{c}	9.35 ± 1.04^{a}
Overall	115				

LS-means with different superscripts between rows were significantly different (last column); LS-means with different superscripts between columns were significantly different (excluding last column); N=number of samples

The high AFB1 contamination level of feeds across the feed value chain was in the order of dairy producers > feed manufacturers > feed retailers (table 4). The reason for higher level of AFB1 in concentrate feeds handled by urban smallholder dairy producers might be due to the poor storage situation employed by them. The lower contamination level of AFB1 of concentrate feeds administered by feed retailers could be related to the small scale of holding and shorter duration of storage because of high market demand. For example in Hawassa, concentrate feeds in feed retailers were not stored for more than a week. [31: 219-226] in their study of AFB1 in animal feeds in Kenya reported that contamination levels for urban smallholder dairy, feed manufacturers and feed retailers respectively was 20.48 ± 29.8µg/kg, 20.62 ± 15.62 and 22.38 ± 17.78 which was much higher than the results found in this study.

Level of aflatoxin M1 (AFM1) in milk across study locations

The overall (average) AFM1 contamination of milk in this study was in the range of 0-0.146; with a mean AFM1 of 0.054 µg/L which was different from the average and range of AFM1 (0.41µg/L and 0.028-4.98 µg/L) in milk samples reported in Ethiopia by [18]. The data also showed variations in AFM1 content of milk samples at different experimental sites (Table 5). Accordingly, milk samples collected from Bishoftu contained relatively higher AFM1 (0.088 µg/l), followed by milk samples with AFM1 contents of (0.057 µg/L and (0.017 µg/L collected from Holetta and Hawassa sampling sites, respectively (Table 5). Although all samples were within the EU and USA permitted levels, the quality of milk seemed to be better at Hawassa than the other experimental sites which may be associated with the lower AFM1 content of feeds in the area (table 5).

Table 5.

Pattern of aflatoxin contamination of milk samples at different sampling sites

Sampling site	*Mean (µg/L)*	*Range (µg/L)*	*Free from AFB1 (n & %)*	*EU standard (0.05 µg/L) (n & %)*	*US standard 0.5µg/L (n & %)*
Bishoftu	0.088	0-0.1403	2 (13)	4 (27)	11 (73)
Holetta	0.057	0.0015-0.146	0 (0)	4 (27)	11 (73)
Hawassa	0.017	0-0.11	11(73)	15 (100)	0 (0)
Quality status (overall)	0.054	0-0.146	29%	58%	42%

Level of aflatoxin in milk in this study in comparison with other countries level

The quality of milk in terms of afltoxin contamination in this study was compared with other countries (Table 6) and the AFM1 contamination level was slightly higher than the AFM1 of milk studied in Turkey where 39% of the samples were free from aflatoxin, and 61% of the samples were within the EU stringent standard reported by [32].

It is interesting to note that the aflatoxin level of milk in this study was in the "low " category (within the Stringent EU standard) similar to most reports from different countries, especially from Egypt [33]; [34]; Iran [35] [36]; Kenya [31]; India [37] Sri Lanka [38] (Table 6). The

AFM1 content of milk in this study was lower than the previous results reported in Ethiopia [18], in Pakistan [39]. However the quality of milk in terms of the level of AFM1 in milk was lower than the milk samples reported from Iran [36] and [32] Tukey (table 6).

Table 6.

Level of AFM1 in milk in this study in comparison with other countries (2009-2016)

Country	*AFM1 in milk (µg/L)*			*Reference*
	Mean	*Range*	*Statius*	
Ethiopia	0.054	0–0.146	Low	This study
Ethiopia	0.41	0.028-4.98	Medium	[18]
Pakistan	0.38	0.01–0.76	Medium	[39]
India	0.5	-	Medium	[40]
Iran	-	0.0021 - 0.131	Low	[35]
Egypt	0.05	-	Low	[33]
Egypt	0.063	0.002-0.11	Low	[34]
Sudan	0.5	-	Medium	[41]
Thailand	0.070	-	Low	[34]
Kenya	0.064	-	Low	[31]
India	0.046	-	Low	[37]
Sri Lanka	0.04	-	Medium	[38]
Iran	0.0004	-	Low	[36]
Turkey	0.0023	-	Low	[32]

Low - within the EU standard; Medium - Within the USA standard

The overall AFB1 and AFM1 levels in feeds and milk in the current study was 5.63µg/kg and 0.054µg/L respectively and the transfer rate of AFB1 in feeds to AFM1 in milk was 0.054/5.63 x 100 = 0.96% which was similar to the findings of □42□ with a transfer rate of 1-2 %.

4. Conclusions

In this study, half of the feed samples were free from aflatoxin contamination; whereas the remaining were within the stringent EU and the more permissible USA standards. The pattern of aflatoxin distribution amongst the different feed sources, showed that concentrate feeds such as oil seed cakes contained more AFB1, followed by cereal byproducts, roughage feeds (grass hay and straws) respectively. The data also showed difference in aflatoxin content amongst sampling sites in that feeds from Bishoftu contained more aflatoxin, followed by feeds from Holetta and Hawassa, respectively.

The aflatoxin contamination level was in the order that feeds belonging to dairy producers contained significantly higher AFB1 than feed retailers, followed by feed manufacturers. All variations in aflatoxin contents amongst feeds and sampling sites were due to different nutritional contents of feeds and the storage situations by the value chains actors in different areas.

Regarding milk samples, aflatoxin was not detected from a reasonable number of samples (29%); whereas 58% of the samples were within the stringent EU standard of (0-0.05); and 42% were within the USA standard set for milk samples indicating that the milk samples were within the permissible level of aflatoxin in

milk. The data also showed the conversion rate of afltoxin from feeds to milk was within the values computed for other milk samples elsewhere. The milk quality of the present study were within the "low category" comparable to studies from several countries. In general, the AFM1 contamination level of feeds and milk samples collected from the study locations were in the order of Hawassa < Holetta < Bishoftu.

5. Recommendations

Further studies are required along the feed and milk production and marketing chains using other techniques such as HPLC, GC and multi-mycotoxin assay using LC-MS-MS by considering different storage conditions such as use of palate, ventilation, and duration of feed storage on aflatoxin and nutrient contents of feeds. The contribution of extreme acidic and extreme alkaline PH conditions on aflaoxin reduction should also be studied. The contribution of cooking, roasting and boiling of agricultural products on reduction of aflatoxin contamination should also be studied. Since, proper handling and management is a key issue for minimizing aflatoxins contaminations on grains and feeds, people engaged in crop production, feed processing, feed marketting and feed utilization should employ better agricultural practices, better grain/feed storage practices and hygienic feeding practices. There is a need to educate and train farmers, feed traders and feed manufacturers on good agricultural practices and good storage practices to produce grains and feeds with low levels or exempt from aflatoxin contamination. Special care and attention is required in storing feeds and grains during rainy season. Feed manufacturers should employ proper screening of spoiled grains and feed ingredients before milling and processing. In addition, dairy producers should follow better feeding practices such as screening of moldy feeds before offering to livestock species.

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