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Aflatoxin Contamination of Milk and Dairy Feed with Quality of Feed in Oromia Special Zone Around Finfinne, Ethiopia

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Abstract
The study was conducted in Oromia special zone around Finfinne with the objective to asses feed quality, detect and quantify the amount of aflatoxine (AFM1) in raw cow's milk and AFB1 in home-mixed dairy feed. For this purpose, 90 milk and 90 feed samples from dairy farmers were collected. Analysis for AFM1 and AFB1 was conducted by high-performance liquid chromatography. The study discovered that the prevalence of AFM1 in all milk samples, and detection level ranged between 0.02ppb and 0.08ppbL. Overall, 64 (71.1%) out of a total of 90 milk samples contained less than or equal to 0.05 ppb of AFM1. Moreover, 26(28.9%) milk samples exceeded 0.05 ppb. All the feed samples were contaminated with AFB1 minimum 12.67ppb and a maximum of 45.67ppb. Overall, out of a total of 90 feed samples collected, about 66 (73.3%) contained AFB1 at a level less than or equal to 20 ppb. At the same time, 34 (26.7%) of the feed samples contained AFB1 at a level exceeding 20 ppb. Linear regression showed significant associations between the presence of AFB1 in the feed and the levels of contamination in AFM1 in milk. The level of aflatoxin contamination found during this study in milk and feed ought to prompt action to spot appropriate interventions. These results recommend that risk mitigation should focus on reducing aflatoxin contamination in raw materials feed which can ultimately minimize AFM1in milk.
Keywords: Aflatoxin, Feed, Milk, contamination, Home-mixed, dairy and Aspegillus fungi
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Introduction

Aflatoxins are the most harmful secondary metabolites of some genus fungi like A. flavus, A. parasiticus and infrequently A. nomius, that square measure present contaminants of animal Feeds and human food (Abdel-Fattah, Kamel, Megalla, & Hafez, 1982). Aflatoxins may be separated into aflatoxins AFG1, AFG2, AFB1and AFB2 (Akiama, Goda, Tanaka, & Toyoda, 2001). Aflatoxin B1 could be a genotoxic and cancer plant toxin that's made by A. flavus and A. parasiticus. AFB1 can be metabolized to aflatoxin AFM1 by the large and small ruminant. Aflatoxin B1 in feeds can decrease milk production, reduce fertility and increase susceptibility to infections (Senerwa D. *et al,* 2016). AFB1 and AFM1 are reflected to be carcinogenic and genotoxic to end-user. When consumed, aflatoxin AFB1 is hydroxylated to AFM1 and secreted in the milk (Applebaum, Brackett, Wiseman, & Marth, 1982). Aflatoxin B1 is of particular importance, as it has been found in most feeds/foods and is highly carcinogenic, initiating liver cancer in humans (Liu, Chang, Marsh, & Wu, 2012). Elevated concentrations of AFB1 in the feed result in elevated levels of AFM1in milk and milk products.

In addition, each AFB1 and AFM1 area unit category field by the International Agency for Analysis on Cancer (IARC) as class carcinogens (International Agency for analysis on Cancer, 2002). This suggests that milk and different milk product might contain toxins that create a threat to humans, significantly youngsters who consume it. Furthermore, exposure to aflatoxins will cause growth impairment (Khlangwiset, Shephard, & Wu, 2011) and immunological disorder (Bondy & Pestka, 2000) in animals and humans. Substantial association between impaired kid growth and biological weapon coverage was reportable from many countries in Sub-Saharan continent as well as Benin, (Gong *et al*., 2004) and African nation (Okoth & Ohingo, 2004).

For these reasons, Ethiopia standard agency collaborated with Veterinary drug and feed administration control authority considered strict parameters for aflatoxin regulation. The maximum level of 20ppb of AFB1 has been established in dairy feed. Also the last Commission Regulation of the European Union (EU) No 165/2010 determine the maximum level of 8 mg/kg of AFB1 has been established in food subjected to sorting or physical treatment before human consumption, and also the corresponding 2 mg/kg of AFB1 for direct human consumption. The maximum level of 0.05 ppb has been set for AFM1 in milk by Ethiopia Food and Medicine control Authority (FMCA) derived from FAO and WHO. The Food and Drug Administration in the USA (USFDA) sets action level for AFM1 in milk and total aflatoxin in animal feed to be 0.5 ppb and 20 ppb respectively (National Grain and Feed Association, 2011). Few study have stated Aflatoxin impurity of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia (D. Gizachew et al 2016). Therefore, there is limited

information about aflatoxine contamination in of dairy feed and milk in Oromia special zone around Finfinne. The present study was therefore designed to quantify and detect the level of aflatoxineB1 contamination in home mixed dairy feed and aflatoxin M1 in milk**.**

MATERIALS AND METHODS

Description of the study areas

The study was conducted on private urban dairy farms in Oromia special zone around Finfinne. It's situated at an altitude ranging between 1700-3600 masl. The common minimum and maximum annual temperatures are 230C and 360C, respectively. With the bimodal rainfall pattern, the mean annual rainfall is between 800-226 mm. The long and heavy rainfall is received from June to September while the short and small shower is received from February to April.

Sampling

Experimental samples were collected from Oomia special Zone around Finfinne, Ethiopia. The data set includes milk samples collected at every dairy long with a home-mixed dairy farm feeds sample for aflatoxin analysis. Three urban centers of Oromia special zone around Finfine (Burayu,Sululta, and Sebeta) were purposively designated for this study based on the Zone Agriculture Office report on the amount of milk production. 90 milk samples of concerning 500 mL and 90 home dairy mixed feed samples of concerning 500 g collected from every city. Milk samples analyzed for aflatoxin level at Bless Agri Food Laboratory Services PLC, (ISO 17025-2005 Accredited) in Lega Dadhi lega xafo, Ethiopia, and feed samples analyzed for aflatoxin level and chemical composition at Animal product, Veterinary drug and feed quality assessment center Laboratory in Addis Ababa. Determination of aflatoxin for both milk and feed was conducted using a very competent method of Higher Performance Liquid Chromatography (HPLC) techniques and for feed chemical composition was used Near-infrared - Nir Technology.

Analysis of AFM1 in Milk

The sample should keep in the refrigerator before the analysis proceeds, take about 100ml of the sample, and warm-up it at 40 degrees centigrade. Centrifuge the warmed sample and remove the upper layer and Repeat the centrifuge if there is a layer that is not thin. Take 50ml of sample and set in the clean-up system (which uses the immunoaffinity column (Aflaclean M1) of Aflatoxin M1) and Eluate by 3ml of Acetonitrile and evaporate. Reconstitute the residue by 1ml of acetonitrile. Inject the reconstituted solution using HPLC System and analyze the chromatogram from HPLC and take the result. This was according to the procedure determination of Aflatoxin M1 (AOAC 2000.08).

Analysis of AFB1 in Home –mixed dairy feed

Determination of aflatoxin B1 Extraction and clean-up procedure: A test portion of 20 g feed sample and extraction solution of 2 g NaCl with 80 mL methanol and 20 mL deionized water was used. Finally, 50 mL of hexane was added to the prepared sample. A pressure pumper was used to extract and the therefore final extract was collected within the column reservoir and therefore the solution was passed undergone filtration. Aflatoxin derivatization: After adding n-hexane (200 µL) within the derivatization vial to re-dissolve aflatoxin, 50 µL of trifluoroacetic acid is added and it's mixed on a vortex mixer for 30 sec. Layers are allowed to separate and an aqueous layer (lower layer) containing aflatoxins is filtered and so injected onto the LC column. Liquid chromatography determination with fluorescence detection: The mobile phase (acetonitrile: methanol: deionized water within the ratio of 20:20:60) is degassed with sonicator before use. Aflatoxin B1 peak is identified in derivatized extract chromatograms by comparing its retention time with the corresponding peak within the standard chromatogram. The number of the aflatoxin was resolve within the derivatized extract (injected) from the respective standard curves.

Chemical Analysis of Feed Samples

Home-mixed concentrate feed samples were collected from each farm and sealed in plastic bags for chemical and aflatoxin analyses. Chemical analyses of the feed samples were performed at the Animal product, Veterinary drug, and Feed quality assessment Center's Laboratory. The DM and ash contents of feed samples were determined by oven drying at 105ºC overnight and by igniting during a muffle furnace at 600ºC for six hours, respectively (AOAC, 1990). Nitrogen (N) content was resolve by the Kjeldahl method and crude protein (CP) was calculated as N*6.25 (McDonald et al., 2002). The two-stage in vitro technique developed by Tilley and Terry (1963) was wont to determine in vitro organic matter digestibility (IVOMD) of the feeds. Metabolizable energy (ME) was estimated from the IVOMD as ME (MJ/kg DM) = 0.016 x (g/kg IVOMD) (McDonald et al., 2002).

Statistical analysis: The level of contamination of aflatoxin in all samples was calculated based on the level of

aflatoxin 0.05ppb. Contamination for aflatoxin in feed was determined based on Ethiopia standard agency limit and Ethiopia veterinary drug and feed administration control Authority, if the concentration of aflatoxin in feed is more than 20ppb it will not be safe to feed the dairy cow. The geometric mean of aflatoxin level and concentration was determined using SPSS 20 statistical software package.

RESULT

Chemical Composition of Home Mixed dairy Feed

The mean chemical compositions of the home-mixed concentrate mixtures feed based on laboratory results are presented in Table1. The overall mean DM and CP content of home-mixed concentrate mixtures in the study area were 898.63±1.92g/kg898.63±1.92g/kg and 160.22± 4.54g/kg, respectively. The mean CP content of the study area was slightly equivalent to the minimum requirements 170g/kg or17% of Ethiopian standard (ES6403:2019) crude protein (CP) content in dairy feed. The overall mean OM and Ash content of home-mixed concentrate feed in the study area was 799.11±2.35g/kg and 99.51± 1.43 g/kg respectively. The mean ME value of the home-mixed concentrate feed on farm where blended to feed crossbred lactating dairy cows was 2428.22± 45.93kcalg /kg of DM. This is lower than the minimum requirements 2500Kcalg/kg of Ethiopian standard (ES6403:2019) metabolic energy (ME) of dairy feed. The mean value of crude fat, crude fiber and Moisture in the study was 39.88± 1.11g/kg,154.15±5.04g/kg and113.33±10.94g/kg respectively which were highly lower than the maximum requirement 1000g/kg, 15g/kg and 1100g/kg (ES6403:2019) of Ethiopian standard respectively. The chemical composition of feed in the study area were no significant different ($p>0.05$) between the study town.

Table: 1 Chemical composition of home-mixed concentrate mixtures in dairy farms Oromia special zone around Finfine

Nutritive value	Burayu N= 30	Sululta N=30	Sebata N=30	Over all mean N=90	p-Value
DM	898.24^{b} ± 3.34	891.14^{b} ±0.77^{b}	906.52^{b} ±1.65	898.63±1.92	0.001
OM	785.46^{b} ±3.73	805.3^{b} ±1.09	806.58^{b} ±2.24	799.11±2.35	0.0002
CP	152.52^{a} ±2.98	166.3^{a} ±5.48	161.84^{a} ±5.17^{a}	160.22± 4.54	0.003
Fat	34.82^{ac}± 1.52	33.15^{ac} ±0.78	51.69^{ac} ±1.04	39.88± 1.11	0.0001
ME kcal g/kg	2415.3^{b} ±50.64	2403.6^{b} ±47.38	2465.8^{b} ±39.8	2428.22± 45.93	0.04
Starch	185.78^{b}± 8.35	325.02^{b} ±15.79	271.3^{b} ±59.85	260.68± 27.99	0.026
Ash	112.78^{bc} ±1.91	85.83^{bc} ±0.96	99.9^{bc} ±1.41	99.51± 1.43	0.0019
Fiber	155.6^{b}±6.26	143.56^{b} ±5.04	163.29^{b} ±3.82	154.15±5.04	0.027
Moisture	106.12^{b}±1.21	131.9^{b}± 30.005	102.01^{b} ±1.63	113.33±10.94	0.008

Aflatoxin B1 Contamination in Home Mixed Dairy Feeds

A total of 90 animal feed samples (30 from each site) were collected for analysis. The samples included all the commonly used home mixed dairy feeds such as wheat bran, nougseed cake, wheat middling, linseed cake, bean hulls, cottonseed meal, brewery by-product and salt. Results revealed that the lowest level of AFB1 contamination was 12ppb and the highest AFB1 level was 46ppb (Table 2). The overall mean of AFB1in the study area, 22.42±1.87pbb was higher than tolerance level of Ethiopia standard 20ppb (ES6403:2019). The mean AFB1 contamination value was significantly ($p<0.05$) higher in Sululta than Burayu and Sebata. There is no statistically significant between Burayu and Sebata.

Table: 2 Aflatoxin B1 contamination of Home mixed dairy feeds in the Oromia special zone Around Finfine

AFB1 in ppb	Burayu N=30	Sululta N=30	Sebeta N=30	Over all N=90	P-value
Mean	21.99^{b}±1.75	23.37^{a}±1.95	21.9^{b}±1.9	22.42±1.87	0.0001
Median	18.00	18.5	18	18.17	
Minimum	12	14	12	12.67	
Maximum	46	46	45	45.67	

It was observed that all the 90 feed samples collected were moderately contaminated with aflatoxin B1 in different level(Fig. 1), 73.3% contained AFB1 at a level less than or equal to 20ppb of the Ethiopia standard (ES6403:2019),mean that it will safe to fed lactating cow. While 26.7% of the feed samples contained AFB1 at a level exceeding Ethiopian standard (20ppb), this is not safe for feeding lactating cow.

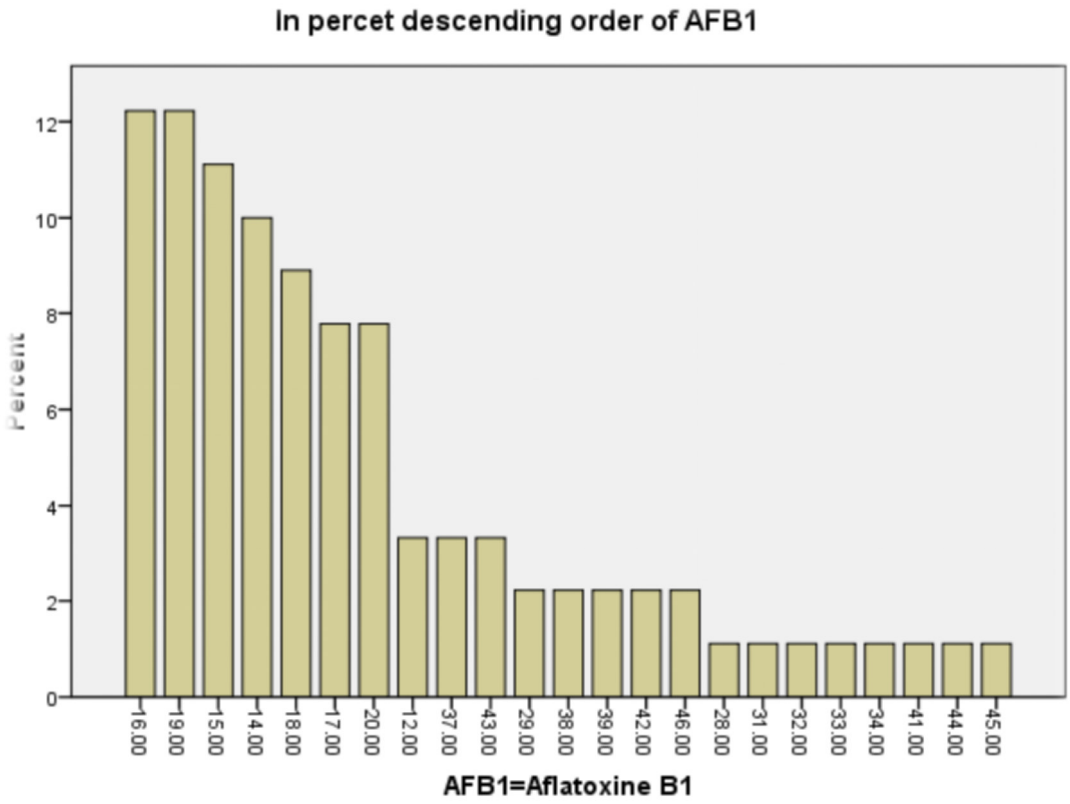

Figure 1 Contamination of feed samples with AFB1 (ppb) in the Oromia special zone around Finfine.

Aflatoxin M1 Contamination in Milk

The results from milk analysis also showed that all the milk samples were contaminated with AFM1 with a median value of 0.042ppb (Table 3). The highest AFM1 content was 0.08ppb from Sululta and Burayu, and the lowest was 0.02 ppb from Sebata. The overall mean value of the study result was 0.044ppb which is moderately less than the Ethiopian standard regulatory limits or FAO/WHO of 0.05ppb aflatoxins M1in milk.

Table 3: Aflatoxin M1 contamination of milk in the Oromia special zone around Finfine

AFM1ppb	Burayu N=30	Sululta N=30	Sebata N=30	Overall mean N=90
Mean	0.0431	0.0454	0.0431	0.044
SD	0.0154	0.017	0.0150	0.016
Medium	0.0420	0.043	0.042	0.042
Minimum	0.02	0.03	0.02	0.023
Maximum	0.08	0.08	0.07	0.0766

Out of the milk samples collected, 64(71.1%) Contained AFM1 at a level less than or equal to 0.05ppb or Ethiopia tolerance level and 26(28.9%) was exceed at the level of Ethiopia limits of Detection (Fig. 2).Even small percent of milk sample is contaminated by AFM1 but the number is significant

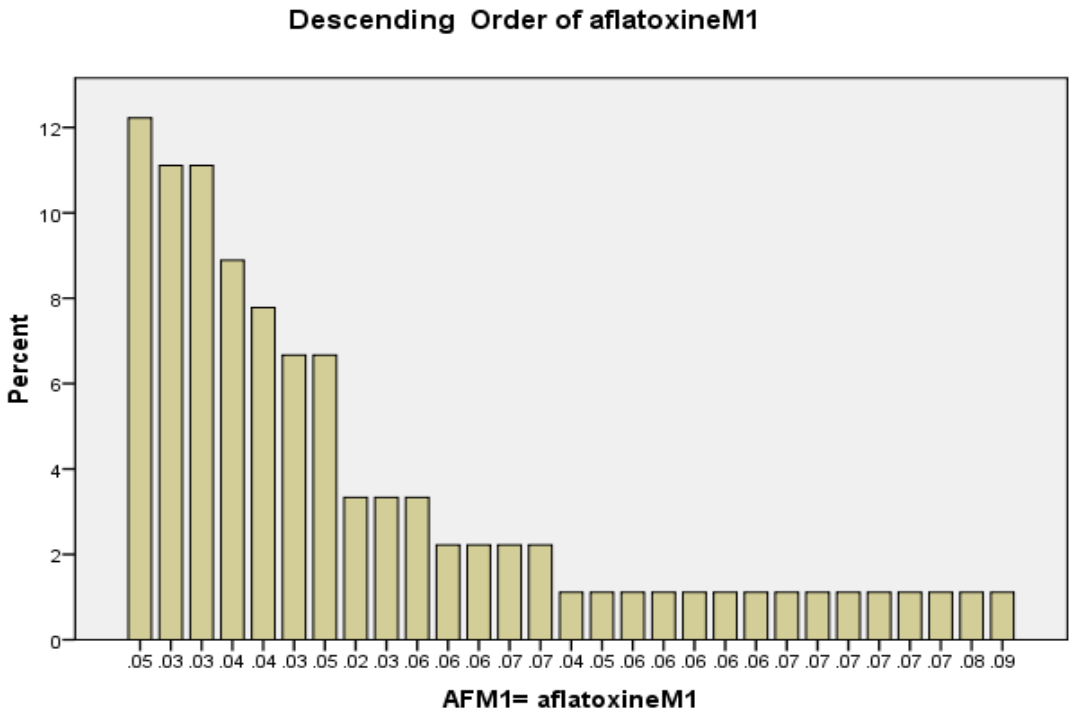

Figure 2 Contamination of milk samples with AFm1 (ppb) in the Oromia special zone around Finfine.

Correlation between AFM1 in milk and AFB1 in feed

The sample regression coefficient is significantly different than zero (Table 4) which means the linear regression model showed a significant association between AFM1 contamination in milk and the presence of aflatoxins B1 in the feed. This indicates that there was a clear association between AFM1 contamination in raw milk and AFB1 contamination in the feed. There was a moderate positive correlation between AFB1 in feed and AFM1 in milk collected from the corresponding sample with a correlation coefficient of 0.932. The correlation between feed samples contained with aflatoxineB1 would have resulted in the contamination of milk with aflatoxins M1. However, some dairy farms had discrepancies between the levels of aflatoxin contamination in their milk and feed.

Table: 4 linear regression model showing association of Aflatoxin B1in feed and level of aflatoxin M1 in milk.

Source	Sum of Squares	Mean Square	Coefficient	95% Confidence interval	F	P-value
Regression	0.019	0.019	0.932	0.001-0.002	581.281	0.0001
Residual	0.003	0.00				
Total	0.012	0.019				

Predictors :(constant), AFB1=AflatoxineB1, Dependent Variable: AFM1=Aflatoxine

DISCUSSION

In the present study, the overall mean CP value was considerably higher than the value (150g/kg DM) of concentrate mixture recommended by Delgado and Randel (1989) for cows grazing tropical grass swards. The mean CP content in the present study was lower than 216.58±20.86 g/kg DM reported by Assaminew (2014) and 260 g/kg DM reported by Mesfin et al. (2013) in the urban and periurban dairy production system of Holeta and home-mixed concentrate feed for lactating crossbred dairy cows in the central highlands of Ethiopia respectively. But it was comparable with research conducted by Negaet al. (2006) in the urban and periurban centers of Central Rift Valley, Ethiopia and who found 163 g/kg DM of CP in the farmers' home-mixed concentrate for lactating crossbred dairy cows. The farmer's home-mixed concentrate mixture for lactating crossbred cows is considerably variable and unbalanced for the CP contents; the ingredients were blended in the concentrate mixture without any standards. The mean metabolic energy (ME) content result in this study was lower than the report of Rehrahie et al. (2003); Mesfin et al. (2013); Tekeba et al., (2013) who showed closer to 2866.8 kcal/kg DM of ME. The present result was comparable to and 2532.34/kg DM of ME content of the finding of Nega et al. (2006) and 2580.12kcal/kg DM of ME with the finding of Mesfin et al. (2013) in farmers' home-mixed concentrate to dairy cows of Central Rift Valley and central Ethiopia, respectively.

The study discovered that all feed samples had detectable with different levels of aflatoxins, this is agreed with the result of Giza chew (et al. (016) for Aflatoxin impurity of milk and dairy feeds in the Greater Addis Ababa milk shed. The current study showed that significant numbers of home mixed dairy feed samples exceeded the limit of detection (20ppb) set by the Ethiopian standard regulated by the Ethiopia veterinary drug and feed administration control authority (VDFACA). Compound feed collected from great Addis Ababa milk shed, with an average concentration of a minimum of7µg/kg(7ppb) and a maximum of 419µg/kg (419ppb) (Gizachew *et al.* 2016), was higher than what was found in this study result. The overall mean contamination in the present study indicated that AFB1 levels were above the Ethiopia limit of detection, this result agrees with Aflatoxin M1 in raw milk and aflatoxin B1 in the feed from household cows in Singida, Tanzania (Joan J.E M.et .al 2016). The high contamination levels of Home mixed dairy feed observed during sample collection at study sites can be attributed to the fact that the storage facilities feed raw materials were very poor. The majority of the farmers were not aware of the presence of aflatoxins in animal feed and their impact on animal health as well as human health. Furthermore, farmers tend to buy the raw materials of feed-in bulk during the low price season and store them for extended periods in poor storage facilities. Inadequate studies have been reported on aflatoxins in dairy feeds in Sub-Saharan Africa with the exception Kenya, where substantial analysis of aflatoxin contamination of maize has been carried out (Kang'ethe, M'Ibui, Randolph, and Lang'at, 2007;Ogana and Muture, 2005). In Ethiopia, young calves are especially susceptible to the harmful effects of aflatoxins before their rumen matures and they consume their mother's milk until weaning. Therefore, the economic losses due to chronic exposure of cattle to aflatoxins could be significant to the urban dairy industry in Ethiopia (.D. Gizachew et al. 2016).

The level of aflatoxin contamination of home mixed feed in Sululta town was significantly higher than Buyayu and Sabeta. Though all dairy farmers of the different towns used similar types of feed raw materials, differences in temperature, moisture, and storage conditions might be the cause for the variation of aflatoxin contamination between areas. In livestock, feeding of very high levels of aflatoxins bases for acute toxicosis and death, while chronic consumption of lower levels can cause liver damage, gastrointestinal dysfunction, and failure in appetite, reproductive role, growth, average daily intake, body weight, and production (Khlangwiset et al., 2011).

This study revealed that all milk samples had detectable with different levels of AFM1. The present study is similar when compared to a previous study conducted on Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia D. Gizachew et al. (2016) but the contamination levels observed in the present study were lower than those reported by the same authors. The majority of milk samples have not exceeded the limit of 0.05 ppb set by the Ethiopia standard or WHO/FAO while a significant percentage of them exceeded the levels of tolerance. The maximum concentration of AFM1 that detected in milk was lower than the studies from urban centers in Kenya has reported AFM1 levels up to 0.68 ppb (Kang ' ethe and Lang' a, 2009), and the levels of AFM1 contamination found in raw milk collected from Khartoum state in Sudan, with an average concentration of 2.07ppb and maximum of 6.9 ppb (Elzupir & Elhussein, 2010). While the Incidence of AFM1 in the present study is slightly similar when compared to a previous study conducted on the Presence of Aflatoxin M1 in Milk, Samples Collected from Jeddah, Saudi Arabia ((Magda A. et al, 2017) were contaminated and the quantity of AFM1 ranged from 0. 09- 0.65 ppb with the mean value of 0.04 ppb which is lower than the Euro-limit (0.05ppb) while 6 samples exceed the USA limit (0.5 ppb).

The study showed there was a moderate positive correlation between AFM1 contamination in milk, and the level of AFB1contamination in the feed. The correlation between feed samples contained with aflatoxineB1 would have resulted in the contamination of milk with aflatoxin M1. The highest level of AFM1 contamination in Sululta farm milk, and the corresponding AFB1 levels in the home- mixed dairy feed. There were some discrepancies between the contamination levels of milk and feed collected from the dairy farms. For example, the four farms in burayu had high levels of AFM1 in the milk while corresponding feed samples were only moderately contaminated with AFB1. Inversely, a high amount of feed contamination was not always reflected in the milk. The cause could be that either at the time when milk samples were being taken, the cattle were fed dissimilar stock of feed, or the feed was not mixed well such that the study analysis didn’t have an exact representation of the different feeds in the mix, this report agrees with previous study Aflatoxin uncleanness of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia D. Gizachew et al. (2016). In livestock, feeding of very high levels of aflatoxins bases for acute toxicities and death, while chronic consumption of lower levels can cause liver damage, gastrointestinal dysfunction, and decrease in appetite, reproductive function, growth, average daily gain, bodyweight, and production (Khlangwiset et al., 2011)

Conclusions: It could be concluded from the present findings that aflatoxin M1 presence in milk is of public health concern and hence all the efforts should be made to keep the levels below the recommended levels. Such efforts need a holistic approach and all the critical control points of entry of aflatoxin B1 into the feed chain has to be monitored and controlled. The bioconversion of aflatoxin B1 to M1 in the liver depends on many factors and in Ethiopia, it is recommended to keep M1 levels below 0.05ppb.

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