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Feed Quality, Prevalence of Aflatoxin Contamination in Dairy Feed and Raw Milk in Oromia Special Zone Surrounding Finfinne, Ethiopia

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ABSTRACT

Background: The study was attended in Oromia special zone around Finfinne with the objective to asses feed quality composition, detect and quantify the amount of aflatoxine (AFM1) in raw cow's milk and AFB1 in home-mixed feed of dairy.

Methods: For this purpose, 90 milk and 90 samples of feed from dairy farmers were collected. Analysis for AFM1 and AFB1 was conducted by high-performance liquid chromatography.

Result: The study discovered that the occurrence of AFM1 in all samples of milk and detection level ranged from 0.02 ppb to 0.08 ppbL. 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 12.67 ppb and a maximum of 45.67 ppb. 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. The Linear regression displayed that the significant associations between the presence of AFB1 in the feed and the levels of adulteration in AFM1 in milk. The level of aflatoxin pollution 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 impurity in raw materials feed which can ultimately minimize AFM1 in milk.

Key words: Aflatoxin, *Aspegillus fungi,* Contamination, Dairy, Feed, Home-mixed, Milk.

INTRODUCTION

A flatoxins are the best hurtful secondary metabolites of some genus fungi like *Aspegillus flavus*, *A. parasiticas* and infrequently *A. nomius*, that square measure present contaminants of animal feeds and human food (Abdel-Fattah *et al*., 1982). Aflatoxins may be separated into aflatoxins AFG1, AFG2, AFB1 and AFB2 (Akiama *et al*., 2001). Aflatoxin B1 could be a genotoxic and cancer plant toxin that's made by *A. flavus* and *A. parasiticas*. AFB1 can be processed to aflatoxinM1 within ruminant livestock. AflatoxinB1 within feeds can decrease production, slow fertility plus to these increase susceptibility to infections (Senerwa *et al*., 2016). These both types of toxins have reflected to be cancer-causing and geno -toxic to end-user. When consumed, aflatoxinB1 is hydroxylate to aflatoxinM1 and secreted within milk (Applebaum *et al*., 1982). Aflatoxin B1 is specific significance, because it has been happened in most feeds/foods and is extremely malignant neoplastic disease, initiating liver disease in humans (Liu *et al*., 2012). Elevated amount of AflatoxinB1 within the food end in elevated stages of aflatoinM1in milk and milk product.

In addition, every aflatoxinB1 and aflatoxinM1 unit of measurement are class field by international Agency for Analysis on malignancy as category cancer-causing agent (Universal Agency for analysis on Cancer, 2002). This implies that milk and completely different milk product would possibly contain toxins that make a threat to humans, considerably kids United Nations agency consume it. Moreover, exposure to aflatoxins can cause growth impairment (Khlangwiset *et al*., 2011) and disorder (Bondy and Pestka, 2000) in animals and humans. Substantial association between impaired child growth and bioweapon coverage was reportable from several countries in Sub-Saharan continent further as Benin (Gong *et al*., 2004) and land (Okoth and Ohingo, 2004).

For these reasons, Ethiopia standard agency collaborated to Veterinary drug and feed administration regulator authority considered strict limitations for aflatoxin regulation. The extreme amounts are 20 ppb of AFB1 has

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been recognized in dairy feed. Also the last Commission Regulation of the European Union (EU) No 165/2010 determine the determined level is 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 determined 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 feed and drug management in the USA (USFDA) sets action level for AflatoxinM1 in milk and total aflatoxinB1 in animal feed to be 0.5 ppb and 20 ppb correspondingly (National Grain and Food Association, 2011). Few research have stated Aflatoxin impurity of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia (Gizachew *et al* 2016). Therefore, there is limited study about aflatoxine impurity of dairy feed and milk in Oromia special zone around Finfinne. The present study was therefore designed to assess feed quality, detect the amount of aflatoxin B1 detoxification of dairy feed and aflatoxin M1 in milk.

Materials and Methods

Description of the study areas

The research has done on private urban dairy farms in Oromia special zone surround Finfinne, Ethiopia. It's situated at an altitude ranging between 1700-3600 m.a.s.l. The common lowest and highest annual temperatures are 12°C and 16°C, respectively. With the bimodal rain fall pattern, the mean yearly rain fall 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 surround 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 surrounding Finfine (Burayu,Sululta and Sebeta) have purposively designated for this work 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 the Laboratory of Animal Product, Veterinary Drug and Feed Quality Assessment Center in Addis Ababa. Determination of aflatoxin for both milk and feed have analyzed using a very competent method of higher performance liquid chromatography (HPLC) techniques and for chemical composition of feed 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°C. Centrifuge the warmed sample and remove the upper layer and repeat the centrifuge if there is a layer that is not thin. Take 50 ml of sample and set in the clean-up system which uses the immunoaffinity column (AflacleanM1) of (AflatoxinM1) and Eluate by 3 ml of Acetonitrile and evaporate. Reconstitute the residue by 1 ml 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 aflatoxinB1 in home-mixed dairy feed

Determination of aflatoxinB1 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 (AOAC, 1997).

Feed quality compositions analysis

At farm mixed concentrate feed samples were collected from every farm and sealed in plastic luggage for chemical 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 105°C overnight and by igniting during a muffle furnace at 600°C for 6 hrs, correspondingly (AOAC, 1990). Nitrogen (N) content was resolve by the Kjeldahl techniques 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 accustomed confirm *in vitro* organic matter digestibility (IVOMD) of the feeds. Metabolizable energy (ME) was calculable from the IVOMD as:

$$\text{ME (MJ/kg DM)} = 0.016 \times \text{(g/kg IVOMD)}$$

(McDonald *et al*., 2002)

Statistical analysis

The stage adulteration of aflatoxinM1 in all samples was designed based on the quantity of aflatoxinM1 0.05 ppb (ESA, 2009). The finding of current study was compared with Ethiopia standard agency limit and Ethiopia veterinary drug and feed administration control Authority, if the concentration of aflatoxin in feed is more than 20 ppb it'll not be safe to fed dairy cattle. The total mean of aflatoxin amount and its concentration was determined using SPSS 20 statistical software package.

RESULTS AND DISCUSSION

Chemical composition of home mixed dairy feed

The mean farm-mixed feed chemical compositions of laboratory test result are given in Table 1. The mean feed mixed at home contents of DM and crude protein (CP) within current study area was 898.63±1.92 g/kg and 160.22±4.54 g/kg, correspondingly. The mean CP content of the study area was slightly equivalent to the minimum requirements 170 g/kg or 17% of Ethiopian standard agency (ES6403: 2019) total crude protein (CP) in dairy cattle feed. The overall mean value of OM and Ash in home-mixed feed in the current study was 799.11±2.35 g/kg and 99.51±1.43 g/kg correspondingly. The overall mean ME value of the home-mixed concentrate feed where mixed to fed crossbred dairy cattle was 2428.22±45.93 kcal g/kg of DM. This is lower than the minimum requirements 2500 kcal g/kg of Ethiopian feed quality 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±1.11 g/kg, 154.15±5.04 g/kg and 113.33±10.94 g/kg respectively which were highly lower than the maximum requirement 1000 g/kg, 15 g/kg and 1100 g/kg (ES6403:2019) of Ethiopian standard correspondingly. The chemical conformation of feed in the study area were no statistically significant different (p>0.05) among the study town.

AflatoxinB1 contamination in home mixed dairy feeds

A total of 90 samples animal feed (30 from every site) was gathered for laboratory 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, salt and brewery by-product. The finding discovered that, the minimum stage of aflatoxinB1 contamination was 12 ppb and the maximum aflatoxinB1 amount was 46 ppb (Table 2). The overall mean of aflatoxinB1 in the study area, 22.42±1.87 pbb was higher than tolerance level of Ethiopia standard 20 ppb (ES6403:2019). The mean aflatoxinB1 pollution value was significantly (p<0.05) higher in Sululta than Burayu and Sebata. There is no statistically significant between Burayu and Sebeta.

It was observed that all the 90 feed samples collected were moderately contaminated with AflatoxinB1 in different level (Fig 1), 73.3% contained AFB1 at a level less than or equal to 20 ppb 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 (20 ppb), this is not safe for feeding lactating cow.

Aflatoxin M1 contamination in milk

The finding of analyzed sample shown that all milk were spoiled with AflatoxinM1 within a median value of 0.042 ppb (Table 3). The uppermost AFM1 content was 0.08 ppb from Sululta and Burayu and the lowermost was 0.02 ppb from Sebata. The overall mean value of the result of study was 0.044 ppb that was moderately lower than the Ethiopian standard regulatory limits or FAO/WHO of 0.05 ppb aflatoxins M1 in milk.

Out of collected samples, 64 (71.1%) sampled occurred aflatoxinM1 at stage of less than or equal to 0.05 ppb or Ethiopia tolerance level (ES 2009) and 26 (28.9%) was exceed at the level of Ethiopia limits of detection (Fig 2). Even small percentage of milk sample is contaminated by AFM1 but the number is significant, since it was above safety tolerance level.

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^a±3.34	891.14^a±0.77	906.52^a±1.65	898.63±1.92	0.001
OM	785.46^a±3.73	805.3^a±1.09	806.58^a±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^a±1.52	33.15^a±0.78	51.69^a±1.04	39.88±1.11	0.0001
ME kcal g/kg	2415.3^a±50.64	2403.6^a±47.38	2465.8^a±39.8	2428.22±45.93	0.04
Starch	185.78^a±8.35	325.02^a±15.79	271.3^a±59.85	260.68±27.99	0.026
Ash	112.78^a ±1.91	85.83^a±0.96	99.9^a±1.41	99.51±1.43	0.0019
Fiber	155.6^a±6.26	143.56^a±5.04	163.29^a±3.82	154.15±5.04	0.027

Table 2: Aflatoxin B1 contamination of home mixed dairy feeds in the Oromia special zone around Finfinne.

AFB1 in ppb	Burayu N=30	Sululta N=30	Sebeta N=30	Overall 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	

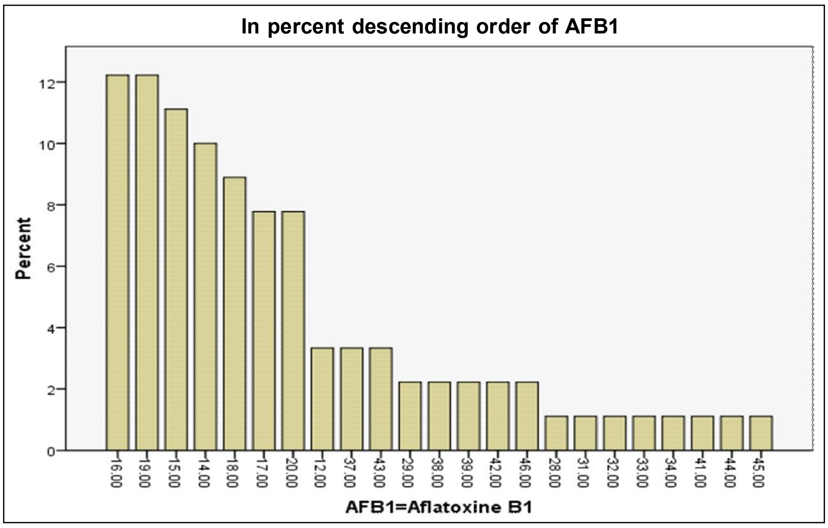

Fig 1: Contamination of feed samples with AFB1 (ppb) in the Oromia special zone around Finfine.

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

Correlation between AFM1 in milk and AFB1 in feed

The parametric statistics of regression coefficient of the study was totally different from zero (Table 4) which advocates that the regression model showed there was clear association among aflatoxinM1 impurity of milk and the occurrence of aflatoxinB1 within the feed. This means that there was a strong positive relationship among aflatoxinM1 adulteration in raw milk and AFB1 within the feed. In this finding within a correlation coefficient 0.932, the feed contained aflatoxineB1 would have resulted milk with aflatoxinsM1. However, some farm farms had discrepancies between the amount of toxin contamination in their milk and feed, sometimes there is the occurrences of high aflatoxin M1 in milk but less aflatoxinB1 in feed and vice versa.

In the present study, the overall mean value of CP was considerably greater than the value (150 g/kg DM) of compound fed mixture recommended by Delgado and Randel (1989) for cows grazing tropical grass swards. The mean crude protein of the present finding was less than 216.58±20.86 g/kg DM stated by Assaminew (2014) and 260 g/kg DM reported by Mesfin *et al*. (2013) in the urban, as well as periurban production system of dairy cattle in Holeta and home-mixed compound feed mixture for cross bred lactating dairy cattle in the highlands of Ethiopia correspondingly. But it has comparable with finding reported by Nega *et al*. (2006) in the urban and periurban area of the Central Rift Valley, Ethiopia who report 163 g/kg DM of CP in the farmer home-mixed concentrate for cross-bred lactating dairy cattle. The home-mixed concentrate mixture for lactating cross-bred cattles 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 result was less 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 similar to and 2532.34 kcal/kg DM of ME content of the finding of Nega *et al*. (2006) and 2580.12 kcal/kg DM of ME with the finding of Mesfin *et al*. (2013) in farmers' home-mixed feed to dairy cows of Central Rift Valley and Central Ethiopia, respectively.

The present study discovered that all feed samples had detectable with different levels of aflatoxinsB1, this finding was agreed with the result of Gizachew *et al*. (2016) for aflatoxinM1 impurity of milk and aflatoxinB1 with in dairy feeds in the greater addis ababa milk shed. The current study showed that significant numbers of home mixed dairy feed aflatoxinB1 contamination exceeded the maximum limit of detection (20 ppb) set by the Ethiopian standard agency that regulated by the Ethiopia veterinary drug and feed administration control authority (VDFACA). In the current study result aflatoxinB1 contamination level was lower than Compound feed collected from great Addis Ababa milk shed, with an average concentration of a minimum of 7 µg/kg (7 ppb) and a maximum of 419 µg/kg (419 ppb) that reported by (Gizachew *et al*. 2016). In the current result, overall mean contamination of AFB1 levels were above the Ethiopia standard limit (20 ppb), this result agreement with Aflatoxin M1 in raw milk and aflatoxin B1 in the feed from household cattle in Singida, Tanzania (Salum *et al*. 2016). The high impurity levels of Home mixed dairy feed perceived during data collection at study sites can be attributed to the fact

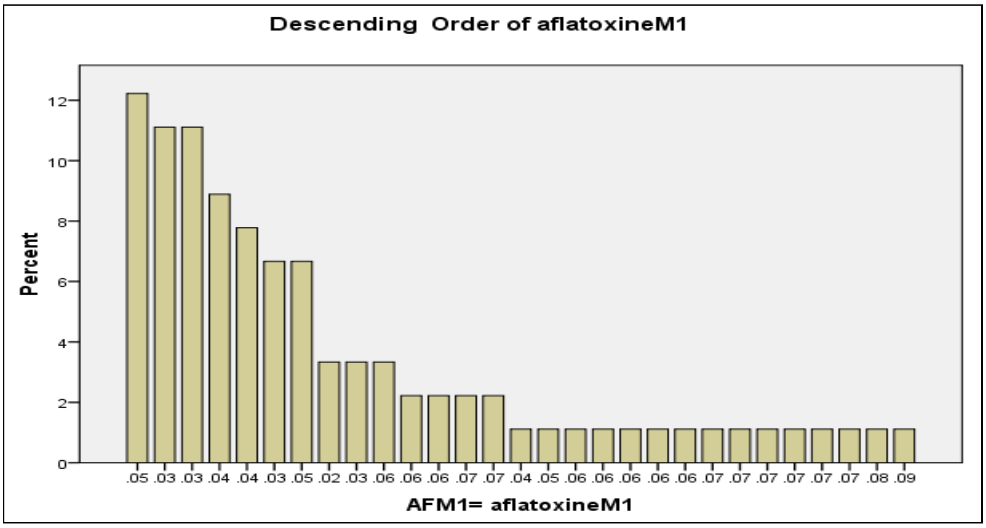

Fig 2: Contamination of milk samples with AFM1 (ppb) in the Oromia special zone around Finfine.

Table 4: Linear regression model showing relationship of AflatoxinB1in feed and level of aflatoxinM1 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= AflatoxinB1, Dependent Variable: AFM1= Aflatoxin.

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 *et al*., 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 (Gizachew *et al*. 2016).

The amount number of aflatoxin contamination of home mixed feed in Sululta town was significantly greater than Buyayu and Sebeta. Though all dairy farmers of the different towns used similar types of feed raw materials, differences in environmental temperature condition, moisture and storage situations might be the cause for the variation of aflatoxin impurity between areas. In dairy cow, feeding of very high levels of aflatoxins bases for critical toxicosis and death, while chronic ingestion of lower levels can cause liver injury, 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 result is similar with a previous finding that conducted on Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia (Gizachew *et al.* 2016) but the detoxification levels perceived in the present study were lower than those stated by the same authors. The more number of milk samples have not exceeded the limit of 0.05 ppb set by the Ethiopia standard or WHO/FAO, however non disregarded percentage of them were exceeded the levels of tolerance. The maximum contamination of AFM1 that spotted in milk was less than the finding from urban centers in Kenya that has reported AFM1 levels up to 0.68 ppb (Kang'ethe and Lang'a, 2009) and the concentration of AFM1 contamination in raw milk collected from Khartoum state in Sudan, with an average level of 2.07 ppb and maximum of 6.9 ppb (Elzupir and 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, data sample Collected from Jeddah, Saudi Arabia (Magda *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 (0.05 ppb) while 6 samples exceed the USA limit (0.5 ppb).

The recent study discovered that, there were a moderate optimistic relation among aflatoxinM1 contamination in milk and aflatoxinB1 in the feed. The high level of aflatoxinB1 in feed correlation with high level impurity of milk with aflatoxinM1. The maximum level of aflatoxinM1 pollution in Sululta farm milk and the corresponding AFB1 levels in the feed. There were some disagreements 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 polluted 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 (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, body weight and production (Khlangwiset *et al.,* 2011).

CONCLUSION

It could be decided that from the present findings that aflatoxinM1 presence in milk is public health concern and hence all the efforts should be made to keep the levels below the recommended levels. Such efforts need all-inclusive tactic and all the serious control points of entry of aflatoxinB1 into the feed chain has to be monitored and controlled. The bioconversion of aflatoxinB1 to aflatoxinM1 in the liver depends on many factors and in Ethiopia, it is recommended to keep M1 levels below 0.05 ppb.

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