Detection of aflatoxin in raw and pasteurized milk by high-performance liquid chromatography (HPLC) in central Ethiopia

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Abstract

Aflatoxins are a group of structurally related mycotoxins produced by the *Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus nomius species* as secondary metabolites. Well-known forms of Aflatoxin are AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2. aflatoxin B1 is the most prevalent one. It can be metabolized in the liver and excreted as aflatoxin M1 in milk. Both forms have mutagenic and carcinogenic effects. In Ethiopia, there is limited information on the occurrence and contamination level of aflatoxin in raw and pasteurized milk. The present study was conducted in the Addis Ababa milk shed area to detect and quantify the level of Aflatoxin M1 in raw and pasteurized milk. A cross-sectional study was conducted from October 2020 to May 2021 on a total of 114 cow milk samples consisting of 60 raw milk and 54 pasteurized milk samples with the aim of detecting and quantifying the amount of aflatoxin M1 in raw and pasteurized milk in central Ethiopia by high-performance liquid chromatography (HPLC) using C18 column with fluorescence detector. AFM1 was detected in 79 (69.3%) of the 114 tested milk samples. The maximum and mean concentrations were 0.893 µg/L and 0.0465 µg/L, respectively. 25.4% of them contain AFM1 above the maximum limit of EU (0.05 µg/L), and 1.8% contained above the maximum limit of CES278 and FDA (0.5 µg/L). 26.7% of the samples from Sebata and 6.7% from Sululta contain above the maximum limit of EU (0.05 µg/L). Higher contamination of AFM1 was detected in pasteurized milk (96.3%) than in raw milk (16.7%). 35.2% of pasteurized and 16% of raw milk contained AFM1 above the maximum limit of EU0.05 µg /L. The study results showed a significant difference in AFM1 occurrence with feed type,

storage time, and milk type. The current AFM1 concentration level in raw and pasteurized milk was not safe for human consumption in the study areas. Due to its heat resistance, AFM1 is found in pasteurized milk and has great health effects. Creating awareness of feed management for feed producers and farmers and developing risk mitigation methods are crucial in order to reduce public health threats.

Keywords: AFM1; Central Ethiopia; Dairy Farm; HPLC; Pasteurized milk.

Introduction

Aflatoxins are a group of structurally related mycotoxins produced by certain species of fungi in the genus Aspergillus, particularly Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius. It was first discovered in 1960 when approximately 100,000 turkeys died in the UK, and the cause was identified as Aspergillus flavus. The name was given from its cause (Bennett et al., 2007). Aflatoxins are produced as a secondary metabolite; the most commonly known are B1, B2, G1, G2, M1, and M2. The letters B and M represent their color under fluorescence detection, where B is blue, and G is green. Those M1 and M2 are metabolites of B1 and B2, respectively. M1 and M2 are found in food of animal origin, such as milk and milk products, meat, and eggs (Jaimez et al., 2000). AFB1 is a well-known and the most prevalent toxin. Its target organ is the liver, which has teratogenic, mutagenic, and carcinogenic effects in animals and humans. The level of toxicity is AFTs-B1 > AFTs-G1 > AFTs-B2 > AFTs- G2 (Ismail et al., 2015). Aflatoxin M1 (AFM1) is four times hydroxylated and produced by hepatic biotransformation of AFB1 in the liver of animals consuming feed contaminated with AFB1 and secreted in milk. The extent of contamination depends on the season (higher in winter than summer), environmental conditions, and genetic conditions of the animals (Fallah *et al.,* 2011; Abyaneh *et al.,* 2019).

About 0.3% to 6.2% of AFB1 is converted to AFM1 and classified as a cause of human liver cancer. Excretion of AFM1 in milk can take 12 - 24 hours after the ingestion of AFB1; however, there is a decrease in concentration after 72 hours (Fallah *et al.*, 2011; Alahlah *et al.*, 2020; Bukari *et al.,* 2020). AFM1 is resistant to autoclaving, pasteurization, and thermal inactivation. It has both acute and chronic effects (Sani and Nikpooyan, 2013). AFM1 is classified as group one causative agent of human liver cancer by the International Agency for Re-

search on Cancer (IARC) (Sharma *et al.,* 2019). It has a potency which is close to that of aflatoxin B1 (AFB1). Milk and milk products are some of the most important diets of humans worldwide. Aflatoxin in milk and milk products is one of the most severe problems of food safety and security (Polak-Śliwińska, 2020). Therefore, it is essential to determine aflatoxin M1 levels in milk to protect children and adults from its potential health hazards (Dehcheshmeh *et al.*, 2020).

Considering its profound health effects, many countries have set limits for its presence in feed and foods. Commission Regulation of the European Union (EU) states that the maximum level of AFM1 in liquid milk should not exceed 0.05 µg/L(ppb). The US Food and Drug Administration (USFDA) standard indicates that Aflatoxin should not be higher than 0.5 µg/L in liquid milk (Markaki and Melissari, 1997; Maqbool *et al.*, 2009).

Ethiopia produces approximately 3.2 billion liters of milk per year (Desalegn, 2018). However, different factors, like the presence of aflatoxin, can affect its safety and pose health risks to the consumer. Gizachew and his colleagues reported a high contamination level of AFM1 in milk in 2016 in the Addis Ababa milk shade area, which was a significant national issue and increased awareness of aflatoxin (Gizachew *et al.*, 2016). After that, there was no report about the status or contamination level of AFM1 in central Ethiopia, including the current study areas (Sebata and Sululta in the North Showa zone of Oromia region, Ethiopia). It is known that aflatoxin is not entirely removed by pasteurization and other thermal treatments. However, there is no report on the contamination level of AFM1 in pasteurized milk in Ethiopia. Therefore, there is a need to study the current status of AFM1 in this area, which is designated as a significant Addis Ababa milk-shed area. The present study attempted to detect and quantify the contamination level of AFM1 in raw milk and pasteurized milk from the Sebata and Sululta areas.

Materials and methods

Study area description

The study was conducted in the significant Addis Ababa milkshed area (Sululta and Sebata). These areas were selected because they are among the major milk supplier sites in Addis Ababa, both for household raw milk consumption and for milk processing plants.

Sebata is located in a special zone of the Oromia regional state in the central highlands of Ethiopia, 24 km west of Addis Ababa, on the main road to Jimma. The average annual rainfall is 1100 mm, more than 85% of which falls in the main rainy season (June to September). The area's altitude ranges from 2200-2600 meters above sea level, and the average annual temperature ranges from 6-21ºC (Desalegn, 2018). According to the Sebeta-Hawas district's livestock agency, there are 310 dairy farms managed by both intensive and semi-intensive farming systems. Daily milk production in the area was about 20,000 liters, of which only half is marketed to Addis Ababa through the formal market. The rest is either consumed at the household level or processed as traditional dairy products (Brandsma *et al.,* 2012).

Sululta district is located between 9° 13′–10° 57′N latitude and 37° 57′–39° 33′E longitude. It is 40 km north of Addis Ababa at an average altitude of 2,550 masl. The annual rainfall is a minimum of 834 mm and a maximum of 1,447 mm. The area's mean minimum and maximum temperatures are 4.4 and 22.5 °C, respectively (Beyecha *et al.,* 2012). According to the Sululta district Livestock Production, Marketing, and Health Agency office, the total cattle population of the district for the year 2019 was 210,211 heads. Intensive and semi-intensive farming systems are practiced in the study area, and there are about 500 dairy farms. From 2018 to 2019, the average annual milk production of the area was 6,694,750 liters, and the average daily milk production was 21,950 liters. From this, about 16,462 liters were marketed formally, and about 5,488 liters were retained at home (Brandsma *et al.,* 2012). More than six milk processing plants receive raw milk from both study areas.

Study design and sample size determination

A cross-sectional study design was used to determine aflatoxin levels in raw and pasteurized milk in central Ethiopia from October 2020 to May 2021. The desired sample size was calculated by using the formula given by Thrusfield (2005) with a 95% confidence interval, 5% precision, and 91.8% expected prevalence based on a previous study by ILRI (Gizachew *et al.*, 2016).

$$n = (Z^2 \, P_{exp}(1-P_{exp}))/d^2$$

Where **n** = required sample size

Z=statistic for the level of confidence at 95% CI, which is 1.96
d= desired absolute precision or margin of error = 0.05

Pexp=expected prevalence, which is 91.8%

$$n = \frac{(1.96)^2[0.918(1-0.918)]}{(0.05)^2}$$

n =<u>113</u>

For this study, 114 samples were collected based on previous prevalence by simple random sampling (60 raw milk samples from farms and 54 pasteurized milk samples from different brands). Simple random sampling was used to select the farms in the districts, and each district's sample size was proportionally allocated.

Sample collection method

About half a liter (500 ml) of pooled raw milk was collected from 60 dairy farms. The sample was taken after the milk was mixed well in a container. This helped to homogenize the milk and take appropriate samples. For the pasteurized milk, 54 packs of 500 ml were purchased from supermarkets from three different brands and transported (at a temperature of +4°C in an ice box) to the Ethiopian Agricultural Authority, Animal Products and Inputs quality testing center, former (VDFACA) and stored at -20°C until analysis. Each farm owner had a questionnaire/interview on the type of feed they used, their feed management practice (storage place, time, and method of storage), the feed source, and their knowledge of aflatoxin.

Sample preparation procedures

The extraction and clean-up procedures for sample preparation were based on the association of official analytical chemists (AOAC, 2002). Frozen milk samples of 100 ml were thawed using a water bath at 40 °C for 30 minutes. After heating and bringing it to room temperature, it was centrifuged at 4500rpm for 15 minutes. This helps to separate the fat from the milk and remove it quickly using a spoon. The fat was then filtered using syringe filters through Whatman No.4 filter paper and transferred into a 50 mL tube. Then, 50 mL of defatted (skim) milk was ultimately passed through the Afla M_1^{TM} Immunoaffinity Column (Afla CLEAN produced by LCTech GmbH Company of Germany) at a rate of about 1-2 drops/second. All samples were allowed to drain through the column until there was no more sample in the column. At this time, antigen-antibody bonds were formed. Then, the column was

washed with 10 ml of distilled water at a rate of 1-2 drops/second. A gentle gas stream or vacuum removes the residual water. Then, 3 ml of acetonitrile was added, and the analyte-antibody bond was waited for 5 minutes to break. After 5 min, the column was opened, transferred into a 10 ml centrifuge tube, and evaporated/concentrated under the nitrogen stream. Finally, the samples were reconstituted in 1 ml of the mobile phase solution of water-acetonitrile-methanol (60:25:15), transferred to amber glass vials, and ready for HPLC detection (AOAC, 2002).

HPLC conditioning and injection procedures

The HPLC machine was conditioned by pumping a mobile phase solution of water-acetonitrile-methanol (60:25:15) at a steady flow rate until a stable baseline developed. Working standard solutions were prepared at concentrations of 0.05, 0.1, 0.5, 1, 1.25, 1.5, 2, and 4 AFM1 µg/L in the mobile phase to construct the calibration curve. The optimal instrument conditions were checked with an aflatoxin M1 calibrant solution before analyzing the test sample. Then, the linearity of the injection of calibrant solutions and the stability of the chromatographic system were checked. A fixed amount of Aflatoxin M1 calibrant solution was repeatedly injected until stable peak areas were obtained. Peak areas corresponding to consecutive injections were within ± 5%. After the HPLC output, the calibration graph was prepared by plotting the peak area against the mass of injected aflatoxin M1. By following the stipulated injection scheme or an ordered sequence, the test samples were injected using the same conditions as for the calibrant solutions (AOAC, 2002).

Once the aflatoxin M1 peak area was determined, the aflatoxin M1 concentration in the test samples was calculated from the calibration graph in µg/L. The formula/conversion factor used to calculate the actual AFM1 was Wm = Wa x (Vf/Vi) x(1/Vs). Where Wm = the numerical value of aflatoxin M1 in the test sample in ng/ml or ug/L, Wa = the numerical value of the amount of aflatoxin M1 corresponding to the area or height of the aflatoxin M1 peak of the test extract (ng), Vf = the numerical value of the final volume of redissolved elute (uL), Vi = the numerical value of the volume of injected elute(uL) and Vs = the numerical value of the volume of prepared test portion passing through the column(ml). The HPLC system was interfaced via network chromatographic software (Agilent Chem Station) to a personal computer for instrumentation control, data acquisition, and processing (AOAC, 2002). The result was interpreted according to the Ethiopian Standard Agency regulatory limit (CES278),

which is 0.5 µg/L in raw/liquid milk, and other country standards were also used for comparison.

Questionnaire survey method

A questionnaire-based survey was used to assess the potential risk factors associated with the contamination level of AFM1 at the individual farm level. The questionnaire was prepared by targeting farm owners concerning the significant risk factors of aflatoxin, like storage time of the feed, moisture content, ventilation of feed storing room, type of commonly used feed, quality of the feed, and the knowledge of farmers on aflatoxin. All necessary information was gathered through this structured questionnaire and was accompanied by direct observation of the farm.

Ethics approval and consent to participate

Ethical clearance was obtained from the animal research ethical review committee of Addis Ababa University College of Veterinary Medicine (Certificate Ref. No: VM/ERC/28/06/13/2021, Date: 28/03/2021). All methods were carried out accordingly.

Data management and analysis

All collected data were organized, coded, and entered into an Excel spreadsheet (Microsoft® Office Excel 2016) and exported to R-statistical software (version R-3.5.1) for analysis. Descriptive statistics (maximum, minimum, mean, SD) were used to present the result of AFM1 contamination level of milk source and milk type. A table of frequency was used to figure out the findings of the questionnaire. Logistic regression was used to analyze the association between AFM1 contamination levels and the considered risk factors. For variables with small positive or negative results, a Fisher exact test was used.

Results

The HPLC analytical results showed high contamination of milk samples with AFM1. The minimum AFM1 was 0, and the maximum was 0.893 µg/L, with mean and SD of 0.0465 and 0.102, respectively. From a total of 114 analyzed milk samples, 1.8% contained AFM1 above the maximum limit of (CES278(0.5 µg/L), and 29(25.4%) of them had AFM1 above the permissible level of the European Community recommended limit, which is 0.05 µg/L in liquid milk. Of

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114 samples, 79(69.3%) were contaminated by AFM1 or contained detectable amounts of aflatoxin M1. Only two samples (1.8%) of 114 analyzed milk samples exceeded the maximum limit set by the USFDA, which is 0.5 µg/L in liquid milk. An example of the HPLC results in ppb is indicated below in Figure 1.
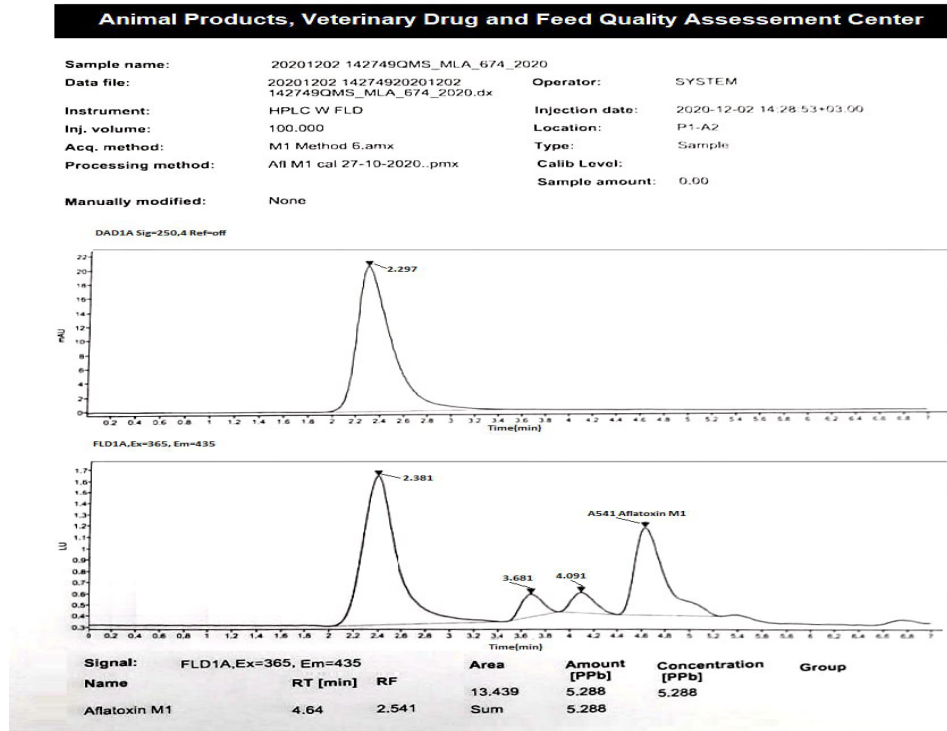

Figure 1. HPLC analytical results in ppb

Raw milk analysis

Of 60 raw milk samples, 10 (16.7%) had AFM1 above the permissible level of 0.05 µg/L. The mean and SD were 0.0469 and 0.1367 respectively. About 50% of the analyzed raw milk samples were positive or had detectable amounts of aflatoxin M1, as indicated in Table 1.

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Table 1. Descriptive statistics of AFM1 level in raw milk in the study area

Location	No sample	Positive	> 0.05 μg/L	min	max	Mean ±SD
Sebata	30	20	8	0	0.893	0.082±0.184
Sululta	30	10	2	0	0.2018	0.012±0.038

The samples from Sebata were more contaminated, with 66.7% positive and 26.7% above the permissible level, than those from the Sululta. Still, it is not statistically significant, with 33.3% of the samples positive and 6.7% above the permissible level. As shown in Table 2 below, the considered risk factors (feed source, presence of noug seed cake in feed, type of milk, and storage time of feed) had a statistically significant difference at 95%CI.

Table 2. Contamination level of AFM1 in raw milk with considered risk factors

Risk factors	Variables	No of sample	Positive	>0.05 μg/L	p value
Location	Sebata	30	20	8	
	Sululta	30	10	2	0.079
Feed source	Graze	37	11	2	
	Not use grazing	23	19	8	0.005
Type of milk	Raw	60	30	10	
	Pasteurized	54	52	19	0.023
Industry byproduct	Without	2634	19	9	0.001
	Without noug		11	1	
Storage time	1week	37	8	1	
	Above1 week	23	22	9	0.001

Pasteurized milk analysis

A total of 54 pasteurized milk samples from different brands were analyzed. Of these,19(35.2%) specimens had AFM1 above the permissible level of 0.05 μg/L. The minimum AFM1 detected was 0, and the maximum was 0.119, with a mean and SD of 0.046 and 0.037, respectively. The toxin (AFM1) was detected in almost all of them (52/54 or 96% of them).

The test statistics showed that there was no statistically significant difference ($p > 0.05$) among the pasteurized milk brands. This means that AFM1 was found in all brands of pasteurized milk included in this study. This can prove the heat-resistant properties of AFM1, as stated in different studies, which

have significant public health effects. Table 3 shows the result of logistic regression of AFM1 for pasteurized milk from different brands.

Table 3. Contamination level of AFM1 in pasteurized milk by their respective brands

Brands	No sample	Positive	>0.05ug/L	Mean	SD	p value
1	13	12	6	0.052	0.032	0.782
2	26	20	6	0.038	0.037	0.148
3	15	14	7	0.055	0.041	0.978

Comparing AFM1 contamination levels in raw and pasteurized milk

There was a statistically significant difference between raw and pasteurized milk contamination by aflatoxin M1 at 95% CI (p-value = 0.0233). As indicated in Table 4, 50% of raw and 96.4% of pasteurized milk were contaminated by AFM1, and 16.7% of raw and 35.2% of pasteurized milk contained AFM1, which is above the maximum limit of 0.05 µg/L.

Table 4. Contamination level of AFM1 by type of milk (logistic regression analysis)

Brands	No sample	Positive	>0.05ug/L	Mean	SD	p value
Raw	60	30 (50%)	10 (16.7%)	0	0.893	0.023
Pasteurized	54	52 (96.4%)	19 (35.2%)	0	0.119	

Farmer's knowledge and awareness of aflatoxin

Survey data were collected during sample collection from the dairy farm owners to obtain complete information; the result of the survey indicates from 60 interviewed farmers, 15(%) were females, and 45(%) were males. Their educational background:6.7% of farmers were illiterate, 41.7% attended primary school, 45% attended secondary school, and 6.6% attended higher education. Indirect observation was also performed on their feed management. The results are summarized in Table 5.

Table 5. Knowledge and practice (KP) of dairy farm owners on aflatoxin contamination.

Knowledge of Aflatoxin	Response	Frequency	Percentage %
Knowledge of mold growth and formation of the toxin No	Yes	41	68.3
	No	19	31.6
Knowledge of favorable conditions for mold growth on animal feed No	Yes	41	68.33
	No	19	31.66
Do you know or heard of Aflatoxin No	Yes	6	10
	No	54	90
Do you know that Aflatoxin causes disease in animals	Yes	6	10
	No	54	90
Do you know Aflatoxin can pass through milk to consumers and have an effect on human	Yes	6	10
	No	54	90
Do you think that Aflatoxin can be destroyed by pasteurization of the milk	Yes	49	81.7
	No	11	18.3
Knowledge and practice on feed management practice			
How do you store the feed	In house	50	83.3
	In shade	8	13.3
	Open field	2	3.4
Is there a ventilator in the feed storing house	Yes	60	100%
	No	0	
Do you control the moisture content of the feed	Yes	45	75
	No	15	25
Do you check the quality of the feed while buying and feeding	Yes	6	10
	No	54	90

Discussion

The present study showed that there is widespread contamination of raw and pasteurized milk by aflatoxin originating from the Sebata and Sululta milk shed areas. From a total of 114 analyzed milk samples, 25.4% exceeded the maximum permissible level of the EU standard (0.05 µg/L). This result is lower than the result of previous studies of Gizachew and his friends in 2016 in the Addis Ababa milk-shed area in which 91.8% of analyzed samples contained above the permissible level of 0.05 µg/L (Gizachew *et al.*, 2016). This difference could be due to changes in the farmers' practices, especially regarding the feed

type used since 2016. Most farmers reduce the amount of noug seed cake in their dairy feed and reduce the time of storage of the feed that is purchased from feed retailers. A study from Injibara reported a low contamination level of AFM1. According to this report, 10% of analyzed samples were above permissible levels, and AFM1 contaminated 15% of the sample (Kassa *et al.,* 2020). This discrepancy could be due to the differences in environmental conditions or climate change, the type of feed used, and the feed management practices of the farmers in Injibara.

The current study showed that 79(69.3%) of 114 analyzed samples were contaminated by AFM1. This result was much lower than the study from Bishoftu (Tadesse *et al.*, 2020), who reported that, from 108 analyzed milk samples, all samples(100%) were found to be contaminated by AFM1 with a mean value of 0.835 µg/l. The result of this study was in line with a study from Kenya by (Anyango *et al.,* 2018), who reported that 26.4% of the analyzed sample exceeded the limit of the EU. A study from Pakistan by (Ahmad *et al.,* 2018) reported that AFM1 contaminated 93% of the analyzed samples, and 69% of the samples exceeded the EU ML (0.05 µg/L), which is much higher than the result of this study.

In the present study, AFM1 was detected in 50% of raw milk samples. Of those, 16.7% were above the permissible level of 0.05 µg/L. This is lower than the study from Kenya by Kagera and his friend (Kagera *et al.,* 2018), who reported that 99% of analyzed milk samples were positive for AFM1 and 64% exceeded the permissible level of EU 0.05 µg/L. The results of this study were also much lower than the study report of Asghar and his colleagues in Pakistan (Asghar *et al.*, 2018), who reported 91.7% contamination and 80.1% above the permissible level from 156 tested raw milk samples.

When comparing the contamination level of raw milk by location of sample collection, raw milk collected from Sebata district had a higher contamination level of AFM1(26.7%) than that of Sululta district (2/30 or 6.7%). This can be due to environmental temperature (high temperature in Sebata), feed type used, and farming system. Most farmers in Sebata district do not have free grazing land for their dairy cattle, so they use purchased feed under an intensive farming system. Farmers from the Sululta district use semi-intensive systems, and they use grazing in their backyard around the home and crop by-

products during harvesting of the crops. This can help to reduce the exposure of dairy cows to Aflatoxin originated from feed.

The present study revealed that high AFM1 contamination was found in pasteurized milk compared with raw milk. However, the maximum AFM1 concentration was found in raw milk. This result was in line with a study in Iran in 2017 (Abyaneh *et al.*, 2019), which reported high AFM1 contamination in pasteurized and heat-treated milk. A report by (Sharma *et al.*, 2019) showed that a high contamination level of AFM1 was found in pasteurized, which indicates thermostability of AFM1, which is in line with this study. An earlier study by (Sani and Nikpooyan,2013) showed a low contamination level of AFM1 in pasteurized milk, but all samples were positive for AFM1. A study report from Iran by(Taherabadi *et al.*, 2016) indicated lower contamination in pasteurized milk (5%) than in raw milk (9.2%). The results of the present study showed that considered risk factors such as storage time, feed type, and grazing have significant effects on aflatoxin production in feed and milk. The storage time of feed has a significant effect on mold growth and aflatoxin production. Feed that was stored for more than one week had more AFM1 than feed stored for less than one week, but in the present study, the toxin was detected at both storage times. This can be due to the moisture content of the feed, climate conditions, ventilation, and other factors. This result was also supported by the study performed by (Abyaneh *et al.*, 2019), who reported that the storage condition of feed can increase the chance of mold growth.

Feed type had a significant effect on aflatoxin production. Some feed can easily be contaminated by AFB1, which can be transformed into AFM1 in lactating cows. In this study, milk from farmers who fed their lactating cows feed containing noug seed cake had a higher contamination level of AFM1 than milk from cows fed other feed types, such as brewery grain and wheat bran. This is in agreement with the previous study by (Gizachew *et al.*, 2016), who reported a high contamination level in feed containing noug seed cake. All (100%) dairy farm owners offered concentrate feed to their lactating cows. The majority (76.7%) of farmers from Sebata district practice zero grazing due to a lack of grazing land in the area, and those farmers were obligated to rely on purchased pasture, fodder, and concentrates, which increases the chance of aflatoxin contamination. Grazing also has a significant effect on the contamination level of AFM1. In this study, a high contamination level was recorded in milk originating from farmers who did not use grazing and low AFM1 con-

tamination in milk originating from farmers who practice an open grazing system in the backyard or grazing field, in addition, to concentrate feeds for their dairy cows. According to the survey results, 68.3% of the farmers were aware of favorable conditions for mold growth and aflatoxin formation. Only 10% of interviewed farmers had heard the word aflatoxin before and knew that it could cause disease in humans through milk from intoxicated cows. 81.7% of the farmers assume that aflatoxin M1 can be destroyed by pasteurization or other heat treatment. This assumption is based on the fact that pasteurized milk is safe from bacteria, but AFM1 is a toxin, not a living organism. The feed management practices of the farmers were observed during the sample collection, and the majority (83.3% of them stored their feed in houses with ventilation, and 75% of them checked the moisture content of the stored feed. Only 10% of them can check the quality of feed by physical observation while buying the feed. They check the physical appearance of the feed and try to buy from good feed retailers in the area.

Conclusions

The present study showed that there is a widespread aflatoxin M1 contamination of milk produced in the study area, with 69.3% of the milk having a detectable amount of AFM1 even though only 1.8% of the milk had a contamination level above the maximum limit (0.5µg/L) as per the compulsory Ethiopian standard (CES)-278 and USFAD regulation level. High AFM1 contamination was found in pasteurized milk compared with raw milk. In this study, risk factors such as storage time, feed type, and grazing have shown a significant association with aflatoxin M1 contamination level in milk. In general, the consumption of milk above the maximum tolerance level might cause a severe public health risk to the community, especially to children who consume milk on a daily basis. Hence, specific regulations and compulsory food safety standards should be put in place by the national regulatory authorities to control or regulate AFB1 in animal feeds and AFM1 in milk and milk products. Awareness and training on feed management, primarily on feed storage and quality feed purchasing, should be provided to farmers. Screening and monitoring of AFB1 in concentrate feed and AFM1 in milk should be performed regularly. Pasteurized milk should be monitored and checked frequently since AFM1 was not removed by pasteurization. A further comprehensive study should be conducted by designing a nationwide survey that encompasses risk mitigation strategies and biodetoxification methods for both AFB1 and AFM1. Moreover,

planned aflatoxin residue surveillance and monitoring activities need to be carried out by the Ethiopian Agricultural Authority.

Declarations

Consent to publication

All authors have agreed to publish the manuscript.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

No conflict of interest.

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Authors contribution

Sitena Kebede was responsible for writing the proposal, collecting data, conducting all laboratory work, performing data analysis, and writing the manuscript. Gezahegne Mamo provided guidance and edited the manuscript. Belachew Bacha and Belachew Tefera contributed by providing comments and editing the manuscript. All authors have thoroughly reviewed and approved the final manuscript.

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