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Kamal M. A. Abdalmahmoud[1], El Tahir S. Shuiep[2], Ibtisam E. M. El Zubeir[3,4] and Omer H. M. Arabi[5]

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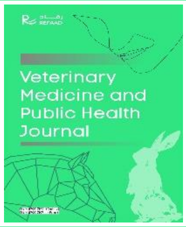

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Kamal M. A. Abdalmahmoud[1], El Tahir S. Shuiep[2], Ibtisam E. M. El Zubeir[3,4], Omer H. M. Arabi[5]

[1] Ministry of Animal Resources and Fishery of Gadarif State, Gadarif, Sudan
[2] University of Gadarif, Faculty of Agricultural and Environmental Sciences, Gadarif, Sudan
[3] Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Khartoum, Sudan
[4] Institute for Studies and Promotion of Animal Exports, University of Khartoum, Khartoum, Sudan
E mail: Ibtisamelzubeir17@gmail.com, Ibtisam.elzubeir@uofk.edu
[5] Department of Basic Science, Faculty of Animal Production, University of Gezira, Sudan
* **Corresponding author:** Ibtisam E. M. El Zubeir (Ibtisamelzubeir17@gmail.com)

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Abstract

Objectives: This study was conducted to analyze milk samples, for chemical composition and detection of aflatoxins M1 in Gedarif town, East Sudan.

Methods: One hundred milk samples were collected randomly; 35 samples from farms, 35 samples from sale points and 30 samples from groceries in Gedarif town. Milk-Kana was used for determination of fat, solids not fat, lactose and protein**,** freezing point, density and added water and Unisensor kits were used as screening method for detection of aflatoxin M1.

Results: The collected milk samples showed lower content of fat (3.4±1.3%, 3.4±1.3% and 3.5±1.25%), solids not fat (7.7±1.1%, 8.1±1.7% and 8±1%), lactose (4.2±0.6%, 4.4±0.8% and 4.3±0.5%) and protein (3±0.4%, 3±0.5% and 3±0.4%). Relatively, the freezing point and density of milk revealed few variations in the examined samples. Moreover, the added water was high in the samples collected from the farms and sale points compared to those from the groceries. The occurrence of aflatoxin M1 was found in 22 (27.50%) of the milk samples. The presence of aflatoxin contamination was high in milk samples collected from sales points (15.0%) followed by farms (11.25%) compared to those obtained from groceries (1.25%).

Conclusions: The presences of aflatoxin in the milk samples might indicate that the cow milk was contaminated with aflatoxins through feed. Hence this study recommended that good hygienic practices should be considered in Gadarif town. Similarly, regulations and lows for selling the milk should be implemented by the official authorities.

Keywords: *Milk; chemical composition; aflatoxins M1.*

1 Introduction

Fresh milk is considered as a complete diet because it contains all the essential nutrients such as lactose, fat, protein, minerals and vitamins in balanced ratio rather than the other foods (Hossain and Dev, 2013). The composition of cows' milk is of the greatest importance for the dairy industry. Since its processability is highly influenced by composition. Knowing the composition of milk also helps to assess adulteration and the quality of the milk for consumers and milk processing industries (Gurmessa Melaku, 2012). On average, bovine milk is composed of 87% water, 4% to 5% lactose, 3% protein, 3% to 4% fat, 0.8% minerals, and 0.1% vitamins (Link mark-Mansson, 2003; Haug et al., 2007; Høstmark et al., 2007).

Aflatoxins are toxic secondary metabolites of moulds; *A. sperigillus flavus* and *A. parasiticus* that are the main mycotoxin, which could be associated with milk (Boudra et al., 2007; Ghorbanian et al., 2008; Ozay et al., 2008; Abbès et al., 2012; Iha et al., 2013; Sartori et al., 2015). Aflatoxins M1 and M2 are major metabolites of AFB1 and AFB2, respectively and are found in the milk of animals that consume feed contaminated with aflatoxins (Zinedine et al., 2007; Asi et al., 2012; Siddappa et al., 2012; Darsanaki et al., 2013; Mohammed et al., 2016). Moreover, Ali et al. (2014) found that the concentration of AFM1 in Khartoum State was affected by the source of concentrated feed but not the farm size. This might be because most of the important milk producing areas in Sudan has no rigid systems of inspection on the farms and most of the products of these farms are sold through venders and groceries (Ahmed and El Zubeir, 2007). Therefore, the incidence of AFM1 contamination in milk from dairy cow must be considered a risk, and raw milk should continually investigated and surveyed with respect to AFM1 contamination worldwide (Ketney et al., 2017).

The incidence of aflatoxin M1 (AFM1) contamination in milk and milk products is a serious health hazard for human worldwide. Thus, the global monitoring of AFM1 in raw milk has been ongoing for decades (Min et al., 2020). Several surveillance and studies showed the occurrence of AFM1 in milk samples from France (Boudra et al., 2007), Portugal (Duarte et al., 2013), Spain (Cano-Sancho et al., 2010), Greece (Malissiova et al., 2013), Italy (Bellio et al., 2016) and Turkey (Unusan, 2006; Kabak and Ozbey, 2012; Sahin et al., 2016). Also, several surveys have been conducted on the occurrence of AFM1 in milk and dairy products was reported in developing countries such as Nigeria (Atanda et al., 2007), Iran (Kamkar et al., 2014), India (Shipra et al., 2004; Sharma et al., 2020), Pakistan (Asghar et al., 2018), Saudi Arabia (Bokhari et al., 2017), Kenya (Kang'ethe and Lang'a, 2009; Kuboka et al., 2019), Tanzania (Mohammed et al., 2016) as well as Sudan (Elzupir and Elhussien, 2010; Ali et al., 2014; Fadlalla et al., 2020; Yousof and El Zubeir, 2020).

Aflatoxins are potent toxic, carcinogenic, mutagenic and immunosuppressive agents produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parsisticus* on a variety of food products (Ozay *et al.,* 2008). The levels of aflatoxins contamination is a cumulative and therefore are associated with acute and chronic toxicities to both humans and animals (Otim et al., 2005; Gavrilova et al., 2014; Patel *et al.*, 2015; El Zubeir, 2023). The higher occurrence of aflatoxin M1 in raw and processed milk samples might have a negative impact on public health, especially infants and children who consumed large amount of fluid milk (Fadlalla *et al.*, 2020; Yousof and El Zubeir, 2020). Because of the harmful effects of the aflatoxin in humans and animals, good agricultural practices and good manufacturing practices thoughout food production chain should be adopted (El Zubeir, 2023). The good agricultural practices needed include appropriate drying techniques, maintaining proper storage facilities and taking care not to expose grains or oil seeds to moisture during transport and marketing (Magan and Aldred, 2007). Hence the aim of the present study is investigating and comparing the chemical composition and occurrence of aflatoxin M1 in milk from farms, sale points and groceries in Gadarif town.

2 Materials and Methods

2.1 Study area

The study was conducted in Gadarif State (Baladia Locality) situated between latitude 12-17° North and longitude 34-36° East, geographically it has a wide variety as well as heavy rains, which range between 600- 900 mm during the year. The livestock is the most important renewable resource in the state and some of the state's population depend on it and comes secondly after agriculture. The state is very rich in livestock, which is estimated to 7% of the total census of livestock in Sudan. The number of livestock in Gadarif State is approximately five million heads of different species, this increase to seven million heads in the rainy season as the result of movement of animals from the traditional pastoral system in the prevailing system. According to the Ministry of Animal Resources and Fishery of

Gadarif State (MARF, 2011), the total number of animals is estimated to be 3.896.134 head. Sheep herds comprise about 48% of the total animal number followed by goats (24%), cattle (24%) and camels are about 4%.

2.2 Source of milk samples

One hundred samples of cows' milk were collected randomly from Gadarif locality from different locations; 35 samples of raw milk were collected from dairy farms; 35 samples of raw milk was collected directly from sellers (sale points) and 30 samples of raw milk were collected from groceries.

2.3 Collection of milk samples

Raw bulk cows' milk samples were collected in the afternoon during May to June 2018. After collection, the milk samples were kept in an ice box till the next morning, and then they were taken to the laboratory of the Faculty of Agricultural and Environmental Sciences for analysis of chemical composition and detection of aflatoxin M1.

2.4 Laboratory examination of milk samples

The raw cows' milk samples were subjected to chemical composition analysis and detection of aflatoxin M1.

2.5 Chemical composition

The chemical composition including fat, solids non-fat, protein, lactose, density, freezing point and added water was performed using Milkana Express Plus Analyzer (Milkana.... 8_10_2 Serial no. 701097 Express plus, Bulgaria), which is based upon ultra-sound technology.

For milk analysis, about 15 ml sample were taken to and every milk sample was first brough to a temperature of 30-35 °C before testing. The milk was then poured into a cylinder and placed under the measuring rod of Milkana and the values for milk contents are automatically displayed in the digital screen in about 45 seconds.

2.6 Test for aflatoxin M1 detection

The Aflasensor kit 041 and its associated accessories (Heatsensor, negative and positive standards, deionized water) that were used in the present study were produced by the Unisensor Company (Liege, Belgium). It is a rapid (10 minutes at 40°C) test that use for the quantification of aflatoxin M1 (AFM1) in raw milk samples. The limit of quantification (LOQ) of the aflsensor is 20 ppt with a range of quantification up to 150 ppt. Aflsensor test requires the use of microwells that containing a predetermined amount of antibody lined to gold particles and a dipstick made up of a set of membranes with specific capture.

The lines for a valid test include the upper red (control line) that should be visible after second incubation time (7 minutes). The test was done by suspending 200 µ1 of milk sample with the reagent from microwell. A specific antibodies will bind the analyses; if present; during the first incubation time (3 minutes). When the dipstick is dipped into the sample, the liquid starts running vertically on the dipstick and passes through capture zones.

The development of a color at the test line indicates that the sample is free of aflatoxin M1. On the opposite, the presence of aflatoxin M1 in the sample will not cause the appearance of the colored signal at the test capture line. The concentration of aflatoxin M1 present in the milk sample is based on the intensity of the line color that should be started from the bottom line of aflatoxin M1. The results were directly interpreted by visual observation. When the test line was darker in color like the control line, the result was negative, which means that at the given sensitivity of the test, the milk samples contain no aflatoxin M1 or aflatoxin M1 at a lower level than the value stated in the enclosed aflatoxin M1 limit of detection. When the line was as the same intensity or lighter in color than the control line, the result is considered positive (+) and the sample should contain higher concentration than 100 ppt. When there was no test line at all, the milk sample should contain higher concentrations of aflatoxin M1 residues and considered as full positive (++) as was described by UNISENSOR (2013).

2.7 Statistical analysis

The data of milk composition was subjected to statistical analysis using SPSS program (SPSS, 2008). The analysis has carried out after obtaining results using ANOVA table of analysis for the presentation of the results.

3 Results

3.1 Chemical composition

A total of hundred milk samples were collected from three sources; namely farms (35), sale points (35) and groceries (30) in Gadarif town. The means and standard deviations of the fat content for the milk samples collected from farms, sale points and groceries revealed 3.4±1.3%, 3.4±1.3% and 3.5±1.2%, respectively (Table 1). However, the minimum and maximum values results were 1.12 and 6.45%, 1.03 and 6.90% and 1.44 and 6.59%, respectively as shown in Table 1. The means for total solids content of milk samples were found as

7.7±1.1%, 8.1±1.7% and 8±1%, respectively. The minimum and maximum values revealed 5.4 and 10.7%, 5.97 and 15.5% and 6.05 and 10.5%, respectively (Table 1).

Table 1: Chemical composition of raw milk samples for farms, sale points and groceries in Gadarif town

Source of samples	N	Fat (%)			Solids not fat (%)			Lactose (%)			Protein (%)		
		M± Sd	Min	Max	M± Sd	Min	Max	M± Sd	Min	Max	M± Sd	Min	Max
Farms	35	3.4± 1.3	1.12	6.45	7.7± 1.1	5.4	10.7	4.2± 0.6	2.81	5.84	3± 0.4	1.97	4.05
Sale points	35	3.4± 1.3	1.03	6.90	8.1± 1.7	5.97	15.5	4.4± 0.8	3.29	6.73	3± 0.5	2.26	4.68
Groceries	30	3.5± 1.25	1.44	6.59	8±1	6.05	10.5	4.3± 0.5	3.33	5.72	3± 0.4	2.29	3.97
Total	**100**	**3.4± 1.3**	**1.03**	**6.90**	**7.9± 1.3**	**5.4**	**15.5**	**4.3± 0.7**	**2.81**	**6.73**	**3± 0.4**	**1.97**	**4.68**

N= number of samples M= Mean
Sd= Standard deviation Min= Minimum Max= Maximum

Milk lactose showed 4.2±0.6%, 4.4±0.8% and 4.3±0.5% for means and standard deviations, while the minimum and maximum values were 2.81 and 5.84%, 3.29 and 6.73% and 3.33 and 5.72%, for the milk samples collected from farms, sale points and groceries, respectively (Table 1). The means for protein content of milk were 3±0.4%, 3.5% and 3±0.4% and the ranges were 1.97-4.05%, 2.86-4.68% and 2.29-3.97%, respectively (Table 1).

The freezing point for milk samples collected from farms, sale points and groceries in Gadarif town was found to range from -.666 to -.328° C (-.503±.079 °C), -.726 to -.373 °C (-.524±.007 °C) and (-.654 to -.383 °C (-.522±.099 °C), respectively (Table 2).

Table 2: Physiochemical composition of raw milk samples for farms, sale points and groceries in Gadarif town

Source of samples	N	Freezing point (° C)			Density (g/cm^3)			Added water (%)		
		M± Sd	Min	Max	M± Sd	Min	Max	M± Sd	Min	Max
Farms	35	-.503± .079	-. 666	- .328	0.025± 0.003	0.016	0.034	10.6± 11.6	0.0	41.1
Sale points	35	-.524± .007	- .726	- .373	0.026± 0.005	0.003	0.040	10.6± 10.4	0.0	32.9
Groceries	30	-.522± .099	- .654	- .383	0.026± 0.003	0.020	0.034	8.7± 8.2	0.0	31.1
Total	**10**	**-.516± .088**	**- .726**	**- .328**	**0.025± 0.004**	**0.003**	**0.040**	**10.3± 10.3**	**0.0**	**41.1**

M= N= number of samples M= Mean
Sd= Standard deviation Min= Minimum Max= Maximum

The density showed ranges of 0.016 to 0.034 g/cm^3 (0.025±0.003 g/cm^3), 0.003 to 0.040 g/cm^3 (0.026±0.05 g/cm^3) and 0.020 to 0.034 g/cm^3 (0.026±0.003 g/cm^3), respectively (Table 2). Table 2 also showed that the mean and maximum values of added water in the milk samples collected from farms, sale points and groceries in Gadarif town were 10.6±11.6% and 41.1%, 10.6±10.4% and 32.9% and 8.7±8.2 and 31.1%, respectively. However, some milk samples showed no detection of the added water (Table 2).

3.2 Aflatoxin M1 detection

The results of positive detection of aflatoxin in milk samples collected from farms, sale points and groceries in Gadarif town showed that about 27.50% of the milk samples were contaminated with aflatoxin M1 (Table 3). The highest occurrence of aflatoxin was found in the sale points (15.0%) followed by those obtained from the farms (11.25%) compared to those obtained from groceries (1.25%) as shown in (Table 3).

Table 3: Occurrence of aflatoxin in raw milk samples collected form farms, sale points and groceries in Gadarif town

Source of samples	Positive (20-150 PPt)		Negative (>20 PPt)		Total	
	Number	Percent	Number	Percent	Number	Percent
Farms	9	11.25	24	30	33	41.25
Sale points	12	15.00	19	23.75	31	38.75
Groceries	1	1.25	15	18.75	16	20
Total	22	27.50	58	72.50	80	100

The milk samples showing strong positive (level 1) contamination of aflatoxins were 6.25% and they belong to the samples obtained from the farms. However, the positive milk samples contaminated with aflatoxins from level 2 were 21.25%, of which 5.0% from the farms, 15.0% from sale points and 1.25% from groceries (Table 4).

Table 4: The levels of aflatoxin M1 in raw milk samples collected form farms, sale points and groceries in Gadarif town

Source of samples	Strong positive (++)		Positive (+)		Total	
	N	Percent	N	Percent	N	Percent
Farms	5	6.25	4	5.00	9	11.25
Sale points	0	0.0	12	15.00	12	15.00
Groceries	0	0.0	1	1.25	1	1.25
Total	**5**	**6.25**	**17**	**21.25**	**22**	**27.50**

Strong positive (100-150 PPt) Positive (20-100 PPt) Negative (>20 PPt)

4 Discussion

This study showed that there was no significant (P>0.05) differences in the results of fat, total solids, lactose and protein content of cows' milk samples collected from the three sources in Gadarif town. This generally indicates the similarity of the milking animals and their management. The chemical composition of milk can be influenced by several factors such as animal species and genetics, environmental conditions, lactation stage, and animal nutritional status (Caroli et al., 2010).

This study revealed that fat content of raw milk samples collected from groceries was 3.5±1.2% (Table 1), which was similar the findings reported by Mohamed and El Zubeir (2007) who found that the means fat content of milk in Omdurman and Khartoum North were 3.75±1.07 and 3.46±1.17%, respectively. However, Ahmed andEl Zubeir (2007) reported higher values; 4.54±0.59% and 4.50±0.47%; for milk samples collected from farms in Khartoum State during winter and summer, respectively. The maximum values reported during this study supported Bashir and El Zubeir (2013) who reported 5.08±1.05% fat content for milk of Baggara cattle (5.08±1.05%) in South Kordofan State. Also, Elsheikh et al. (2015) found that milk samples collected from Khartoum North and Omdurman were 4.72±0.67% and 5.02±0.60%, respectively. Moreover, Warsama et al. (2017) showed significantly (P≤0.001) higher fat (5.03%) content of cows' milk samples obtained during winter in Khartoum State. The reported variations could be attributed to difference in cows' breeds, location and feeding strategy. Similarly, Shuiep et al. (2016) reported that variations between milk fat content could be due to different management, feeding regimes, production systems and breed of cattle. They indicated that the local cows are significantly (P<0.05) capable to produce higher milk fat throughout their lactations. On the other hand, Mirzadeh et al. (2010) found that fat content was 3.90±0.97% in Iran, while, Eckles and Combs (2004) reported that the average percent of fat in milk was 3.8% in India and concluded that the milk fat is the most valuable constituent of milk and should be considered as the food value of the milk. Also, Pavel1 and Gavan (2011) reported that nutrition, climatic conditions and regional differences can be regarded as important sources of variation in the composition of milk.

The solids not fat of milk samples collected from farms (7.7±1.1%) showed lower values than those obtained from sale points (8.1±1.7%) and groceries (8±1%) as shown in Table 1. Similarly, Pavel1 and Gavan (2011) reported 8.70% for SNF content of milk during summer period in lactating dairy cows. Mirzadeh et al. (2010) in Iran reported that the average SNF content in raw milk produced by dairy cow at different lactations was 8.67±0.69%. However, higher values were reported by Czerniewicz et al. (2006); Bille et al. (2009); Landi et al. (2011). Moreover, Bashir and El Zubeir (2013) reported 9.19±0.78% for SNF content of milk of Baggara cattle in Sudan. The solids not fat of milk samples collected from different sources (11.52%) in Khartoum State also revealed non significant differences (Warsama et al., 2017). The SNF content of milk from local cows and crossbred cows was found to be influenced significantly (P≤0.05) by stage of lactation and parity order (Shuiep et al., 2016). They concluded that the variation of breed, feeding and management could be the reasons. The SNF content of the milk (8.09 to 9.03%) generally follow the variation of the fat content, the higher the fat content the higher was the SNF but lower the density (Bille et al., 2009). In Ethiopia, Gemechu et al. (2015) found that the total solids were 12.87%.

The average protein content of milk samples (3±0.4%) collected from the three sources in Gadarif town (Table 1) was comparable to that reported by Ahmed and El Zubeir (2007) who found the mean value of protein content was 3.73±0.587% in dairy farms located in Khartoum State. Also, Elsheikh et al. (2015) found protein content was 3.58±0.38% for milk samples collected from Khartoum and 3.57±017% for the samples obtained from Khartoum North. The result of protein content of milk samples were also in line to those (3.5±0.9%) obtained by Warsama et al. (2017) in Khartoum State and those collected from Baggara cattle (3.62± 0.31%) in South Kordofan State (Bashir and El Zubeir 2013). Similarly, Rafig et al. (2016) found 3.57% in milk collected from Faisalabad. However, Mohamed and El Zubeir (2007) found that the mean value of protein content in milk collected from Khartoum North (3.08±0.59%) was higher than that obtained from Omdurman (2.93±0.47%).

The study showed the lactose content of milk samples collected from sale points were (Table 1) was in accord to the findings reported by Ahmed and El Zubeir (2007) for lactose content of milk samples collected during summer and winter in Khartoum State, which were 3.95±0.561% and 4.06± 0.618%, respectively. Also, Elsheikh et al. (2015) found that the mean of lactose content in milk samples from Omdurman was 4.72±0.4% and those obtained from Khartoum showed a mean of 4.86±0.24%. Similarly, the mean of milk lactose from Baggara cattle in South Kordofan State, Sudan was 4.89±0.33% (Bashir and El Zubeir, 2013). Nateghi et al. (2014) found that the lactose content of milk during summer was 4.61%. The lactose of the milk samples collected from different sources and during different seasons showed high significant differences (Warsama et al., 2017). The lactose content was found in a range of 5.21 to 5.15% and 5.33 to 5.02%, in local and crossbred cows, respectively (Shuiep et al., 2016). This might be due to the fact that the lactose content of milk is affected by the different locations and feedstuff that animals utilized (Kittivachra et al., 2007). On the other hand, Eckles and Combs (2004) reported that lactose has an important relation to the manufacture. However, there is some evidence that lactose is the least cariogenic of the common dietary sugar. In addition, various other components of milk have been considered to be protective against dental caries (Bánóczy et al., 2009).

There was no significant difference between the results of milk sources in Gadarif town as this study revealed that the freezing point in sale points were -0.524±-0.007° C (Table 2). Similarly, Ahmed and El Zubeir (2007) reported that the freezing point of raw milk were -0.519±-0.0251° C and -0.533±-0.013° C in the samples collected during summer and winter, respectively and the average was -0.535±0.033° C. Marshall (1992) stated that a freezing point of -0.517° C is considered normal for milk and milk that freezes at or below this value is presumed to be free of added water.

In the present study, the mean density was 0.026±0.005 g/cm^3 (Table 2), which was lower than those reported by Abd Elrahman et al. (2009); Bashir and El Zubeir (2013); Elsheikh et al. (2015); Warsama et al. (2017) who found the average density of milk was about 1.031 g/cm^3. This indicated addition of water or subtraction of fat as was shown in Table 1 that lower values were found for fat, lactose and solids not fat. Similarly, El Zubeir et al. (2008) reported lower levels for the chemical content (fat, protein, lactose, SNF, and total solids) of the pasteurized milk compared to the raw milk samples obtained from different milk producing companies in Western Cape, South Africa.

The added water in the milk samples collected from the farms was estimated as 10.6±11.6% (Table 2). When comparing the present results, it was observed that the level of the added water was relatively high. This may be due to adulteration by adding water to milk in Gadaref town. Elsheikh et al. (2015) reported the adulteration by water in some of the milk collected from Omdurman and Khartoum towns. Also, the percentage of the added water was very high in the processed milk compared to the samples from herd raw bulk milk in South Africa (El Zubeir et al., 2008). Tasci (2011) stated that addition of water and ice affected the physical and chemical quality of milk by adulterant proportion different constituents of milk in Western Cape, South Africa. Similarly, Faraz et al. (2013) reported 97 and 93% of the milk samples in canteens of educational institutes and public places of Faisalabad had added water.

The raw milk samples tested during this study (Table 3) showed that 22 (27.5%) of the samples were found positive for aflatoxin M1 with the highest occurrence in the samples obtained from sale points and farms (15% and 11.5%, respectively). Moreover, all strong positive (level 1; 0.05 to 0.1) contaminated samples were obtained from the farms (22.72%). Aflatoxin M_1 (AFM1) is a hydroxylated metabolite of aflatoxin B1 (Zinedine et al., 2007). Hence the high level of contamination in raw milk samples from the farms might be due to the contamination of dairy cow rations with aflatoxins B1 (Elteib et al., 2012; Ali et al., 2014). Higher occurrence were also reported in India, the range of contamination with AFM1 was 28- 164 µg/l and that 99% of the contaminated milk samples exceeded the European Communities recommended limit (Shipra et al., 2004). Also, Kang'ethe and Lang'a (2009) detected 99% of milk samples were contaminated with aflatoxin in Kenya. Bokhari et al. (2017) in KSA, tested 160 milk samples and found that 74.47% of milk samples were contaminated with aflatoxin. Ali et al. (2014) concluded that the levels of AFM1 in the raw milk samples indicated that the feeds offered to the cows were contaminated with aflatoxin B1 in such a level that might cause a serious public problem. Aflatoxins are absorbed in the gastrointestinal tract but not been biotransformed in the liver can also be excreted (Scaglioni et al., 2014). Thus aflatoxin can accumulate through the food chain posing a serious health concern to both humans and animals (Otim et al., 2005; Gavrilova et al., 2014; Patel et al., 2015). However, according to the survey conducted throughout North western Italy between 2012 and 2014, the overall AFM contamination rate was 2.2% (36 samples out of 1668 samples) and less than 1% of milk samples were non-compliant with EU limits (Bellio et al., 2016). Furthermore, the positive samples with level 2 (0.1 to 0.15) were detected in the samples collected from sale points (15.0%) compared to those collected from farms (5.0%) and groceries (1.25%) as shown in Table 4. This might indicate that the higher concentration of the contaminated milk from the farms was dilated to a lower level in the sale points, because in the sale points and groceries they bulked the milk from different farms. Similarly, Sharma et al. (2020) was able to detect AFM1 contamination in the milk sold by local traders (14/50) and vendors (16/50) in India. This study suggested the presence of aflatoxin in animal feed, which supported that of Omer et al. (2004); Elteib et al. (2013) who showed the presence of aflatoxin content in groundnut seeds and cakes, respectively. Aflatoxins are generally classified into B1, B2, G1 and G2, which metabolized to aflatoxins M1 and M2 (Boudra et al., 2007). Aflatoxins B1 is a potent mutagenic and carcinogenic agent found in numerous agricultural and dairy products consumed by humans (Madrigal-Santillan et al., 2007). Moreover, aflatoxins are highly carcinogenic and mutagenic in nature (Ehrlich et al., 2003; Williams et al., 2004; Ozay et al., 2008). Aflatoxins contaminated corn and cotton seed meal in dairy rations has resulted in aflatoxins M1 contaminated milk and milk products (Van Eijkeren et al., 2006; Zinedine et al., 2007). Hence, regular monitoring of AFM1 is necessary for evaluating their contamination and improvement status. Simultaneously, more precautions could be implemented on hygiene controls in order to limit AFM1 contamination in dairy products (Min et al., 2020).

Furthermore. in Khartoum State, higher prevalence was found compared to the present study and the percentage of AFM1 contamination has been found in 42/44 (95.45%) samples with contamination level ranging between 0.22 and 6.90 lg L_1 and average concentration of 2.07 lg L_1 (Elzupir and Elhussien, 2010). Also, Ali et al. (2014) showed that the average concentration for AFM1 in raw milk samples ranged between 0.1 and 2.52 ppb with 100% exceeding the limits of European countries. The presence of AFM1 was detected in a concentration that ranged between 20150- ppt and that 88.7% of the processed milk samples were found to be contaminated with aflatoxin M1 compared to 92% of the raw milk samples (Fadlalla et al., 2020). The average concentration for AFM1 in Nigeria revealed 2.04 µg/k (Atanda et al., 2007), although Nigeria set a limit of 1.0 µg kg-1 (Iqbal et al., 2015). However, the European communities and Codex Alimentarius recommend limits of 0.05µg/kg and 0.5µg/kg, respectively. Kemboi et al. (2020) stated that despite their regulation being stricter, the EU is a major destination of trade for most African countries, and hence the EU regulatory and guidance values are used for comparison since they may negatively impact trade and in addition they cover a wide variety of feeds for different species. In China, the situation of AFM1 contamination in milk proved the improvement of surveillance (Zheng et al., 2017; Xiong et al., 2018).

The variations in AFM1 levels among milk samples from farms and distributors (Table 3 and 4) could be attributed to forage and feed quality, cow's diet, genetic variation in dairy cows, and geographical and seasonal variations (Mohammed et al., 2016; Sahin et al., 2016). On the other hand, the mean concentration of AFM1 in milk samples collected in summer ((96.3%) was significantly ($P<0.05$) higher than that obtained in winter ((89.0%) in Karachi, Pakistan (Asghar et al., 2018). They added that seasonal variations tend to increase the growth rate of fungi and AFs contamination, ultimately resulting in higher AFM1 contamination in summer when compared to winter. Based on 171 different milk samples, the results showed that all age's categories, especially children were exposed with high risk related to presence of AFM1 in milk in Serbia (Kos, 2014). However, Ahmad et al. (2019) found that the dietary exposure data of AFM1 among six different groups was indicated that the male children population was the most vulnerable group to AFM1, up to 6.68 ng L^{-1} per day and the least affected one was the female group above 20 years of age with 1.13 ng L^{-1} per day. The economic impact of aflatoxins leads directly to crop and livestock losses as well as indirectly to costs of regulatory programmers designed to reduce risks to animal and human health (Martins et al., 2007).

5 Conclusion

The results of the chemical composition of cow's milk showed lower content of fat, solids not fat, protein and density. Moreover, the added water was high. It was also observed that the highest percentages of most of the investigated compositional content were occurred in the sale points. On the other hand, the presence of aflatoxin in raw cow's milk samples need further monitoring and control, although the present frequencies are low compared with the previous studies. The presences of aflatoxin in milk samples might indicate that the cow milk was contaminated with aflatoxin through feed because all samples showing strong positive were detected in the milk samples collected from the farms. The present study recommended that good practices should be adopted for dairy cows' feeding, and the sale points of milk should be improved. Also, education and awareness should be conducted especially among farmers and livestock producers on the health hazards of aflatoxins. Moreover, strict lows and legislations should be implemented for the milk producers in order to minimize occurrence of aflatoxins and to ensure the quality of milk and dairy products in country.

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