PRINT: ISSN 0970-9274 ONLINE: ISSN 2456-6608
J Hum Ecol, 56(1,2): 183-187 (2016)
DOI: 10.31901/24566608.2016/56.1-2.23

Determination and Quantification of Aflatoxin M_1 in Fresh Milk Samples Obtained in Goats and Cattle in Selected Rural Areas of the Limpopo Province, South Africa

Mwanza Mulunda

Department of Animal Health, Faculty of Agriculture, science and Technology, Mafikeng Campus, North West University, Private Bag X2046 Mmabatho 2735, South Africa
E-mail: 24059676@nwu.ac.za

KEYWORDS Carcinogenicity. ELISA. Subsistence Farming. Commercial. Goat. Cow

ABSTRACT In this study, the Enzyme Linked Immuno-sorbent Assay (ELISA) was used to detect aflatoxin M1 (AFM_1) detection in 118 milk samples from subsistence farms in selected rural areas in the Limpopo Province, South Africa. This was designed to evaluate the possible health risks on individuals who are exposed to this mycotoxin either through contact or consumption of milk that is contaminated. A further objective was to quantify the levels of AFM_1 using the RIDASCREEN® kit and to assess the effect of climatic conditions on AFM_1 contamination in milk from two areas—Nwanedi, which is relatively dry and hot compared to Mapate, which is a mountainous, hot and humid area. Results from this study showed that all the samples (100%) from cattle and goats in both Nwanedi and Mapate were contaminated with AFM_1. In addition, it was noted that 90.6 percent and 62.1 percent of the milk samples from cattle and seventy-six percent and 53.8 percent of those obtained from goat's milk in Mapate and Nwanedi respectively, had AFM_1 concentrations $\geq$ 0.05 µg/l. These results show that animal nutrition did seriously influence the quality of milk in regard to AFM_1 contamination. It was noted that animals that were poorly fed had highly contaminated milk as compared to other animals. In addition, climatic conditions did influence the quality of milk collected in both areas. Chronic exposure of the population and particularly children to this contaminated milk, would have negative impacts on their health.

INTRODUCTION

Aflatoxin contamination in animal feed and human food remains a major concern because it has carcinogenic properties and is found worldwide, especially in warmer and humid climatic regions (Whitlow et al. 2010). The most common toxins that are frequently detected include aflatoxins B_1 and B_2, which contaminate mainly cereals and aflatoxin M_1 (AFM_1), which is a hydroxylated metabolite of AFB_1 and is excreted in milk from cow that fed on diet, which is naturally contaminated with aflatoxin B1 (AFB1) (Sorensen and Elbaek 2005). However, reference is frequently made to aflatoxin B_1, which is the most prevalent and presents to be in the most toxic form (Whitlow et al. 2010).

Aflatoxins are known to be mainly produced in food and feed materials by *Aspergillus flavus* and *A. parasiticus*, and at low levels by *A. tamarii* and *A. nomius*, as well as other emerging fungal spp. including *A. ochraceoroseus, A. rambellii, Emericella astellata* and *E. venezuelensis* (Klich 2002; Vargas et al. 2002). *Aspergillus flavus* and *A. parasiticus* (AF producers) mainly contaminate cereals (maize) and nuts (peanuts) and their by-products including animal feeds (Pitt and Hocking 1997; Klich 2007; Mwanza 2007). AFM_1 was initially classified by the International Agency for Research on Cancer (IARC) as a group 2B agent that is carcinogenic to humans (IARC 1993) due to lack of data. However, following further investigations that demonstrated *in vivo* the genotoxicity and cytotoxicity of AFM1 (Caloni et al. 2006), the toxin has since been classified as a group 1 human carcinogen (IARC 2002).

The importance of AFM1 can be evaluated after considering the quantity of milk and milk products consumed daily. Moreover, they are of primary importance in infants' diet around the world (EC 2002). In South Africa, the agricultural sector is divided in two and these include the commercial farming that mainly constitutes rich farmers who are most often supported by the government. The rest is small-scale farming wherein ninety-five percent of the rural Black population is involved. There is no clear boundary between these two types of farming and activities. For example, farmhouse cheese making is not strictly a formally regulated activity. The basis of the test was the antigen-antibody reac-

tion. The wells in the microtiter strips were coated with specific antibodies to AFM_1 and after a washing step, the enzyme conjugate is added. Free AFM_1 and AFM_1 enzyme conjugate competed for the AFM_1 antibody binding sites (competitive enzyme immunoassay). Any unbound enzyme conjugate is then removed in a washing step. Substrate or chromogen was added to the wells and incubated. Bound enzymeconjugate converts to colorless chromogen into a blue product. The additions of the stop solution lead to a color change from blue to yellow. The measurement was made photometrically at 450 nm and the absorption is inversely proportional to AFM_1 concentration in the sample.

The aims and objectives of this study were to evaluate the level of AFM_1 contamination in milk obtained from cows and goats consumed in selected rural areas of the Limpopo Province and to evaluate their possible health risks in regards to current legislations.

METHODOLOGY

Quantitative analysis was performed to detect AFM_1 using a competitive ELISA test kit (RIDASCREEN® Aflatoxin M1 30/15) obtained from R-Biopharm AG, Darmstadt, Germany. A total of 118 fresh milk samples that comprised 55 from Mapate (30 and 25 samples from cattle and goats, respectively) and 63 from Nwanedi (37 from and 26 samples from goats, respectively) were collected directly from animals and placed in sterile containers. The samples were stored in a deep freezer until further analysis. Aflatoxin M_1 analysis was performed according the manufacturer's instruction.

The percentage absorbance was calculated using the formula:

$$\frac{\text{Absorbance of standard (or sample) x100}}{\text{Absorbance of zero standard}}$$

The zero standards were co-samples concentrations were calculated using the calibration curve (Fig. 1) equal to one hundred percent and the absorbance values were quoted in percentages of the aflatoxin M_1 concentration of controls obtained from controls concentrations and absorbance.

Data obtained from the geographic regions was analyzed and compared by a t-test using Sigma Stat 3.10. Mean values were deemed to be significantly different if the level of probability was ≤ 0.05. The graphs were plotted using Sigma Plot 10.0.

RESULTS

Results from this study showed the incidence of one hundred percent in all samples analyzed for being contaminated with AFM_1 for both cat-

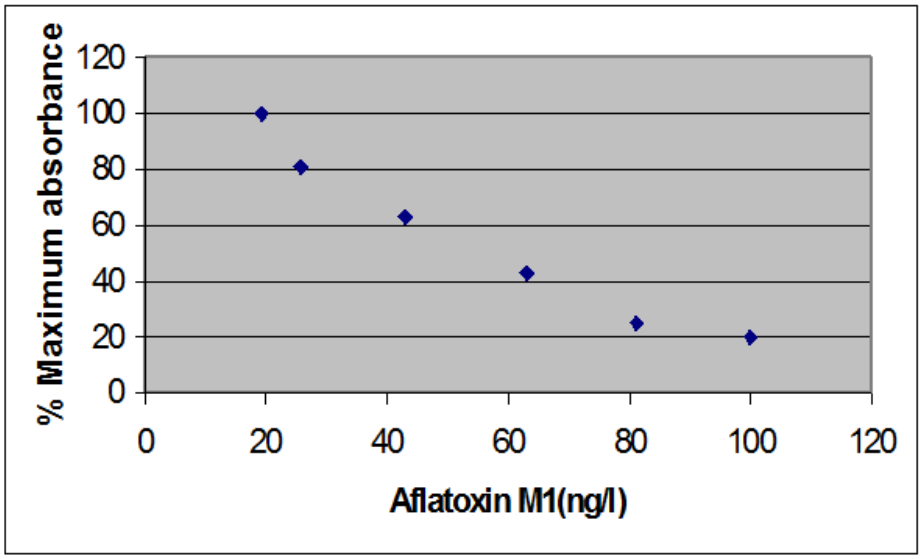

Fig. 1. Calibration Curve for the determination of aflatoxin M_1 in milk samples

tle and goats from both Nwanedi and Mapate areas (Table 1). Mean concentrations of AFM_1 were higher in milk obtained from cattle with 0.092 and 0.073 µg/l and for goat's milk 0.064 and 0.061 µg/l respectively, from Mapate and Nwanedi areas (Table 1). In addition, it was noted that 90.6 and 62.1 percent for cattle and seventy-six and 53.8 percent for goat's milk obtained respectively from Mapate and Nwanedi had AFM_1 concentrations $\geq$ 0.05 µg/l. Significant differences ($P \leq 0.05$) were observed between cattle and goat's milk concentrations within and between both sampled areas (Table 2).

DISCUSSION

The main aim of this study was to determine the level of contamination with AFM_1 in milk samples obtained from cattle and goats in two rural communities. Aflatoxin M_1 (AFM_1) was detected in milk samples from both sample areas (Nwanedi and Mapate). It is therefore suggested that presence of this toxin (AFM_1) in the samples from these areas might result from the fact that the animals may have been exposed to feed contaminated with *Aspergillus flavus* and *A. parasiticus* (Mwanza 2007, 2012). *Aspergillus* contamination is regarded as a storage problem (Pittet 1998) and may also contaminate plants on the field (Pitt and Hocking 1997). This is more common during drought stress and low soil moisture content (Klich 2002). *Aspergillus parasiticus* and *A. flavus* are known to be producers of aflatoxins B_1, B_2, G_1 and G_2 (Pitt And Hocking 1997, Egbuta et al. 2015). Figure 1 illustrates the standard curve of AFM_1 standards using the competitive ELISA, in which a concentration dependent decrease in percent maximum absorbance at 450 nm was observed. These results show a sensitivity of the ELISA immunoaffinity method to AFM_1 (Table 1) with detection of AFM_1 in all milk samples tested. Results obtained in this study are in accordance with those of a previous report in which the toxin was detected in milk from dairy cows in a rural community (Sassahara et al. 2005). Results obtained in this study are similar to those reported by Rastogi et al. (2004), Torkar and Vengus (2008) Dashti et al. (2009), Nuryono et al. (2009), Fallah (2010) Iha et al. (2011), Buldu et al. (2011) and Mwanza et al. (2015), who used the same method and found that eighty-seven, ten, 56.9, 57.5, 67.1, eighty-four and sixty-three percent respectively, of positive to AFM_1 contaminated samples among investigated raw milk. Similar results have been also reported in raw dairy milk in Bakirci (2001), Sarmelnetoglu et al. (2004) and Siddappa et al. (2012). The difference of results obtained from these two areas might be explained by the weather difference between these two regions characterized as hot, humid with high rainfalls area for Mapate area whereas the weather in Nwanedi is hot but dry with low rainfalls (Mwanza 2007). These results are also confirmed by Cano-Sancho et al. (2010) and Mwanza et al. (2014) who in a study done on milk samples col-

Table 1: Incidence and ranges of aflatoxin M1 contamination in raw milk using the ELISA Methods

Area of collection	*Animal species*	*Number of samples (n)*	*Incidence (%)*	*Range (µg/l)(Mean concentrations)*	*(%) samples ≥ 0.05 µg/l*
Mapate	Cattle	30	100	0.02-0.15(0.092)	23 (90.6)
	Goats	25	100	0.02-0.10(0.064)	19 (76.5)
Nwanedi	Cattle	37	100	0.03-0.11(0.073)	23 (62.1)
	Goats	26	100	0.02-0.09(0.061)	14 (53.8)

Detection limit 0.2 µg/l

Table 2: Summary of aflatoxin M_1 contamination in raw milk samples based on the ELISA assay

Area of collection samples	*Specie tested*	*Sample (n)*	*Level of positive (%)*	*Distribution of samples (n)*		
				<0.05	*0.05-0.1*	*>0.1 (µg/l)*
Mapate	Cattle	30	100	7 (23.3%)	20 (66.7%)	3 (10.0%)
	Goats	25	100	6 (24.0%)	19 (76.0%)	0
Nwanedi	Cattle	37	100	14 (37.8%)	12 (32.4%)	11(29.7%)
	Goats	26	100	12 (46.2%)	14 (54%)	0

lected in the North West Province showed a very low concentration of AFM1 (< 0.05 µg/l) and the reason was more due to climatic conditions characterized by hot and dry with as consequence low fungal contamination. This situation affects mostly feed quality in Mapate, because the high humidity often leads to crop contamination by *Aspergillus flavus, A. graminearum* and *A. Parasiticus*, which were found to produce AFB_1 and AFB_2. Aflatoxin B_1 subsequently metabolized into AFM_1 (Klich 2007; Egbuta et al. 2015a). In addition, the presence of AFM_1 in milk samples might be explained by the consumption by animals of feed naturally contaminated with aflatoxin B_1 mycotoxins, as mentioned above but also the attitude of farmers who feed poor quality feed to animals (Mwanza 2007). Animal consumption of non-treated feed may lead to higher toxicity than the feeding of diets containing equivalent amount of purified mycotoxins (Whitlow et al. 2010). The natural contamination of the feed is probably due to the late harvesting or improper storage of the crops used for preparing of animal feed as was observed in the study.

The higher contamination levels of AFM_1 in cattle samples as compared to goat samples might be explained by the amount of feed consumed by cattle in comparison to goats. In addition, the mean values of AFM_1 obtained in this study were above the levels of 0.05 µg/l for aflatoxin M_1 as regulated by the European Union and South Africa (Price et al. 1993; Mwanza et al. 2013). So far, no official legislation for AFM_1 has been established in South Africa. Regardless of there being a government regulation number R313 dated 16th February 1990, which gives some limitations at 10 µg/kg upper limits for aflatoxins, but with lower limit of 5 µg/kg for AFB_1 in foodstuffs (Mwanza et al. 2013). This high contamination level of milk with AFM_1 is a risk for rural populations in Limpopo province and South Africa regarding to chronic diseases such as liver cancer and kidney failure and primarily in kids. It is known that aflatoxin B_1 is the most important contaminant of crops, and AFM_1 is a biotransformation product of AFB_1 (Stoloff 1971; Mwanza et al. 2013) and the results obtained in this study indicate high levels than the prescribed limit. It is important to mention that studies conducted have shown that the amount of AFM_1 excreted in cow milk varies from one to three percent of the amount of AFB_1 ingested (Sassahara et al. 2005; Mwanza et al. 2013). This allows estimating from the AFM_1 results obtained in this study the possible amount of aflatoxin B_1 to which animals were exposed.

The statistical evaluations showed that there were significant differences ($p<0.05$) between the means concentrations of AFM_1 in milk samples tested with the ELISA methods. No statistical differences were found between samples collected in Mapate and Nwanedi or between samples from cattle and samples from goats ($p<0.05$).

CONCLUSION

This study reveals the level of aflatoxins exposure of rural populations and animals and suggests a need to introduce safety measures in rural areas in terms of the harvesting period and proper storage conditions. The study shows that highest incidences of Aflatoxins M_1 were observed in cattle milk samples from Mapate as compared to Nwanedi because of high humidity, which favor fungi growth. The presence of fumonisin B_1 in milk samples reveals that either animals are exposed to high concentrations of contaminated feed or they are exposed to contaminated feed for long periods. This is the case in rural areas of the Limpopo Province. It has been noted that rural populations consume this milk daily and this is hazardous in case of consumption of more than 5 µg/l of aflatoxin M_1 with consequences of nephritic lesions and kidney failure especially in children or hepatotoxicosis among the adult population. Also, the presence of fumonisin B_1 in milk samples, which is unusual, is an indicator of either long period of exposure to this mycotoxin or high levels of feed contamination.

ACKNOWLEDGEMENTS

The researchers would like to thank the National Research Foundation (NRF) for funding this project.

REFERENCES

Bakirci I 2001. A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. *Food Conrol,* 12: 47-51.

Buldu HM, Koc AN, Uraz G 2011. Aflatoxin M1 contamination in cow's milk in Kayseri (Central Turkey). *Turkish Journal of Veterinaires and Animal Science,* 35: 87-91.

Caloni, F. Stammati, A, Friggé G De Angelis I 2006. Aflatoxin M1 absorption and cytotoxicity on human intestinal in vitro model. *Toxicon,* 47: 409-415.

Cano-Sancho G, Marin S, Ramos AJ, Peris-Vicente J, Sanchis V 2010. Occurrence of aflatoxin M1 and exposure assessment in Catalonia, Spain. *Revista Iberoamericana de Micologíia,* 27: 130-135.

Dashti B, AL-Hamli S, Alomirah H, AL-Zenki S, Abbas AB, Sawaya W 2009. Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. *Food Control*, 20: 686-690.

Dutton MF, de Kock S, Khilosia LD, Mwanza M 2012. Mycotoxins in South African foods: A case study on aflatoxin M1 in milk. *Mycotoxin Research,* 28: 17-23.

Egbuta MA, Mwanza M, Dutton MF 2015a. Evaluation of five major mycotoxins co-contaminating two cereal grains from Nigeria*. International Journal of Biochemistry Research and Review,* 6: 160-169.

European Commission 2002. Commission Directive 2002/27/EC of 13 March 2002 amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs. *Official Journal European of the Commission*, 75: 44-45.

Fallah AA 2010. Assessment of aflatoxin M1 contamination in pasteurized milk and UHT milk marketed in central part of Iran. *Food Chem and Toxicol*, 48: 88-991.

IARC 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. From <http://monographs.iarc.fr/ENG/Monographs/vol82/> (Retrieved on 15 June 2014).

IARC 1993. *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Human: Some Naturally Occurring Substances. Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins.* IARC, Lyon, pp. 397-444.

Iha MH Barbosa CB Okada IA, Truckses MV 2011. Occurrence of aflatoxin M1 in dairy products in Brazil. *Food Control,* 22: 1971-1974.

Klich MA 2002. Identification of common *Aspergillus spp*, Ponson and Looijen, Wageningen. *The Netherlands*, 1-107.

Klich MA 2007. Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycosciencem,* 48: 71-80.

Mwanza M 2012. A Comparative Study of Fungi and Mycotoxin Contamination in Animal Products from Selected Rural and Urban Areas of South Africa with Particular Reference to the Impact of this on the Health of Rural Black People. From <https://ujdigispace.uj.ac.za/handle/10210/7880> (Retrieved on 20 April 2014).

Mwanza M 2007. A Survey of Fungi and Mycotoxins with Respect to South African Domestic Animals in Limpopo Province. Dissertation Submitted in Partial Fulfilment for the Degree of Magister Biotechnology. From <http://152.106.6.200:8080/dspace/bitstream/10210/884/1/Mwanza% 20M% 20tech%20dissertation.pdf> (Retrieved on 5 June 2014).

Mwanza M, Ngoma, L, Nyirenda, M, Lebohang M, Bakunzi F 2013. *A Decade of Aflatoxin M1 Surveillance in Milk and Dairy Products in Developing Countries (2001-2011): A Review. Agricultural and Biological Sciences » "Mycotoxin and Food Safety in Developing Countries."* ISBN 978-953-51-1096-5. Rijeka, Croatia: Published: April 10, 2013 under CC BY 3.0 License.

Mwanza M, Abdel-Hadi A, Ali A, Egbuta M 2015. Evaluation of analytical assays efficiency to detect aflatoxin M1 in milk from selected areas in Egypt and South Africa. *Journal of Dairy Science,* 98: 660–6667.

Nuryono N, Agus A, Wedhastri S, Maryudani YB, Sigit Setyabudi FMC, Bohm J, Razzazi-Fazeli E 2009. A limited survey of aflatoxin M1in milk from Indonesia by ELIZA. *Food Control,* 20: 945-946.

Pitt JI, Hocking AD 1997. Fungi and mycotoxins in foods. In: AE Orchard (Ed.): *Fungi of Australia. Volume1B, Introduction Fungi in the Environment.* Canberra, Australia: Aust Bio Research Study, pp. 315-342.

Pittet A 1998. Natural occurrence of mycotoxins in foods and feeds: An updated review. *Revue de Médecine Vétérinaire*, 149: 479-492.

Price WD, Randall RA, McChesty DG 1990. Naturally occurring toxins in feedstuffs: Centre for veterinary medicine perspective. *Journal of Animal Science*, 71: 2556-2562.

Rastogi S Dwivedi PD Khanna SK, Das M 2004. Detection of aflatoxin M_1 contamination in milk and infant milk products from Indian markets by ELISA. *Food Control*, 15: 287-290.

Sarmalnetoglu B Kuplulu O, Celik TH 2004. Detection of aflatoxin M_1 in cheese samples by ELIZA. *Food Control,* 15: 45-49.

Sassahara M, Netto PD, Yanaka EK 2005. Aflatoxin occurring in foodstuffs supplied to dairy cattle and aflatoxin M_1 in raw milk in the North of Paraa State. *Food Chemistry Toxicol*ogy, 43: 981-984.

Siddappa V, Nanjegowda DK, Viswanath P 2012. Occurrence of aflatoxin M (1) in some samples of UHT, raw and pasteurized milk from Indian states of Karnataka and Tamilnadu. *Journal Food Chemical Toxicology,* 50(11): 4158-4162.

Sorensen LK and Elback TH 2005. Determination of mycotoxims in bovine milk by liquid chromatography tanden mass spectrometry. *Journal Chromatography, B*, 820-196.

Stoloff L, Neshein S, Yin L, Rodricks JV, Stack M, Campbell AD 1971. A multimycotoxin detection method for aflatoxins, ochratoxins, zearalenone, sterigmatocysth and patalin. *Journal Association Official Analytical Chemistry*, 54: 91-97.

Torkar KG, Vengus T 2008. The presence of yeast, mould and aflatoxin M1 in raw milk and cheese in Slovenia. *Food Control*, 19: 570-577.

Vargas J, Due M, Frisvad JC, Samson RA 2002.Taxonomic revision of Aspergillus section Clavati based on molecular, morphological and physiological data. *Studies Mycology,* 2007(59): 89-106.

Whitlow LW, Nebel RL, Hagler WM Jr 2010. The association of deoxynivalenol in grain with milk production loss in dairy cows. In: GC Llewellyn, WV Dashek, CEO Rear (1994)(Eds.): *Biodeterioration Research 4.* New York: Plenum Press, P. 131.