

DETERMINATION OF AFLATOXIN M1 IN RAW MILK PRODUCE AT YOBE STATE UNIVERSITY FARM DAMATURU, NIGERIA

*¹Gide S., ¹Mohammed B., ¹Galal G. H., ¹Iliyah A. A., ¹Mabu I. M., ¹Lawan M. S., ¹Muhammad Y. and ²Sheriff W.,

¹Desert Research and Monitoring Control Centre, Yobe State University, Damaturu

²Department of Microbiology, Yobe State University, Damaturu.

Corresponding Author's email: gidesule@ysu.edu

ABSTRACT

The study was carried out to determine the contamination of aflatoxin_M1 (AFM1) in samples (n=66) of raw milk, from three distinctive animal species (cow, n = 30; goat, n = 20; sheep, n = 16) at Yobe State University farm Damaturu in 2018. The analytical strategy utilized was high-performance liquid chromatography (HPLC). Immunoaffinity columns were used to achieve clean-up step during HPLC and fluorometric determination. The outcomes demonstrated that 36 (54.54%) samples out of the 66 samples are debased with AFM1. The sullying rates of AFM1 in dairy animals, goat milk and sheep milk were 80.0%, 25.0% and 46.75% respectively. The mean concentration for the cow, goat and sheep milk was 0.1333µg/l, 0.0462µg/l and 0.0519µg/l respectively. The general mean convergence of AFM1 levels for positive samples from the three distinctive species was 0.0727 µg/l and there was no huge contrast (p = 0.3624) in fixation levels between the three species. The estimated intake (EDI) of AFM1 from consumption of cow milk products by teachers and the students was 0.00158g/kg b.w/day based on one-day recall methods, while hazard index was recorded to be 1.58 x10⁻⁴. The high levels of AFM1 concentration recorded in this study is an indication of contamination by the fungus during storage of feeds, this may have negative effects on the human and animal's health since it's proven to be carcinogenic, causes growth impairment and immune suppression. Measures should be enforced on the storage of feeds which will consequently decrease the odds of aflatoxin in milk of the animals.

Keywords: Aflatoxin, raw milk, cow, sheep, goat, HPLC.

INTRODUCTION

Mycotoxin signifies “poison from fungi”, however, not all harmful compounds produced by the fungus is viewed as mycotoxins (Hussain, 2009). Mycotoxins are secondary metabolites, basically produced by filamentous fungi, for example, *Aspergillus flavus*, *A. parasiticus* and *A. nomius* that possess a genuine risk for humans and animals (JECFA, 2001). In domestic animals such as cows, ingested AFB1, usually metabolize into a harmful carcinogenic substance which is released through the milk (Bellio *et al.*, 2016). These molds affect a wide scope of horticultural items, such as maize, millets and groundnuts, both in the pre-harvest and post-harvest seasons (Sarimehmetoglu *et al.*, 2004). Most mycotoxins are found in grain, typically developed in a dry season condition. Meanwhile, aflatoxins are found in corn and cottonseed, and uncommon in soya beans or distiller's grain. Shelled nut items might be polluted with aflatoxins and bring about toxin in milk items--Additionally, climate changes and poor agribusiness practices may affect the expansion levels of Aflatoxin M1 (AFM1) in dairy products as well as AB1 in food products (Mulunda *et al.*, 2013).

The Chemistry of AFB1 and AFM1

AFM1, the 4-hydroxylated metabolite of aflatoxin B1 (AFB1) is found in animals, humans bosom milk as well as dairy products (ICRC, 1993). The presence of AFM1 in milk is considered as a potential hazard for human wellbeing due to its cancer-causing nature. (Mulunda *et al.*, 2013). It was first set for research on malignancy (IARC) as group 2B agent cancer-causing to people (IARC) which revealed the genotoxicity and cytotoxicity of AFM1 (Caloni *et al.*, 2006), the poison is currently been delegated as group 1 human cancer-causing agent (IARC).

In the year 2004, Martins and Martins reported that around 1-2% of AFB1 in animal feed is changed to AFM1 in milk with differences from one to another, and over time from one milking to the next. Additionally, it was revealed from the research that admission of AFB1 correspond to AFM1 value in the milk when AFB1 is halted, AFM1 concentration diminishes to an imperceptible level after 3 days. AFB1 in the feed to AFM1 in the milk are highly correlated, a mean carry-over rate at oral administration of feeds containing the toxins after a week appears to increase exponentially. (Shlosberg *et al.*, 2013). The presence of AFM1 in milk has been determined to be within 15minutes to 60 minutes after utilization and come back to baseline within two to three days after removal from diets (Henry *et al.*, 2001). Studies on AFM1 metabolism have demonstrated that the rate between the measure of AFB1 ingested by dairy animals and the amount discharged in milk is generally 0.2 to 4% (Henry *et al.*, 2001, Sassahara *et al.*, 2005). It takes 72-144 hours of the consistent day by day ingestion of aflatoxin B1 before unfaltering state discharge of AFM1 in milk can be accomplished (Wagacha and Muthomi_2008).

AFB1 and AFM1 Standard Limits

Standard limits tolerated worldwide on AFs vary between 10-20 ppb for AFB1 and 0.05 ppb for AFM1 in Europe and South Africa and 0.5 ppb in the United States (Whitlow *et al.*, 2010). Research has shown that concentrations of 20 ppb of AFB1 in the complete blended proportion of a lactating dairy steer could result in AFM1 levels in milk underneath the FDA set up a limit of 0.5ppb. European Union and a few different nations, for example, South Africa have set up a worthy degree of AFM1 in milk and milk

products at 0.05 ppb (Henry *et al.*, 2001, Whitlow *et al.*, 2010). The examination of the sum 126 test of raw milk, pasteurized milk, and powdered milk demonstrated that 80% of the test was contaminated with various levels of AFM1 ranging from 0.020 µg/l to 0.765 µg/l. The AFM1 contaminated samples exceed the US, Syrian, and EU satisfactory cut-off points with 22%, 38%, and 52% respectively. Most developing countries in Africa have not yet set bearable points

of confinement on feed contamination levels of AFs as well as AFM1 in milk. Mulunda *et al.* (2013) stated that very little work has been done with regards to AFM1 the world. The information in Table 1 demonstrated that only a few African nations apart from South Africa (Dutton *et al.*, 2012), Egypt (Motawee *et al.*, 2009) and Morocco (Zinedine *et al.*, 2006) were engaged with the overview for AFM1.

Table 1 Survey of AFM₁ in developing countries

Country	Sample size	Method of detection	Positive (%)	Ranges (µ/L)
Egypt	175	ELISA	86 (49)	0.01-0.250
Libya	49	ELISA	35 (71)	0.03-3.13
South Africa	90	ELISA/IAC/HPLC	85 (94.5)	0.02-1.50
Morocco	54	IAC/HPLC	48 (88.8)	0.001-0.0117

The information demonstrated that the review in African nations has a large amount of contamination (0.05 µ/l >) in many milk tests analyzed.

Multiple factors determine the contamination of agricultural commodities with mycotoxins, probably the two most important environmental components favouring mold growth and AF production are hot and humid conditions. The climate change plays a major role in production of aflatoxin from *Aspergillus* in food crop. (Magan *et al.*, 2011). The temperature interacts with moisture content (a_w) and influences the ratio of regulatory genes in *A. flavus*, which directly proportional to the production of AFB1 (Schmidt-Heydt *et al.*, 2010). Increasing temperature to 37°C and water stress significantly reduces the production of AFB1 produced, despite the growth of *A. flavus* under these conditions. According Gallo *et al* (2016), fungal biomass and AFB1 production were reported to be highest at 28°C and 0.96 a_w , while no fungal growth or AFB1 production was seen at 20°C with value of 0.90 a_w and 0.93 a_w .

The ecological condition in term of humidity and temperature of an environment are factors that could favours the growth molds that may affect carbohydrate-rich cereals such as rice straws, sorghum stalks, corn stalks (at curing stage), when stored poorly and serves as feeds for livestock, thus, AFB1 contamination in the cereals by-products, then it's carryover in livestock products, such as meat, egg, and milk, thereby compromising the safety of public and animal health.

Therefore, the present study aimed to access the level of AFM1 in milk produced by three different species of animals and to evaluate the human risk associated with it.

MATERIALS AND METHODS

Chemical and reagents

Standard solution of AFM1 (Sigma Aldrich, Steinheim, Germany) was used for the analysis. A day by day working arrangements were set up in acetonitrile/water of 0.004 µg/ml (25/75 v/v) were utilized to spike the samples as indicated by Cammilleri *et al* (2018).

Deionized water was purchased (Milli Q, Millipore, and Bedford, MA, USA). The Acetonitrile (ACN) and methanol used in the research are specific for HPLC, were obtained from BDH (British drug house).

Sample collection

A total of 66 raw cow milk, sheep milk, and goat milk samples were collected from the University farm from April to May, 2018. Goats and sheep (breeds) involved in this study are Red Maradi and Yankasa. The sampling was carried out during the dry season. Details of the samples collected and types of breeds have appeared in Table 1. Each milk sample of 200 mL, were collected by hand expression into glass tubes following accumulation, was moved under the sterile condition to the science exploration research laboratory in the icebox at a temperature of about 4 °C and where then stored at -20°C until examination.

Table 2: Milk samples material under study

Sample category	Breeds	Milk samples
Cow	F1	30
Goat	Red maradi	20
Sheep	Yankasa	16
Total	3	66

Table 3: Temperature, rainfall and humidity from January-December (2018) in Damaturu Yobe State

Months	Temperature (max/min)	Humidity (out/in) %	rainfall (mm)
January	30.1/29.2	26/27	0.0
Feb	30.9/30.2	20/22	0.0
March	32/31.3	10/11	0.0
April	33.2/32.7	11/12	2.1
May	34.2/34.1	41/37	86.5
June	35.8/34.8	56/152	121.5
July	36.9/35.3	78/69	99.7
August	30.9/30.1	80/71	99.7
September	33.9/31.7	76/62	122.7
October	30.9/31.7	57/44	0.0
November	30/30.1	32/30	0.0
December	29.2/27.4	21/23	0.00

Source: Desert Research, Monitoring and Control Centre 2018

Determination of Aflatoxin M1 by HPLC

The strategy utilized for assurance of AFM1 was the AOAC Official Method (AOAC 2010).

Preparation of milk sample for high performance liquid chromatography (HPLC)

After warming the milk sample at around 37°C in the water bath, the liquid milk was then centrifuged at 2000rpm for 15min to separate the fat layer and then filtered through a glass microfibre filter paper (Whatman Schleider & Schuell, Maidstone, England, product number 934-AH). Later, 20 ml of filtered extract was transferred to a 50 ml capacity vial and 20 ml of sodium acetate buffer (pH 5.0) was added. The pH of the mixture was measured and corrected to 5.0 using an appropriate volume of a 0.1 M glacial acetic acid solution. The mixture was directly passed through an immunoaffinity column (Neocolumn, Neogem Europe, UK) at a flow rate of approximately 1.0–1.5 ml min⁻¹. After adding the mixture the column was washed with 40 ml of ultrapure water (Milli Q, Millipore, and Bedford, MA, USA). The column was dried by applying positive pressure with a syringe and bound AFM1 was eluted with 2.0 ml of HPLC-methanol which was recovered in a 4 ml vial previously treated with acid. The eluate was evaporated under nitrogen gas and reconstituted with 500 µl of the mobile phase before liquid chromatography analysis.

The Detection and quantification of sample extracts were performed by high-performance-liquid chromatography (HPLC) with a liquid chromatography system equipped with a LC-10AT Shimadzu pump (Kyoto, Japan), a Shimadzu RF-10AXL fluorescence detector (excitation 365 nm and emission 435 nm), an injection volume of 100µl, and a reverse phase column (250- 4.6mm, particle size of 3 µm) and pre column (Synergi Fusion, Phenomenex Inc., Torrance,CA, USA) kept at room temperature. The mobile phase consisted of an isocratic mixture of water and acetonitrile at a volume ratio of 75:25 and a flow rate of 1.0 ml min⁻¹. A calibration curve was prepared using standard AFM1 solutions in mobile phase at concentrations of 0.05, 0.01, 0.02, and 0.03ng ml⁻¹. The standard obtained (Sigma, St. Louis, MO, USA, and product code 6428, 10 lg) as purified crystalline AFM1 was dissolved in HPLC-grade acetonitrile and its concentration was determined by spectrophotometer according to Trucksess (2009).

Analytical performances

The Limit of detection (LOD) for AFM1 was assessed as 10 ng/ml and Limit of quantification (LOQ) was 50 ng/ml respectively, the linearity of the curve was 10 to 50 ng/ml. The calibration curve for AF M1 had a linear equation of $y = 4147.142x - 230.3028$; Figure 1 gives the correlation coefficient of $R^2 = 0.993$ and retention of 10 min.

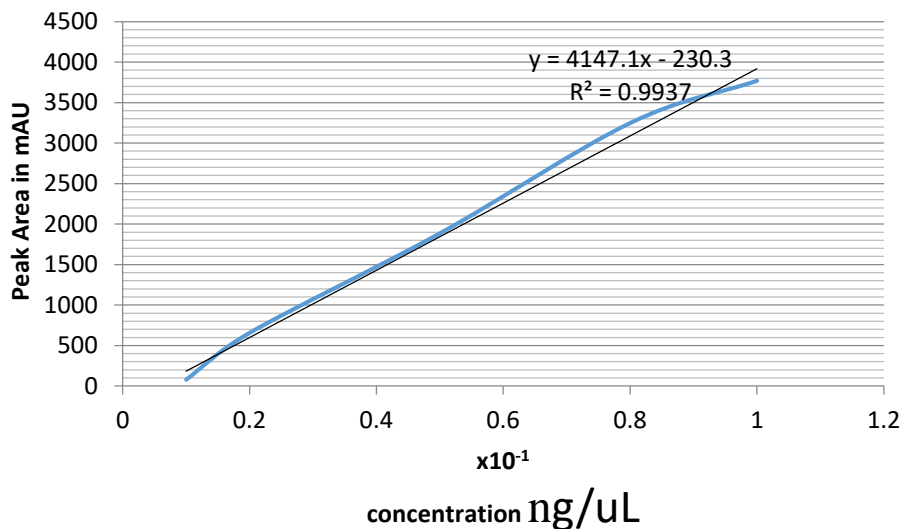


Figure 1: gives calibration curve of AFM1 with correlation coefficient

Human hazard appraisal to presentation to add up to aflatoxins utilizing the utilization of milk.

Dietary admission assessment

The estimate dietary intake of AFM1 was estimated following the technique for Hung *et al* (2016). The consumption of different milk by individual per day, was estimated based on a food frequency

questionnaire by European Food Safety Authority (EFSA) guidelines on information for national food consumption, direct interviews has been made, (n=116) including 116 individuals (table 4), asking them what type of milk do they consume per day and the quantity they devoured.

Table 4: Daily intakes of various milk produce in YUF and Demographic profile of participant samples

Milk category	Daily intake (Kg/person/day)	Demographic characteristic	
		Male (%)	Female (%)
Cow milk	0.375	47 (40.51)	69 (59.48)
Sheep milk	0.000	0 (0.00)	0 (0.00)
Goat milk	0.000	0 (0.00)	0 (0.00)

Exposure estimation

Calculation of the Estimated Daily Intake (EDI) was done by using the mean levels of aflatoxins obtained in cow, goat and sheep milk samples, and the daily intakes of each sample and the average body weight were recorded. The EDI for mean aflatoxins was calculated according to the following formula and expressed in µg/kg of body-weight/day (µg/kg b.w/day). (Taghizadeh *et al.*, 2018).

$$EDI = \frac{\text{daily intake of milk} \times \text{mean level of AFM1}}{\text{Average body weight (kg)}}$$

Estimation of Hazard index (HI)

The Hazard Index (HI) was calculated according to the below-mentioned formula, by dividing the EDI by TD50, divided by the safety factor of 50,000. TD50 is the dose (ng/kg/body/weight/day) required to induce tumors in half of the test animals that would have remained tumor-free at zero doses as described by Ismail *et al* (2016).

$$HI = \sum_{n=0}^1 \left(\frac{EDI/TD50}{50000} \right)$$

Analytical Package (Software) ^{SPSS}

Analysis of the data generated was performed using SPSS version 24. The generated data was analyzed using simple linear correlation to evaluate the relationship between AFM1 concentrations. The p-value of < 0.05 was considered statistically significant.

RESULTS

Table 4 shows the occurrence of AFM1 in the milk samples collected (30 cow milk, 20 goat milk, and 16 sheep milk respectively, 36 (54.54%) of the 66 samples were found to be contaminated with AFM1. The contaminated rate of AFM1 in diary milk of cow, goat and sheep was found to be 80%, 25%, and 43.75% respectively.

The range of AFM1 level in the milk samples was found to be

between 0.01 and 0.18 µg/l. The range of AFM1 level in milk samples from cow, goat, and sheep was 0.03-0.81 µg/l, 0.01-0.07µg/l and 0.03-0.08µg/l. 54.54% of the contaminated samples had AFM1 level exceeding the limit (0.05µg/l) set by the European Commission. The highest concentration AFM1 was found cow (F1) with tale code 063 (0.81 µg/l, sixteen times higher than the European Union maximum level. This is to note that the concentration at 0.81 µg/l was higher than the value set by US food and drug administration. About 6 samples of cow milk had

concentration (0.05 µg/l>) with values between 0.03-0.04 µg/l. Goats and sheep had concentration below 0.05 µg/l at 75% (15 samples) and 56.25 (9 samples) with levels of AFM1 ranging from 0.01-0.04 µg/l and 0.03-0.04 µg/l respectively. There is no significant difference in AFM1 means concentration found between the cow milk, goats milk and sheep milk samples (p = 0.3624), most of cow milk samples had concentration higher than the limit imposed by EC Reg. 1881/2006.

Table 5: Occurrence of AFM1

(Milk Category)	No of samples	Incidence of AFM1 (%)	Range of AFM1 (µg/L)
Cow	30	24(80)	0.03-0.81
Goats	20	5(25)	0.01-0.07
Sheep	16	7(43.75)	0.03-0.08
Total	66	36(54.54)	0.01-0.81

Table 6: Estimated daily intake (EDI) and Hazard indices of Aflatoxin M₁ via consumption of milk from different animal species at Yobe State University Farm

(Milk Category)	Mean total Aflatoxin (µg/L)	Age (yrs)	Average body weight (kg)	EDI(µg/Kg.bw/day)	HI
Cow milk	0.1333	17 -65	63.15 ^a	0.00158	1.58 x10 ⁻⁴
Sheep milk	0.0519	NS	NS	NS	NS
Goat milk	0.0462	NS	NS	NS	NS

Key: EDI estimate daily intake HI hazard index NS not significant (not consumed)

TD₅₀ = 0.2ng/Kg.bw (Kuiper-Goodman, 1990)

Ing=0.001µg

A = Average body weight of an adult in Nigeria (Akinpelu et al., 2015)

Average Daily Intake (Kg/person/day) = 0.75

DISCUSSION

The findings revealed a high occurrence of AFM1 sullyng in the samples examined, and a high level of positive tests with AFM1 level surpassing the level of 0.05 µg/l. 54.54% of samples indicated discernible degrees of toxin (>0.05 µg/l); most of the positive samples were estimated to contains AFM1 between 0.06 µg/l and 0.19 µg/l with the exception of raw milk from cow milk which is above (>0.80 µg/l). Milk samples obtained from Sheep and Goats showed concentration between (0.03-0.04 µg/l) 56.25% and (0.01-0.04µg/l) 75% lower (<0.05 µg/l) AFM1 content. Similar study by Cammilleri et al. (2018) in southern Italy recorded the low level of the toxin (just two) samples at 6%, (sheep milk) that slightly exceeded the EU maximum levels of 0.05 µg/l, He further asserted that the low degree of the toxin recorded in his study maybe be due to the high amount of proteins and their affinity with AFM1. The low level of the toxin in the goat and sheep milk in this study is accordance with other studies that reported that local breeds may have more resistance to aflatoxin (Malissiova et al., 2013). This outcome additionally, conforms with the outcome of similar research reported by (Hussain et al., 2010; Fallah et al., 2011) that

goat milk is less contaminated than cow milk, and this is a result of the distinctive stomach related contraption and mechanism of AFB1 assimilation of animals, or the diverse feeding proportion utilized in cows breeding contrasted with ovine and caprine species.

These findings are in contrast to that recently obtained by Okeke et al. (2012) which indicated sullyng of dairy cattle milk samples with AFM1 in the northern part of Nigeria (Bida) at mean concentration of (0.575-0.924 µg/l) with about 100% prevalence surpassing the standard level of 0.05 µg/l for diary milk and other milk product. AFM1 has been found in most dairy items with a high fixation slope than the worthy furthest reaches of detection (>0.05 µg/l) in Nigeria (Makun et al., 2009, Okeke et al., 2012). The findings by these researchers with an abnormal state of the toxin in milk and milk products maybe because of the administrative body (National Agency for Food and Drug Administration and Control) in Nigeria with poor regulatory enforcement on the nature of feeds. Hence, the state of poor enforcement and sanctions has left most projects unchecked. Raw milk sample of dairy animals had 80% with mean AFM1 concentration of 0.1333 µg/l levels which is unsafe for

drinking. These cows are generally kept inside bound munching territory and some of the time nourished utilizing biomass of harvests (sorghum, maize, millets, and groundnut) put away at the ranch house, it means that these bovines were presented to the abnormal state of AFB1 in their feeds which is processed to aflatoxin M1 and discharged into milk. Sassahara *et al.* (2005) revealed that the measure of AFB1 ingested by cows and the amount of AFM1 discharged in milk is generally 0.2 to 4%. Han *et al.* (2013) in their overview of AFM1 in crude milk produce in china recorded just three samples containing AFM1 at the level surpassing the EU lawful point of confinement. High sullied tests with AFM1 discovered during this overview might be because of regular variety in AFM1 contamination in milk, bovine gets increasingly concentrated feed in the dry season. Tajkarimi *et al.* (2008), saw that the period of production is significant in deciding AFM1 in cheese and showed that milk used to produced cheese in winter is contaminated than those produced in summer. In this work, cow milk, sheep milk, and goat milk samples were collected in dry season, then animals were fed using stored composite feeds. With this, we expect that the animals were nourished with feeds contaminated with AFB1 and subsequently, produced milk with high level of AFM1 in the samples analyzed. Another investigation in Italy on cheese samples produced with milk from dairies during summer showed that cheese has less contamination of AFM1 (Anfosi *et al.*, 2011); cheese belongs to milk from grazing animals which has lesser defilement than that during winter and spring which have a place with animals fed with composite and stored fodder.

This study is in line with the data reported in Morocco by Zinedine *et al.* (2006) that AFM1 in pasteurized milk from dairies had contamination levels ranging from 0.001-0.117 µg/l.

The estimated intake of AFM1 from consumption of cow milk products by the teachers and the students was 0.00158ug/kg.bw/day based on one-day recall methods, while the hazard index was 1.58×10^{-4} . According to Cano- Sancho *et al.* (2013) estimated the exposure of the adult Catalonian population (20 to 65 years old) to be 0.039 ng kg⁻¹ BW day⁻¹. The mean milk intake was 305 mL day⁻¹ (750 mL day⁻¹ for 95th percentile). Early estimation of AFM1 intake performed in 2001 by the joint FAO/WHO expert committee on food additives (JECFA) was calculated to be 6.8 ng per person per day (approximately 0.11 ng kg⁻¹ BW day⁻¹) for the European type diet (2001).

CONCLUSION

The seasonal variation ought to be assessed in Nigeria as it influences AFM1 formation in milk, on the presumption that animals fed on pasture have less exposure to AFB1 ingestion and therefore less AFM1 contamination in milk. Studies based on local and exotic breeds need to be done with the speculation that local breeds may have more prominent protection from aflatoxin, given that no accessibility of such research discoveries in Nigeria. Moreover, the Nigerian administrative body (NAFDAC) should carefully force control on the animal feeds which consequently decreases the odds of aflatoxin in milk and milk products of ruminant animals.

REFERENCE.

A. O. A. C (2010). Official Methods of Analysis of Chemistry, Washington, D.C. Association of Official Analytical Chemists (18th Ed.).

Akinpelu A. O, Oyewole O. O and Adekanla BA. (2015). Body size

perceptions and weight status of adults in a Nigerian Rural Community. *Ann Med Health Sci Res*; 5:358-64

Anfosi L., Baggiani C, Giovannoli C and Giraudi G. (2011). The occurrence of Aflatoxin M1 in Dairy Products Italy. *Anal. Bional. Chem*

Bellio A., Bianchi D., Gramaglia M., Loria A., Nucera D., Gallina S., Gill M. and Decastleli L. (2016). Aflatoxin M1 in cow's milk: Method validation for milk sampled in Northern Italy. *Toxins*, 8 57.

Caloni F., Stammati A., Frigge G., De Angelis. I (2006). Aflatoxin M1 absorption and cytotoxicity on human intestinal in vitro model. *Toxin* 47, 409-415

Camilleri G., Graci S. and Collura, R. (2018). Aflatoxin M1 in cow, sheep, and donkey milk produced in Sicily, Southern Italy. *Mycotoxin Res* 35, 47-53

Cano-Sancho, G., Sanchis, V., Marín, S. and Ramos, A.J. (2013). Occurrence and exposure assessment of aflatoxins in Catalonia (Spain). *Food Chem. Toxicol.* 51, 188–193.

Dutton M.F, Mwanza M, de Kock S and Khilosia LD. (2012). Mycotoxins in South African foods: a case study on aflatoxin M1 in milk. *Mycotoxin Research*; 28: 17-23.

EFSA (2009). General principles for the collection of National food consumption data in the view of pan-European dietary surveys. *EFSA J.* 7, 1435.

Elzupir A.O and Elhussein A. M. (2010). Determination of aflatoxin M1 in dairy cattle milk in Khartoum State, Sudan. *Food Control*; 21: 945-946

Fallah A. A., Rahnama M., Jafari T. and Saei-Dehkordi S. S. (2011). Seasonal variation of aflatoxin M1 contamination in industrial and traditional Iranian dairy products. *Food Control* 22 1653–1656.

Gallo A., Solfrizzo M., Epifani F., Panzarini G., Perrone G. (2016). Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *Apergillus flavus* on almond medium. *Int J. food Microbial.* 217 162-169. 10.1016/j.ijfoodmicro.2015.10.

Henry S. H, Whitaker T, Rabbani I, Bowers J, Park D, Price W, and Bosch F. X. (2001). Aflatoxin M1. Joint FAO/WHO Expert Committee on Food Additives (JECFA); 47

Hussain, Z., Khan, M.Z., Khan, A., Javed, I., Saleemi, M.K., Mahmood, S. and Asi, M.R. (2010). Residues of aflatoxin B1 in broiler meat: Effect of age and dietary aflatoxin B1 levels *Food and Chemical Toxicology* 48 (12): 3304-3307.

Hussain, Intiaz, and Institue of chemistry (2009). "Micro-Analysis of Aflatoxin M 1 in dairy products at trace levels and its elimination."

International Agency for Research on Cancer (IARC) (2011). Monographs on the evaluation of the carcinogenic risk of chemicals to man, 40, 83, *in INCHEM*

Ismail A., M. Riaz, R.E. L., Akhtar S., Gong Y.Y., and Hameed A. (2016). Seasonal prevalence level of aflatoxin M1 and its Estimated

Daily intake in Pakistan, *Food Control* 60 461–465 [SEP]

JECFA (2001). Safety evaluation of certain mycotoxins in food. Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on food additives. In FAO Food Nutric Paper; FAO: Rome, Italy.

Joint FAO/WHO Expert Committee on Food Additives (2001). "Safety evaluation of certain mycotoxins in food," in 56th meeting of the JECFA-FAO Food and Nutrition Paper 74/WHO *Food Additives Series* 47, Geneva

Malissiova E., Tsakalof A., Arvanitoyannis I. S., Katsaflika A., Katsioulis A., Tserkezou P., Koureas M, G. Govaris A. and Hadjichristodoulou C. (2013). Monitoring aflatoxin M1 levels in ewes and goats milk in Thessaly, Greece; Potential risk factors under organic and conventional production Schemes. *Food Control* 34: 241-248.

Malka B., Shmulik F., Joshua M., Ran S., Olga C., Jakob A.S., Stefan S., Rina A., Sima A. and Alan S.T. (2013). Carry-over of Aflatoxin B1 to Aflatoxin M1 in High Yielding Israeli Cows in Mid- and Late-lactation 5, 173-183; doi: 10.3390

Martins, M. L., Martins, H. M. and Bernardo, F. (2001). Aflatoxins in spices marketed in Portugal. *Food additives and contaminants* 18 (4), 315-319.

Makun H. A., Gbodi T. A., Akanya H. O., Salako E. A. and Ogbadu G. H. (2009). Fungi and some mycotoxins found in moldy sorghum in Niger State, Nigeria. *World J. Agric*; 5(1): 5-17

Motawee M. M., Bauer J., and McMahon D. J. (2009). Survey of Aflatoxin M1 in Cow, Goat, Buffalo and Camel Milks in Ismailia-Egypt. *Bull Environ Contam Toxicology*; 83: 766-769

Mulunda, M., Lubanza N., Mathew N., Lebohang M. and Frank B. (2013). "A Decade of Aflatoxin M 1 Surveillance in Milk and Dairy Products in Developing Countries (2001-2011): A Review."

Okeke K. S, Abdullahi I. O, Makun H. A. and Mailafiya S. C. (2012). A Preliminary Survey of Aflatoxin M1 in dairy cattle products in Bida, Niger State, Nigeria. *Afri. J. Food Sci. Tech* vol 3(10) pp 273-276.

R.W. Han, N. Zheng, J.Q. Wang, Y.P. Zhen, X.M. Xu, S.L. Li (2013). Survey of aflatoxin in dairy cow feed and raw milk in China, *Food Control* 34 pp 35-39

Taghizadeh S.F., Rezaee R., Davararynejad G., Asili J., Nemati S.H., Goumenou M., Tsakiris I., Tsatsakis A.M., Shirani K. G. K. (2018). Risk assessment of exposure to aflatoxin B1 and ochratoxin A through consumption of different Pistachio (*Pistacia vera* L) cultivars collected from four geographical regions of Iran, *Environmental Toxicology and Pharmacology*; 61: 61-66.

Sarimehmetoglu, B., Kuplulu, O. and Celik, T. H. (2004). Detection of aflatoxin M1 in cheese samples by ELISA. *Food Control*, 15, 45-49.

Sasahara M. Netto P. D and Yanaka E. K (2005). Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Paraa state. *Food Chemistry and Toxicology*; 43: 981-984.

Schmidt-Heydt M., Rufer C.E., Abdel-Hadi A., Manga N., Geisen R. (2010). The production of aflatoxin B1 or G1 by *Aspergillus parviticus* at various combination of temperature and water activity is related to the ratio of aflS or aflR expression. *Mycotoxin Res.* 26 241-246. 10.1007/s12550-010-0062-7

Substances: Food Items and Constituents, Heterocyclicaromatic Amines and Mycotoxins. In:

IARC monographs on the evaluation of carcinogenic risks to humans. pp. 245–395, International Agency for Research on Cancer, ISBN 928321256-8, Lyon, France

Kuiper-Goodman T. (1990). Uncertainties in the risk assessment of three mycotoxins: Aflatoxin, ochratoxin, and zearalenone *Can. J. Physiol. Pharmacol.* 68: 1017–1024.

Tajkarimi M., Aliabadi-Sh F, Nejad A. S., Poursoltani H., Motallebi A. A., and Mahdavi H. (2008). Aflatoxin M1 contamination in winter and summer milk in 14 states in Iran, *Food Control*, vol 19, no. 11, pp, 1033-1036

Trucksess M.W., Whitaker T.B., Weaver C.M., Slate A., Giesbrecht F.G., Rader J.I., Betz J.M., USDA ARS. (2009). Sampling and analytical variability associated with the determination of total aflatoxins and ochratoxin A in powdered ginger sold as a dietary supplement in capsules. *J Agric Food Chem* 57(2): 321– 5

Wagacha J. M. and Muthomi J. W. (2008). Mycotoxin problem in Africa's current status implications food safety and health and possible management strategies. *International Journal of Food Microbiology*; 124:1-12.

Whitlow W. M, Hagler J. R and Diaz (2010). Mycotoxins in feeds. Quality feed Mycotoxins. *Feedstuff*. Feedstuffs 83,

Zinedine A., Brera C., Elakhdari S., Catano C., Debegnach F., Angelini S., De Santis B. F. M., Benlemlih M., Minardi V. and Miraglia M. (2006). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*; 17: 868–874.



©2020 This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International license viewed via <https://creativecommons.org/licenses/by/4.0/> which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is cited appropriately.