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Survey of Aflatoxin M_1 in Cow, Goat, Buffalo and Camel Milks in Ismailia-Egypt

Mahmoud M. Motawee · Johann Bauer · Donald J. McMahon

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Abstract Milk from buffalo, cow, goat and camel species was collected in Ismailia in Egypt. Aflatoxin (AFM_1) levels were lower than previous surveys, and were influenced by feeding practices. Cows and buffaloes are fed prepared rations and had highest incidence of AFM_1. Camels forage freely on available pasture and had lowest AFM_1 in their milk. Goats are fed a combination of prepared ration as a supplement to pasture grazing. Most milks (80%, 74%, 66% and 52% of the camel, goat, cow and buffalo milks, respectively) were below the European Union maximum of AFM_1 <50 ng/L and all milk samples were <500 ng/L.

Keywords Aflatoxin · Milk · ELISA · Egypt

Aflatoxin M_1 (AFM_1) is the main aflatoxin B_1 (AFB_1) metabolite found in milk of lactating animals that have ingested feed contaminated with some common molds such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. Early studies (Stoloff 1980) suggested that only 0.30%–0.62% of AFB_1 is transformed to AFM_1 by the hepatic microsomal mixed function oxidase system and excreted in milk. Price et al. (1985) determined that 1.6% of ingested AFB_1 is converted to AFM_1, and this average rate of conversion has been used frequently by other researchers (Unusan 2006). Battacone et al. (2003) showed that the relationship between amount of AFB_1 ingested and AFM_1 excreted in milk varies between 1% and 2% and it can be as high as 6% in high milk-producing cows (Veldman et al. 1992). High levels of AFM_1 in milk and other dairy products is considered undesirable because it has toxic, teratogenic, and carcinogenic properties (Creppy 2002). *Aspergillus* species molds can often be found in legumes such as peanuts, copra and soya beans, and in cereals such as maize, rice and wheat that are often used in preparing rations for dairy animals (Creppy 2002). Aflatoxin M_1 is resistant to thermal inactivation and not destroyed completely by pasteurization, autoclaving and other food processing procedures (Sadeghi et al. 2009).

The maximum allowable concentration of AFM_1 in food varies by country. The European Union has the lowest maximum allowable level for AFM_1 in milk of 50 ng/L (Commission Regulation 2006) while the action level for AFM_1 in fluid milk in the United States is ten fold higher at 500 ng/L (FDA 2005). This survey was conducted to evaluate the prevalence of AFM_1 in milk of four different dairy animals (cow, goat, buffalo and camel) in the Ismailia in Egypt, during the summers of 2003 and 2004.

Materials and Methods

One hundred seventy-five samples of animal milk from four species (50 buffalo, 50 cow, 50 goat and 25 camel) were collected from different areas in the Ismailia Governorate in Egypt during the summers of 2003 and 2004 at random during normal milking times. Samples were stored frozen until analyzed. All milks were analyzed for total

M. M. Motawee (✉)
National Organization for Drug Control and Research, P.O. 29, Cairo, Egypt
e-mail: mahmoud.motawee@yahoo.com

J. Bauer
Lehrstuhl für Tierhygiene, Technische Universität München, 85354 Freising, Germany

D. J. McMahon
Western Dairy Center, Utah State University, Logan, UT 84322, USA

Table 1 Composition of milk collected from Ismailia Governorate in Egypt

Species	Total solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	pH
Buffalo	16.6–16.9	5.0–7.5	4.0–4.3	4.7–4.9	0.80–0.82	6.6–6.7
Cow	12.8–13.0	3.5–4.0	3.0–3.6	4.7–4.8	0.70–0.72	6.5–6.7
Goat	12.5–13.0	3.9–4.1	3.0–3.4	4.3–4.4	0.77–0.8	6.5–6.6
Camel	13.3–1 3.5	3.1–3.3	3.9–4.1	5.5–5.8	0.80–0.84	6.6–6.7

solids, fat, protein, lactose, and ash by using International Dairy Federation method No. 141, C: 2000.

Aflatoxin M_1 levels in milk were measured using an enzyme-linked immunoassay test kit (RIDASCREEN, r-biopharm, Darmstadt, Germany) that included microtiter plates with immobilized AFM_1-antibody, AFM_1-enzyme conjugate, enzyme substrate (urea peroxide), and chromogen (tetramethyl benzidine), and stop reagent (1 M H_2SO_4). Analytical reagent grade methanol, *n*-heptane and dichloromethane were obtained from Merck (Darmstadt, Germany). Phosphate buffer saline at pH 7.2 was prepared by mixing 0.55 g $NaH_2PO_4 \cdot H_2O$ with 2.85 g of $Na_2HPO_4 \cdot 2H_2O$ and 9 g NaCl and then filled up to 1,000 mL with distilled water.

Milk samples (4 ml) were chilled to 4°C, centrifuged for 10 min at 3500 rpm, (Heraeus Megafuge 1.0 – Hettich model Universal 16 R, Germany), and then the upper cream layer was completely removed by aspiration through a Pasteur pipette. The samples were further diluted 20 times (v/v) with deionized water.

Pure AFM_1 standard from Sigma–Aldrich (Deisenhofen, Germany) was used to prepare six standard solutions (0, 50, 100, 200, 400, and 800 ng AFM_1/L) for making a calibration curve. Samples (50 µL) in microtiter plate wells (in duplicate) were incubated for 60 min at room temperature (~22°C) in the dark, to allow antibody-binding sites in the wells to be occupied proportionally to AFM_1 concentration. The liquid was then removed completely from the wells, which were washed twice with 250 µL of washing buffer and distilled water. In the next step, any remaining free binding sites were occupied by adding 100 µL of enzyme conjugate to the microtiter plate wells and incubated for another 60 min at room temperature in the dark. Any unbound enzyme conjugate was then removed in a washing step. This was followed with 50 µL of urea peroxide and 50 µL of tetramethyl benzidine and 30 min incubation at room temperature in the dark, followed by addition of 100 µL of stop reagent. Yellow color was measured at 450 nm against an air blank within 60 min, with AFM_1 concentration being inversely proportional to A_{450}. The mean absorbance from the duplicate subsamples of the standard solutions and the milk samples were calculated and evaluated by the instrument software (Ridavin.exe version 1.2 for windows; r-biopharm GmbH). Recovery rate of AFM_1 in the standard solutions with 10–80 ng/L of AFM_1 was 95% with a mean coefficient of variation of 15%. Similar recovery was observed when milk was spiked with AFM_1.

Results and Discussion

Milk composition is shown in Table 1 and was comparable to our expectations for these four species. It is important to realize milk composition depends on a variety of factors including species, genetic variation, lactation period, individual animal variability, animal nutrition and type of feed consumed. The range of AFM_1 levels in buffalo, cow, goat and camel milks collected from the Ismailia in Egypt during the summers of 2003 and 2004 are shown in Table 2.

Some differences were observed based on the feeding practices followed for each species. It is customary in the region from which the milk was sampled, that cows and buffalos are kept in enclosed areas and fed a prepared ration with little, if any, exposure to grazing on pasture. In contrast, camels are allowed to roam freely on available wild pasture and forage for their feed requirements without any supplemental feeding. Goats occupy an intermediate feeding pattern and are released onto pasture for grazing each morning, and then brought back into an enclosed area in the evening for milking, and provided a prepared ration.

All milk samples in this study had AFM_1 levels less than the United States maximum allowable level (i.e. <500 ng/L). Half of the samples (51%) tested negative for AFM_1 and were below the level of quantification of 10 ng/L. Some milk samples were above the European limit (i.e. >50 ng/L) although most were <150 ng/L. Milk from

Table 2 Level of aflatoxin M_1 (AFM_1) in different milk samples collected from Ismailia-Egypt during the summers of 2003 and 2004

Species	Number of milk samples							
	Total	AFM_1 level (ng/L)						
		<10	10–50	51–100	101–150	151–200	201–250	>250
Buffalo	50	18	8	10	6	4	3	1
Cow	50	24	9	9	4	2	2	0
Goat	50	32	15	6	3	3	1	0
Camel	25	16	4	2	1	1	1	0

Table 3 Occurrence of AFM_1 in milk in Egypt and some neighboring countries

Type of milk	Country	Samples tested	Limit of Quantification (ng/L)	Samples testing positive for AFM_1 (%)	Reported AFM_1 levels (ng/L)	Reference
Raw milk	Iran	111	15	77	15–280	Kamkar (2005)
UHT[a] milk	Turkey	129	10	58	108–117	Unusan (2006)
Buffalo's milk	Egypt	10	10	30	220 (average)	Motawee et al. (2004a)
Cow's milk	Egypt	10	10	40	250 (average)	Motawee et al. (2004a)
Cow's milk	Libya	49	10	71	30–3013	Elgerbi et al. (2004)
Liquid milk	Iran	100	5	78	52–113	Oveisi et al. (2007)
Pasteurized milk	Morocco	54	10	89	186 (average)	Zinedine et al. (2007)
Camel's milk	Egypt	24	30	25	30–850	Balata and Bahout (1996)
Pasteurized milk	Turkey	85	5	88	127 (average)	Celik et al. (2005)
Buffalo's milk	Egypt	25	10	32	228 (average)	Motawee et al. (2004b)
Cow's milk	Egypt	25	10	20	312 (average)	Motawee et al. (2004b)

[a] Ultra-high temperature processed

camels, which are fed only on free-range pasture without any prepared ratios had the lowest AFM_1 levels with 80% being ≤50 ng/L compared to 74%, 66% and 52% for goat, cow, and buffalo milks, respectively. Even so, 84% of the buffalo milk samples had ≤150 ng/L AFM_1 levels, and only one sample had 270 ng/L. The highest concentrations of AFM_1 were 210, 220 and 230 ng/L found in camel, cow and goat milk, respectively, and 92% of the camel milk samples had AFM_1 levels ≤150 ng/L.

When comparing the levels of AFM_1 in milk detected in this survey with previous research (Table 3) the levels in cow and buffalo milk were similar to what had been observed earlier in Egypt (Motawee et al. 2004a, b) although slightly more samples tested positive (i.e. >10 ng/L). Balata and Bahout (1996) had reported AFM_1 levels in Egyptian camel milk up to 850 ng/L but the highest observed in our study was 250 ng/L found only in one sample.

It is important to realize that AFM_1 levels in milk are dependent on the level of AFB_1 in the food consumed, so both type of feedstuffs used and environmental factors will influence AFM_1 levels in milk. Salem (2002) investigated occurrence of aflatoxins in animal feed and raw milk from six dairy farms in Assuit-Egypt and found that 93% of feed samples were contaminated with AFB_1 and 59% of raw milk samples tested positive for AFM_1. In our study, the highest levels of AFB_1 in feeds consumed by the animals was calculated to be 13–17 μg/kg, based upon a 1.6% conversion rate of AFB_1 to AFM_1 as reported by Price et al. (1985).

The specific Egyptian climatic conditions prevalent during the summers of 2003 and 2004, when our study was conducted, was ideal for promotion of *Aspergillus* fungal growth in cereal feedstuffs with consequent production and accumulation of AFB_1. Even so, only 10% of all milk samples collected had AFM_1 levels >150 ng/L. Camels are primarily grazed on pasture and are not given prepared rations and so in our study would probably have ingested less *Aspergillus*-contaminated feed, thus, giving camel milk the slightly lower AFM_1 levels in their milks observed in this study.

References

Balata MA, Bahout AA (1996) Aflatoxin M_1 in camel's milk. Vet Med J Giza-Egypt 44(2A):109–111

Battacone G, Nudda A, Cannas A, Capio Borlino A, Bomboi G, Pulina G (2003) Excretion of aflatoxin M_1 in milk of dairy ewes treated with different doses of aflatoxin B_1. J Dairy Sci 86:2667–2675

Celik TH, Sarmehmetoglu B, Küplülü O (2005) Aflatoxin M_1 contamination in pasteurized milk. Veteinarski Arhiv, Ankara. Turkey 75(1):57–65

Commission Regulation (2006) (EC) No. 1881/2006 of 19 December 2006. Setting maximum levels for certain contaminants in foodstuffs. Off J European Union 364:5–24 L077:1–13

Creppy EE (2002) Update of survey, regulation and toxic effect of mycotoxins in Europe. Toxicol Lett 127:19–28

Elgerbi AM, Aidoo KE, Candlish AAG, Tester RF (2004) Occurrence of aflatoxin M_1 in randomly selected North Africa milk and cheese samples. Food Addit Contam 21:592–597

FDA (2005) Sec. 527.400 Whole milk, low fat milk, skim milk – aflatoxin M1 (CPG 7106.10). Available from http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg527-400.html. Accessed 20 Apr 2009

Kamkar A (2005) A study on the occurrence of aflatoxin M_1 in raw milk produced in Sarab city of Iran. Food Control 16:593–599

Motawee M, Meyer M, Bauer J (2004a) Incidence of aflatoxin M_1 and B_1 in raw milk and some dairy products in Damietta. Egypt J Agric Sci Mansoura Univ 29(2):711–718

Motawee M, Meyer M, Bauer J (2004b) Occurrence of aflatoxin M_1 and B_1 in milk and some milk products in Mansoura. Egypt J Agric Sci Mansoura Univ 29(2):719–725

Oveisi MR, Jannat B, Sadeghi N, Hajimahmoodi M, Nikzad A (2007) Presence of aflatoxin M_1 in milk and infant milk products in Tehran, Iran. Food Control 18:1216–1218

Price RL, Paulson JH, Lough OG, Gingg C, Kurtz AG (1985) Aflatoxin conversion by dairy cattle consuming naturally contaminated whole cottonseed. J Food Prot 48:11–15, 20

Sadeghi N, Oveisi MR, Jannat B, Hajimahmoodi M, Bonyani H, Jannat F (2009) Incidence of aflatoxin M_1 in human breast milk in Tehran, Iran. Food Control 20:75–78

Salem DA (2002) Natural occurrence of aflatoxin in feedstuffs and milk of dairy farm in Assuit Province, Egypt. Wien Tierarztl Monatsschr 89:86–91

Stoloff L (1980) Aflatoxin M_1 in perspective. J Food Prot 43:226–230

Unusan N (2006) Occurrence of aflatoxin M_1 in UHT milk in Turkey. Food Chem Toxicol 44:1897–1900

Veldman A, Meijst JAC, Borggreve GJ, Heeres-van der Tol JJ (1992) Carry over of aflatoxin from cows' food to milk. Anim Prod 55:163–168

Zinedine A, Gonzalez-Osnaya L, Soriano JM, Molto J, Idrissi L, Manes J (2007) Presence of aflatoxin M_1 in pasteurized milk from Morocco. Int J Food Microbiol 114:25–29