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Aflatoxin M2 concentrations in raw milk of different dairy animal species in Egypt

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Abstract
Previously we reported aflatoxin M1 (AFM1) concentrations in dairy milk of different animal species. Yet, there are no reports of aflatoxin M2 (AFM2) concentrations in raw milk and milk products of dfferent animal species. Aflatoxin M2 levels are usually very low or un-detectable using ELISA or chromatography techniques. The reasons of low concentrations of AFM2 in milk and milk products still remain obscure and controversial. In this study, variable AFM2 concentrations are being reported in milk of different animal species with the highest levels reported in goats milk.

Keywords: *raw milk; dairy products; AFM2; food animals.*

1. Introduction

Mycotoxins are toxic metabolites produced by varios species of fungus in human and animal feed material and could have serious consequences affecting the health of humans and animals. Aflatoxin M2 (AFM2) is a fungal metabolite reported in milk and related milk products (Henry et al., 1997; Shepherd et al., 2012). Aflatoxin M2 is a hydroxylated form of aflatoxin B2 which is secondary product of *Aspergillus flavus* and other species. The degradation of aflatoxins in the rumen remained the important element that affect further distribution to blood and then secreted in milk(Kuboka et al., 2019). Also, the lactating animal that consumed aflatoxins B1 and B2 contaminated rations secreted the hydroxylated metabolites into milk respectively known as aflatoxins M1 and AFM2 (Peraica et al., 1999; Sartori et al., 2015). Similarly, after ingestion, AFB1 and AFB2 are metabolized mainly by the liver to their hydroxylated metabolites M1 (AFM1) and M2 (AFM2), which can be excreted through the urine, feces, and milk (Fink-Gremmels, 2008; Tozzi et al., 2016).

Aflatoxin M2 can be detected, by HPLC. In raw milk in El- Minufia Governorate, Egypt, 8.33% of samples were found to have AFM2 in the range of 0.010- 0.034 µg/kg (Kuboka et al., 2019). However, in another study, all milk samples analysed were not contaminated with aflatoxin M_2 in the commercial milk and/or pasteurized milk collected from supermarkets in Ribeirooão Preto-SP, Brazil(Garrido et al., 2003).

Notably, the carryover from AFB2 to AFM2 was reported to be 0.31%in donkeys milk. There are no reports of aflatoxin M2 (AFM2) concentrations in raw milk and milk products of different animal species. Thereffore, the objective of this study was to reort the concentrations of AFM@ in raw milk of dairy cow, buffalo, sheep and goats.

2. Material and methods

Collection of the samples

A total of 120 raw dairy milk samples (30 samples from each of cows, buffalo, sheep and goats) were collected randomly from Aga City, Dakahlia Governorate, Egypt. Each sample was analysed for the detection of aflatoxin M2 using Ultra Performance Liquid Chromatography XEVO-TQ (UPLC). All collected milk samples were transferred to the laboratory in clean, dry, and sterile tightly closed bottles and kept in an ice box until analysed.

Chemicals for mycotoxin extraction

HPLC-grade methanol (MeOH), Hexane, Acetonitrile (ACN) (Sigma Aldrich;Cairo, Egypt) were used in the extraction of mycotoxins from the raw milk. Preparation, extraction and detection of AFM2 in the raw milk samples was carried out and preparations of standard combined stock solutions were performed as described earlier (Kamal et al., 2019).

3. Statistical analysis

Statistical analysis was carried out using Oneway ANOVA. Values were expressed as mean ± standard error of mean (SEM). Minimum (Min), maximum (Max) and range values were also calculated (18). Computerized SPSS program version 13 was used in the evaluation of data.

4. Results

Aflatoxin M2 ranged from zero to few ng/kg in different dairy milk of cows, buffalo, sheep and goats. Notably, the highest level of AFM2 recorded in goats milk (Fig. 1 and Table 1).

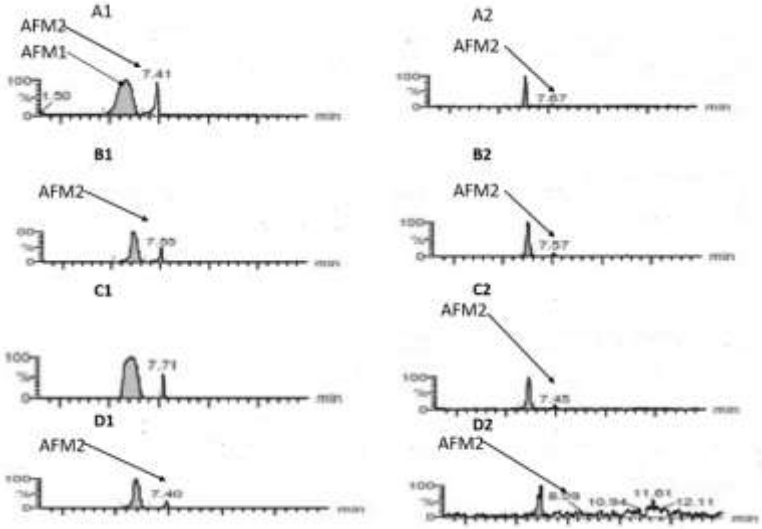

Fig (1): The depicted figure shown levels of AFM2 (ng/kg) in dairy milk of cow (A1 high, A2 low), buffalo (B1 high, B2 low), sheep (C1 high, C2 low) and goat (D1 high, D2 low)

Table (1): Means and standard error and ranges of AFM2 concentrations (ng/kg) **in dairy milk samples.**
a,b,c significant at p ≤0.05

Dairy milk	N	Mean	SD	SE	95% Confidence Interval		Minimum	Maximum
					Lower Bound	Upper Bound		
Cow	30	7.46	0.09	0.05	7.23	7.69	7.41	7.57
Buffalo	30	7.55	0.01	0.00	7.52	7.58	7.55	7.57
sheep	30	7.53	0.15	0.08	7.16	7.90	7.45	7.71
goat	30	7.86	0.39	0.23	6.87	8.84	7.40	8.09
Total	120	7.60	0.24	0.07	7.44	7.75	7.40	8.09

5. Discussion

AFM2 is produced from hydroxylation of the fourth carbon in the AFB2 molecule (Agag, 2004). Aflatoxin M2 was detectable with very low in dairy milk of different species for many reasons; firstly, AFM2 is secreted in milk after hydroxylation of AFB2 (Fink-Gremmels, 2008; Tozzi et al., 2016) and this form was recorded to be low level in animals feed relative to AFB1 (Agag, 2004; Goto et al., 1982). Secondly, there was another possibility that AFB2 could convert to AFB1 by post-mitochondrial supernatant reactions in ducks, rats, mouse, and human livers reported in an *in vitro* study (Roebuck et al., 1978). Thirdly, ruminal degradation of AFB2 (An, 2010; Ji et al., 2016; Kuboka et al., 2019; Wu et al., 2009) was also reported and so the level of absorption and conversion to AFM2 could be at very low levels. However, the conversion rate of AFB2 to AFM2 was high than of AFB1 to AFM1 (Tozzi et al., 2016). Fourthly, the fungi, *Aspergillus parasiticus*, itself could convert mycotoxin metabolites into AFM2 rather than AFB1(Cleveland et al., 1987) and this could explain that the level of AFM2 was reported in milk products than raw milk with evidence of fungi detection in raw milk (Delavenne et al., 2011; Lavoie et al., 2012). Taken collectively, various concentrations of AFM2 were recorded in all dairy milk samples in this study. Farmers and food safety authorities should note that AFM2 could convert to AFM1 which is considered a carcinogenic agent.

Conflict of interest: All author had no conflict of interest

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