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CONFERENCE PAPER

SEASONAL VARIATIONS OF AFLATOXIN M_1 IN THE FARM MILK IN ALBANIA

EDMOND PANARITI

Institute of Veterinary Research, Tirana, Albania

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This paper presents monitoring data on levels of aflatoxin M_1 in the farm-gate milk in Albania. The monitoring included 120 evenly distributed samples collected in winter and summer from various farms all over the country. The levels of aflatoxin M_1 were determined using the quantitative thin layer chromatography. On average, the winter milk samples revealed higher concentrations of aflatoxin M_1 than the summer samples. Thirteen percent of the winter samples resulted above the 0.5 µg/kg level, as compared to 3% of the summer samples exceeding that level. Skimmed and semi-skimmed milk from the same source contained comparatively lower levels of aflatoxin M_1 than whole milk.

Key words:
animal feed, TLC determination of AFM_1

Poor agricultural practices in Albania and inappropriate storage of animal feedstuff constitute a favourable medium for the growth of toxic moulds. It has been indicated that the ensilaged maize mostly used for feeding lactating cows during winter is heavily contaminated with moulds (1). The mould contamination is related to inappropriate storage conditions and high humidity which is characteristic for this kind of feed (>17%).

Aspergillus and *Penicillium* species prevail over other present mycotic species. Literature data have pointed to the overwhelming presence of toxicogenic *strains Aspergillus flavus* and *Aspergillus parasiticus*, known for producing aflatoxins (2). Aflatoxins are a group of structurally related difuranocoumarin derivatives of which 18 types have been identified by now (3, 4). Aflatoxin B_1 (AFB_1) is the most frequent in food. This compound has been found at relatively high levels in animal feeds in Albania, sometimes exceeding the concentration of 400 μg/kg (5). Research of labo-

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ratory animals has shown that aflatoxins are liver carcinogens in animals and there is evidence to suggest that they are also human carcinogens, with AFB_1 being the most potent and the aflatoxin M_1 (AFM_1) less so (6, 7). As a matter of fact, lactating cows exposed to AFB_1-contaminated feeds convert the toxin into the hydroxylated version of the primary compound, the resulting metabolite AFM_1. Therefore the main concern in milk should be the AFM_1 levels.

The goal of the screening survey described below was to assess the levels of AFM_1 in the farm milk of Albania and investigate their seasonal fluctuations using the quantitative thin layer chromatography (TLC) method.

MATERIALS AND METHODS

Materials

The investigation involved 120 milk samples obtained from farms throughout Albania. Samples were obtained in equal numbers during summer (when cows were at pasture) and winter (when cows consumed more concentrated feedstuffs).

Method of Analysis

The screening for AFM_1 was performed at the Institute of Veterinary Research in Tirana, using the quantitative thin layer chromatography. The limit of detection was 0.05 μg/kg. The analysis used spiked samples to assess recoveries; the acceptable recovery range was 65–100% and all spiked milk samples fell within that range. The winter samples recovery range was 67–99%, whereas the summer samples range fell within 73–92%.

The coefficients of variation calculated for the recoveries were 9.7% and 5.8%, respectively. The average recovery value for AFM_1 in farm milk was 87%. All results were corrected using recovery values determined day by day.

Extraction of Samples

The extraction of milk samples involved the blending of a 20-ml milk sample with 80 ml of methanol and 50 ml of water. The mixture was additionally treated with 50 ml of petroleum ether in a 250-ml separator for removal of fat. After the layers were separated by vigorous shaking, the petroleum ether phase was discarded (8, 9). The mixture was then submitted to extraction with 100 ml chloroform and consequently evaporated to the residual volume of 0.5 ml.

Thin layer chromatography

Aflatoxin M_1(Sigma) standards were used at a concentration of 0.2 μg/ml. The procedure involved TLC plates Kieselgel 60 without the fluorescence indicator. The spiking of blank and fortified sample extracts (with 5 μl standard addition) as well as of the standards was performed by an automatic spiking sample device (Camag). One 20 μl

aliquot of sample extract and 2, 4, 6, 8, and 10 μl aliquots of M_1 standards were spotted on each TLC plate. The plates were previously soaked in diethylether in a saturated chamber to avoid the interference of potential impurities. The Plates (20x20 cm) were developed at ca 12 cm into a chamber containing chloroform/trichlorethylene /n-amylalcohol/ formic acid (80+15+4+1 v/v).

A TLC scanning analytical system Camag II, equipped with the data elaboration software Cats 3 was used for the quantitative determination of AFM_1 in milk samples. The detection was performed at the excitation wavelength of 365 nm and emission wavelength of 430 nm.

RESULTS AND DISCUSSION

Relatively higher concentrations of AFM_1 were determined in the winter milk samples than in the summer ones (Table 1). Almost 13% of the screened winter milk samples contained AFM_1 levels higher than 0.5 μg/kg, with the maximum reaching 0.85 μg/kg. The respective summer values were 3% and 0.65 μg/kg. Skimmed and semi-skimmed milk contained comparatively lower levels of AFM_1 than the whole milk, indicating a partial removal of AFM_1 residues through skimming. The relatively higher levels of AFM_1 levels in milk in winter should be attributed to the dominant diet of stored feedstuffs associated with the season. This is an indication of greater exposure risk for consumers during this period. On the other hand AFM_1 is rather heat-stable and once inside the product its levels remain virtually unaffected by heating such as pasteurisation and sterilisation (10, 11). It should be noted that the determined residual levels of AFM_1 in milk should be considered compatible with the reported relatively high

Table 1 *Aflatoxin M_1 levels in farm-gate milk in Albania*

Milk type	No. of samples	Number of contaminated samples				Maximum level (µg/kg)
		<0.05 µg/kg	0.05–0.1 µg/kg	0.1–0.5 µg/kg	>0.5 µg/kg	
Whole (summer)	30	8	8	12	2	0.65
Skimmed and semi-skimmed (summer)	30	21	7	2	–	0.2
Sub-total	60	29 (48)	15 (25)	14 (23)	2 (3)	
Full (winter)	30	4	14	4	8	0.85
Skimmed and semi-skimmed (winter)	30	17	13	–	–	0.08
Subtotal	60	21 (35)	27 (45)	4 (6)	8 (13)	
Total	120	50 (42)	42 (35)	18 (15)	10 (13)	

Percentages are given in parentheses
The results are corrected for recovery. Limit of determination=0.05 µg/kg

levels of aflatoxin B_1 in animal feedstuffs. The AFM_1 levels found in milk samples issue concern because of their potential impact on the consumer's health. As AFM_1 is not included in the Albanian regulations, the risk is assessed with reference to the EU regulatory limit of 0.05 μg/kg. For the aflatoxins in general, only a zero level would ensure absolute safety for consumers (12, 13). As thie zero level is not attainable in practice, the risk associated with the residual levels of AFM_1 in milk should be weighed and assessed by health authorities and become a matter of concern for the animal breeders.

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Sažetak

SEZONSKE VARIJACIJE AFLATOKSINA M_1 U MLIJEKU SKUPLJENOM NA FARMAMA U ALBANIJI

U ovom radu prikazani su rezultati praćenja razina aflatoksina M_1 u mlijeku skupljenom na farmama u Albaniji. Analizirano je 120 uzoraka skupljenih ravnomjerno tijekom zime i ljeta na različitim farmama diljem zemlje. Za određivanje aflatoksina M_1 primijenjena je kvantitativna tankoslojna kromatografija. U uzorcima skupljenim tijekom zime uglavnom su određene više razine aflatoksina M_1 nego u uzorcima skupljenim tijekom ljeta. U 13% uzoraka skupljenih tijekom zime nađeno je više od 0,5 µg/kg, dok je samo 3% uzoraka skupljenih tijekom ljeta prelazilo navedenu granicu. Obrano ili poluobrano mlijeko iz istih izvora sadržavalo je niže razine aflatoksina M_1 u usporedbi s punomasnim mlijekom.

Ključne riječi:
mikotoksini, mlijeko

Requests for reprints:

Edmond Panariti, Ph. D.
Institute of Veterinary Research
Tirana 10, Albania
E-mail: *panariti@icc.al.eu.org*