

Presence of Aflatoxin M₁ in Commercial Ultra-High-Temperature-Treated Milk

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Forty-seven samples of commercial ultra-high-temperature-treated milk from a dairy facility in the northwest part of Spain were analyzed for the presence of aflatoxin M₁. A total of 14 samples (29.8%) were positive for aflatoxin M₁ (4 in May, 3 in November, 3 in December, 1 in January, 1 in April, 1 in July, and 1 in August), 29 (61.7%) were negative, and 4 (8.5%) were doubtful, i.e., they showed trace quantities of aflatoxin M₁. The range of aflatoxin M₁ content was 0.02 to 0.1 ng/ml.

Several studies have been undertaken in different countries to determine the presence of aflatoxin M₁ in milk and dairy products. In view of these works, there is an evident seasonal influence on the presence of M₁ in milk. Various investigators discuss results which group the samples on the basis of the season in which they were obtained, generally observing in milk samples taken during winter a higher contamination level than in those sampled in summer (1, 4-12, 14; J. L. Blanco et al., *Abstr. 23rd World Vet. Congr.*, 1987, 4.2.6, p. 92; J. M. Freymy et al., *5th Int. Union Pure Appl. Chem. Symp.*, Vienna).

The province of León was chosen to carry out the testing for M₁ contamination of milk originating in the northern area of Spain, which has a humid climate similar to that prevailing in central Europe; this humid climate might give rise more easily to the molding of feeds for dairy cattle and consequently to contamination of milk with aflatoxins.

In our studies, we analyzed a total of 47 samples of commercial ultra-high-temperature-treated (130 to 150°C for 2 to 10 s) milk from a dairy facility in León, a province in the northwest region of Spain. Samples were collected throughout 1985, on days 1, 7, 14, 21, and 28 of each month except during June and September, when no sampling was done.

Each sample was analyzed by the technique described by us previously (3), with a recovery rate of 94% and a detection limit of 0.02 ng/ml. In this technique, aflatoxins are dissolved in the aqueous phase of milk after the milk is alkalized with a sodium bicarbonate solution. Bicarbonate is precipitated with methanol-acetone, leaving the aflatoxin M₁ in the methanol-acetone-water layer. Centrifugation at a low temperature precipitates proteins and lipids. Finally, aflatoxins are extracted with chloroform, and the extract is purified by adsorption chromatography. Aflatoxin M₁ is quantitated by thin-layer chromatography, and its presence is confirmed by the technique of Van Egmond et al. (13).

Samples were classified as positive, negative, or doubtful. We defined doubtful samples as those in which the presence or absence of M₁ could not be clearly confirmed after two analyses; we assumed that these samples corresponded to those in which M₁ was present in trace quantities. This circumstance may be due to an aflatoxin level in the samples below 0.02 ng/ml (detection limit) and the interference of certain substances in the detection of aflatoxin.

The results obtained on the distribution by month of the positive, negative, and doubtful samples and on the level of contamination in the positive samples are shown in Table 1.

Four samples positive for M₁ were registered during May, three positive samples each were found in November and December, and only one positive sample each was found in January, April, July, and August. We found one doubtful sample each in January, April, July, and October.

In terms of seasonal distribution, we found the highest number of positive samples in spring (five samples) and autumn (four samples), while in winter we detected three and in summer only two positive samples (Table 2).

The highest concentration of aflatoxin was registered in one December sample; it contained 0.1 ng/ml. Except for one sample in May with 0.08 ng/ml, another with 0.05 ng/ml in November, and the December sample mentioned above, the remaining positive samples had levels ranging between 0.02 and 0.04 ng/ml.

Milk samples were taken throughout 1 year to study a possible seasonal influence. Our experiments confirmed this influence, since the highest numbers of positive samples were found in spring and autumn. These data reflect the fact that during autumn and winter, animals are fed mixed feeds, the most common vehicle for aflatoxin B₁. The number of positive samples detected by us in winter is low compared with data from other countries.

There are no laws in Spain which regulate the allowable levels of aflatoxins in products for human consumption. However, legislation dictates a limit of 20 µg of aflatoxin B₁ per kg in feeds for dairy cattle (*Boletín Oficial del Estado* de 6 September 1976, p. 17,366). In view of the low levels of M₁ in milk detected by us, we may conclude that the feeds ingested by the milking animals did not exceed the lawful B₁ content, but we could affirm it only if it had been milk from a specific farm. Because it was commercial milk, the dilution process of aflatoxins has to be considered, since contaminated milk is mixed with uncontaminated milk. This suggests that the toxic milk could come from animals fed products in which the aflatoxin B₁ concentration is above 20 µg/kg.

Only two of the positive samples exceeded the limit of 0.05 ng/ml for commercial milk which is dictated in Switzerland (*Ordonnance du Departement Federal de l'Interieur* no. 817.024), probably the country with stricter legislation with respect to aflatoxins.

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TABLE 1. Distribution by month of commercial milk samples and aflatoxin M₁ concentration

Mo	No. of samples:				Contamination level(s) (ng/ml)
	Tested	Negative	Doubtful ^a	Positive	
January	5	3	1	1	0.02
February	5	5			
March	5	5			
April	5	3	1	1	0.025
May	5	1		4	0.02, 0.025, 0.032, 0.08
July	5	3	1	1	0.04
August	5	4		1	0.032
October	3	2	1		
November	4	1		3	0.02, 0.025, 0.05
December	5	2		3	0.022, 0.04, 0.1

^a Trace quantities of aflatoxin M₁.

Finally, there have been two previous follow-ups on the presence of aflatoxin M₁ in milk in Spain, as follows. Jodral et al. (7) did not detect any M₁ contamination in 330 commercial sterilized milk samples. However, M₁ was present in raw milk sampled on different farms in southern Spain (region of Andalusia) at levels slightly lower than ours; positive samples were found only in winter and spring. These differences may be due to the different climates prevailing in the south and north of Spain.

Burdaspal et al. (2) found an M₁ contamination rate in commercial milk of around 7.3%, with amounts, similar to ours, between 0.02 and 0.04 ng/ml.

These investigators attributed the low amounts to the low level of contamination in cattle feeds, while, as we have

TABLE 2. Seasonal distribution of commercial milk samples and aflatoxin M₁ concentration

Season	No. of samples:				Contamination levels (ng/ml)
	Tested	Negative	Doubtful ^a	Positive	
Winter	16	12	1	3	0.02, 0.022, 0.1
Spring	11	5	1	5	0.02, 0.022, 0.025, 0.032, 0.08
Summer	10	7	1	2	0.032, 0.04
Autumn	10	5	1	4	0.02, 0.022, 0.04, 0.05

^a Trace quantities of aflatoxin M₁.

previously mentioned, we believe it is a consequence of the effect of dilution of contaminated milk in the dairy plant when it is mixed with uncontaminated milk from different locations.

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LITERATURE CITED

1. **Blanc, M., and A. Karleskind.** 1981. Données sur la contamination par l'aflatoxine M₁ du lait et des produits laitiers en France. *Lait* 61:481-493.
2. **Burdaspal, P. A., T. M. Legarda, and I. Pinilla.** 1983. Incidencia de la contaminación por aflatoxina M₁ en leche. *Rev. Agroquim. Tecnol. Aliment.* 23:287-290.
3. **Domínguez, L., J. L. Blanco, E. Gómez-Lucía, E. F. Rodríguez, and G. Suárez.** 1987. Determination of aflatoxin M₁ in milk products contaminated at low levels. *J. Assoc. Off. Anal. Chem.* 70:470-472.
4. **Fremy, J. M., T. Cariou, and C. Terrier.** 1981. Evaluation de la contamination en aflatoxine M₁ dans le lait en poudre par HPLC en phase inversée. *Ann. Falsif. Expert. Chim.* 74:547-554.
5. **Fremy, J. M., and A. Gaymard.** 1980. Recherche d'aflatoxine M₁ dans les poudres de lactosérum. Evaluation saisonnière de la contamination. *Lait* 60:635-644.
6. **Heesch, W., A. Blüthgen, A. Tolle, and G. Engel.** 1981. Untersuchungen zum Vorkommen von Aflatoxin M in Milch und Milchpulver in der Bundesrepublik Deutschland. *Milchwissenschaft* 36:1-4.
7. **Jodral, M., G. Zurera, R. Jordano, L. M. Polo, and R. Pozo.** 1984. Investigación de aflatoxinas en leche natural, esterilizada y en polvo. *Arch. Zootec.* 33:189-198.
8. **Kiermeier, F., G. Weiss, G. Behringer, and M. Miller.** 1977. Über das Vorkommen und den Gehalt von Aflatoxin M₁ in Käsen des Handels. *Z. Lebensm. Unters. Forsch.* 163:268-271.
9. **Patterson, D. S. P., E. M. Glancy, and B. A. Roberts.** 1980. The "carry over" of aflatoxin M₁ into the milk of cows fed rations containing a low concentration of aflatoxin B₁. *Food Cosmet. Toxicol.* 18:35-37.
10. **Pfleger, R., and E. Brandl.** 1980. Aflatoxinrückstände in Trockenmilch österreichischer. *Wien. Tierärztl. Monatsschr.* 67:101-106.
11. **Polzhofer, K. P.** 1977. Aflatoxinbestimmung in Milch und Milchprodukten. *Z. Lebensm. Unters. Forsch.* 163:175-177.
12. **Sieber, R., and B. Blanc.** 1978. Zur Ausscheidung von Aflatoxin M₁ in die Milch und dessen Vorkommen in Milch und Milchprodukten. Eine Literaturübersicht. *Mitt. Geb. Lebensmittelunters. Hyg.* 69:477-491.
13. **Van Egmond, H. P., W. E. Paulsch, and P.L. Schuller.** 1978. Confirmatory test for aflatoxin M₁ on a thin layer plate. *J. Assoc. Off. Anal. Chem.* 61:809-812.
14. **Zimmerli, B., and O. Blaser.** 1979. Vorkommen von Aflatoxin M₁ in Milch. *Mitt. Geb. Lebensmittelunters. Hyg.* 70:287-293.