Partitioning Behavior of Aflatoxin M in Dairy Products

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When cows ingest contaminated feeds containing sufficient aflatoxin B_1 to yield a dose of 0.6-0.9 mg/day, they excrete in their milk a metabolite called aflatoxin M (1). The latter is nearly as toxic as aflatoxin B_1 and causes the same liver pathology in ducklings.

We have conducted surveys to determine the frequency of aflatoxin contamination in molded silage pits and in milk at local dairy farms. Twenty five percent of the silage samples contained fluorescent materials having chromatographic properties of aflatoxins B and G. Furthermore, five percent of milk samples examined contained a fluorescent entity with the R_f value of aflatoxin M. The presence of aflatoxin M in some milk samples from several dairy farms may have public health significance under two circumstances: 1) long term home consumption of toxic milk by families of dairymen; and 2) exposure of the general public to increased aflatoxin in milk products resulting from preferential solubility or adsorption of toxin in individual milk fractions (4,7).

The purpose of this study was to determine the distribution of aflatoxin M in cream, butter, and curd prepared from toxic milk. The findings may be of value in assessing the probable extent of human exposure to aflatoxin M during ingestion of dairy products obtained from the milk of cows feeding on aflatoxin-contaminated silage or other fodder.

MATERIALS AND METHODS

Aflatoxin M was produced by biological transformation of aflatoxin B_1 (Calbiochem, Los Angeles) administered as a single oral dose (0.2 mg/kg) to a barren ewe. Aflatoxin M was recovered from the urine by exhaustive extraction with chloroform and was purified by preparative channel layer chromatography on silica gel G in a solvent system of chloroform:methanol (97:3). Identification of aflatoxin M was based on its reported

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mobility(1) and on its toxicity in the brine shrimp bioassay(2). The method for biological transformation of aflatoxin B_1 to M was based on the studies of Nabney et al.(3).

Samples of whole milk, skim milk, or cream were spiked with known amounts of aqueous aflatoxin M for determining its distribution in the cream, butter, and curd fractions. Extraction of aflatoxin M from milk fractions and quantitative chromatographic analyses were performed with the methods of Roberts and Allcroft(1).

The partitioning behavior of aflatoxin M in a milk-cream system was determined by mixing equal volumes of skim milk and cream previously spiked with known amounts of toxin. The mixtures were placed on a rotary shaker at 4C for 2 hours to permit equilibration of the system. The samples were then held unshaken at 4C to allow creaming to occur. Skim milk was collected by siphoning for analysis.

The aflatoxin M contents of buttermilk, skim milk, whey, curd, and wash fractions were measured by analytical chromatography(1); the amounts remaining in butter and cream were estimated by difference. Overall recoveries of aflatoxin M added to samples of whole milk were 80-100 percent.

Laboratory procedures for the production of butter and cottage cheese were as follows:

Butter- Raw milk samples were pasteurized at 55-62C for 30 minutes. The pasteurized milk was placed at 4C for creaming and the skim milk was withdrawn by siphon. Churning was accomplished by placing 50 ml portions of cream in screw-cap dilution bottles mounted horizontally on a rotary shaker at 25C. After 16 hours agitation, the bottles were chilled in ice and the buttermilk collected by decanting. The butterfat was washed twice with 20 ml portions of ice water and kneaded with a spatula to aid removal of residual buttermilk.

Cheese- Pasteurized skim milk samples were placed in battery jars (200 ml per 2 liter jar). The milk was inoculated with 10 percent (v/v) of a commercial cottage cheese starter culture and incubated for 24 hours at 28C. The curd was cut into 1/4 inch cubes and heated for 30 minutes at 52C. The whey was collected by aspiration. The curd was allowed to drain thoroughly and was washed twice with 25 ml portions of cold water.

The aflatoxin B_1 chromatographic standard used for establishing R_f value and fluorescent intensity of aflatoxin M extracted from milk fractions was obtained from the Southern Utilization and Development Division of the United States Department of Agriculture, New Orleans, Louisiana.

RESULTS

When aflatoxin M was partitioned between equal volumes of skim milk and cream, 75 percent of the added amount was recovered in the skim milk fraction. Therefore, during normal creaming of milk contaminated with aflatoxin M, the cream fraction would contain only about 10 percent of the toxin initially present in the whole milk. However, if the milk were heavily contaminated with aflatoxin M, substantial amounts could still occur in batches of cream used for buttermaking.

Portions of pasteurized cream were spiked with known quantities of aflatoxin M. Following churning, the buttermilks and washings were analyzed for toxin content. Approximately 60 percent of the added aflatoxin M was recovered in the buttermilk; the washings contained 30 percent of the added toxin. Thus, the amount of aflatoxin M remaining in the butterfat was not greater than 10 percent of that present in the cream.

Cottage cheese was produced from skim milk samples to which known amounts of aflatoxin M had been added. The cheese-making process was similar to commercial methods except that no rennin was added for accelerating curd formation. The curd fraction, comprising 20 percent by volume of the beginning material, contained approximately 50 percent of the added aflatoxin M. The whey contained 40 percent of the initial toxin; the washings contained only 10 percent.

DISCUSSION

It can be calculated from the experimental data that cream obtained from milk contaminated with aflatoxin M would contain approximately 10 percent of the toxin initially present. If butter were produced from such cream, only 10 percent of the toxin present would remain associated with the butterfat. Therefore, the fat fraction obtained from toxic milk would rarely contain more than a trace of aflatoxin M, a finding in agreement with experiments described by Allcroft(7).

The partitioning behavior of aflatoxin M between skim milk and cream reflects an affinity for the casein fraction, a characteristic already noted by other investigators(6). In fact, statements in the literature(4,5,7) lead to the prediction that virtually complete adsorption or occlusion of aflatoxin M would occur during curd formation. However, we found that when curdling and subsequent processing were carried out with simulated commercial procedures for cottage cheese manufacture, about 50 percent of added aflatoxin M was recovered in the whey and washings. The aflatoxin M content, per

unit volume, was increased by a factor of 2.5 rather than the predicted value of 5 during manufacture of cottage cheese from contaminated milk. It is unlikely that human exposure to aflatoxin M is disproportionately increased during normal consumption of cottage cheese prepared from toxin-contaminated milk. However, the occurrence of any aflatoxin in milk and in the silage fed to dairy cows is a disturbing finding in view of the possible cumulative effects in humans of continuous exposure to low levels of aflatoxin M. It appears essential to exercise simple preventive measures such as the use of covered rather than open silage pits at dairy farms, and the discarding of visibly molded surface layers of silage to minimize biotransfer of aflatoxins to humans.

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