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# Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin $M_1$ in raw milk in the North of Paraná state

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**Abstract**

Moulds occur in a great variety of foods, including the concentrated and roughage destined for animal feeding, and can produce mycotoxins under certain conditions. The ingestion of mycotoxin $AFB_1$ by dairy cattle leads to the biotransformation of that substance, which is eliminated via milk as $AFM_1$, and also causes damage to human health. The present study aimed to analyze the presence of aflatoxins in foodstuff destined for dairy cattle and in the milk produced by these animals. The contamination of the foodstuff by aflatoxins happened mainly in the feeds and the silages did not present contamination by aflatoxins. Out of the 42 samples of milk analyzed, 10 (24%) were contaminated by $AFM_1$, and 3 (7%) were above the 0.5 μg/l limit.


*Keywords:* Mycotoxin; Aflatoxin; $AFB_1$; $AFM_1$; Dairy cattle

## 1. Introduction

Aflatoxins are toxic metabolites produced mainly by the moulds *Aspergillus flavus* (*A. flavus*) and *A. parasiticus* and can be found in several types of foods destined both for human and animal consumption. They present carcinogenic, teratogenic and mutagenic potential, causing great damage to the human health and high economic damage in the revenue of domestic animals (Lazzari, 1997). Aflatoxin $B_1$ ($AFB_1$) presents the highest degree of toxicity for animals, followed by the aflatoxins $M_1$ ($AFM_1$), $G_1$ ($AFG_1$), $B_2$ ($AFB_2$) and $G_2$ ($AFG_2$) (Gourama and Bullerman, 1995). $AFM_1$ is a product of the biotransformation of $AFB_1$, and can be found within 12–24 h after the first ingestion of $AFB_1$ (Stoloff, 1977). Studies show that the amount of $AFM_1$ excreted in cow milk varies from 1 to 3% of the amount of $AFB_1$ ingested (Masri et al., 1969; Polan et al., 1974), although values of 0.43% have been reported by McKinney (1973), and up to 6% by Pitet (1998). A recent study by Battacone et al. (2003) in lactating ewes shows that $AFM_1$ can be found 12 h after first ingestion of $AFB_1$, and last up to three days after last ingestion of the mycotoxin. The authors also observed a linear relationship between $AFB_1$ and $AFM_1$ concentrations, but the carryover was lower than those reported for dairy cattle, suggesting sheep have a better ability to degrade $AFM_1$.

The maximum limit of aflatoxins allowed in grains varies according to the legislation of each country. The limits allowed in Brazil for foods destined to human consumption are 20 μg/kg of total aflatoxin ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) for corn in grain (whole, parted, kneaded and crushed), flours or cornstarch, peanut and by-products, and 0.5 μg/l of $AFM_1$ in fluid milk

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(National Agency of Sanitary Surveillance—ANVISA, 2002). The Ministry of Agriculture, Livestock and Provisioning of Brazil adopts the limit of 50 μg/kg for foods destined for animal consumption (direct ingestion or raw material of rations). (Ministério da Agricultura, Pecuária e Abastecimento, Brasil, 2002.)

The common legislation of countries constituting the MERCOSUL follows the limits determined by ANVISA, where the maximum limit of aflatoxins allowed is 20 μg/kg.

The objective of this study was to evaluate the occurrence of aflatoxins in foodstuff supplied to dairy cattle on farms producing type B milk in the North of Paraná state, Brazil, and the presence of $AFM_1$ in the milk produced by these animals. The dairy industry is an important economic sector of the North of Paraná State, and although several studies regarding the occurrence of $AFM_1$ throughout Brazil are available, none of them were conducted in this region.

## 2. Material and methods

### 2.1. Sampling

From July 2001 to November 2002, 98 samples of animal feeds, roughage (silage) and concentrated meals (feeds and grains) supplied to dairy cattle were collected for the analysis of aflatoxins B and G, and 42 samples of raw milk for the analysis of detection of $AFM_1$, on 40 farms producing type B milk, located in 12 municipal districts in the North of Paraná State.

The silage samples were collected at nine different points in the silo: three points in the upper third of the silo, three points in the middle third and three points in the lower third. The collected material from the nine points was homogenized and analyzed in an aliquot of 100 g, representative of the exposed face of the silo. The samples were placed in plastic bags, and transported in styrofoam boxes with recyclable ice and stored in freezer (−20 °C) until processing.

The collection of concentrated meal samples was conducted in the barns where the meals were stored, or in grain silos. In the case of concentrated meals stored in sacks, random samples from the sacks destined for the next feeding time of the animals were collected, and in the case of grain silos, the samples were collected by the "moving flow" method, with a recipient passed every 2 min through a flow of grains for a period of 10 min. In both cases, the sub-samples were mixed and an aliquot of 100 g was taken, which was placed in a paper bag and maintained at room temperature until processing.

The raw milk samples were collected directly from the cooling tanks of the farms. After homogenization of the content of the cooling tank, aliquots of 30 ml were removed, placed in amber glass flasks and maintained refrigerated (4 °C) until processing and analysis. The samples were centrifuged for 10 min to 3500 g at 10 °C. After centrifugation, the superficial layer of fat was removed with a Pasteur pipette and the skimmed milk was frozen (−20 °C) for subsequent analysis.

### 2.2. Analytic methodology

The samples of foodstuffs collected were analyzed with the thin layer chromatography (TLC) methodology described by Soares and Rodriguez-Amaya (1989) and adapted by the Adolfo Lutz Institute/São Paulo (1996).

The standards used for the TLC technique were $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ (Sigma Inc.—USA) at the concentrations 2.55, 2.62, 2.45 and 4.55 μg/ml, respectively, prepared according to the methodology of the Association of Official Analytical Chemists (AOAC) also adapted by the Adolfo Lutz Institute (1996).

The detection limit for aflatoxins B and G of the method is 2 μg and the determination limit is 4 μg. The evidence for the presence of aflatoxins was found through a chemical reaction test with trifluoroacetic acid, according to the methodology described by the Adolfo Lutz Institute/São Paulo (1996).

The milk samples were analyzed in duplicate with the immunoenzimatic assay Ridascreen® Fast Aflatoxin $M_1$, produced by R-Biopharm/Germany. The reading was accomplished with the use of a 450 nm wavelength spectrophotometer, and the result expressed by the means of the two analyses.

The absorbance values obtained for the standards and samples were divided by the absorbance of the first standard (zero standard) and multiplied by 100. Therefore, the zero standard is considered 100%, and the absorbance values are expressed in percentage. The detection limit of the test is 245 ppt (ng/l).

## 3. Results and discussion

The result of the analyses of aflatoxins in foodstuff destined for animal consumption can be observed in Table 1. The contamination by aflatoxins could be detected in seven (26%) of the 27 samples of commercial feed, eight (53%) of the 15 samples of prepared feed and two (100%) of the samples of corn bran analyzed. None of the samples of roughage presented contamination by aflatoxins.

Table 2 presents the levels of $AFM_1$ found in the positive samples. Out of 42 samples of raw milk analyzed, ten (24%) were contaminated by $AFM_1$, being three (7%) of these 42 above the maximum limit allowed of 0.5 μg/l established by ANVISA. These results differed from those found by other authors in Brazil. Corrêa et al. (1997) did not verify any positive sample among the 144 samples of raw milk analyzed in four areas of