J. Dairy Sci. 90:3197–3201
doi:10.3168/jds.2006-565

Aflatoxin M_1 and Ochratoxin A in Raw Bulk Milk from French Dairy Herds

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ABSTRACT

Mycotoxins in milk are a public health concern and have to be regularly monitored. A survey on the presence of aflatoxin M_1 (AFM_1) and ochratoxin A (OTA) in raw bulk milk was conducted in 2003 in the northwest of France, the main French milk-producing basin. Randomly selected farms (n = 132) were characterized by a diet based on corn silage and containing a large proportion of on-farm produced cereals, feeding sources that are frequently contaminated by mycotoxins. Farms were surveyed twice in winter and in summer. At each sampling time, a trained surveyor completed a questionnaire recording farm management procedures and production traits. The AFM_1 was found in 3 out of 264 samples but at levels (26 ng/L or less) that are below the European legislation limit of 50 ng/L. Traces of AFM_1 (less than 8 ng/L) were also found in 6 other samples. The OTA was detected in 3 samples also at low levels, 5 to 8 ng/L. Farms that tested positive to the presence of mycotoxins, 12 in total including 6 farms that had traces of AFM_1, differed from negative farms by a more extensive use of total mixed rations, 58 vs. 27%. In addition, the positive farms tended to have lower milk yields. Although the incidence of milk contamination with AFM_1 and OTA at the farm level was low during the period studied, production and management data from the surveyed farms suggest a link between feeding management practices and mycotoxin contamination.

Key words: mycotoxin, milk safety, French dairy herd, feeding management

INTRODUCTION

Aflatoxins and ochratoxins are highly toxic secondary metabolites produced by several *Aspergillus* and *Penicillium* species that can be found in cow's milk. Aflatoxin M_1 (**AFM_1**), the major metabolite of aflatoxin B_1 (**AFB_1**), and ochratoxin A (**OTA**) are classified by the International Agency of Research on Cancer as class 2B, possible human carcinogens (IARC, 1993). Aflatoxin M_1 level in milk and milk products is regulated in several countries; the European Union limit of 0.05 μg/kg being one of the lowest in the world (EFSA, 2004). In contrast, no regulation for OTA in milk exists, even though it has been suggested that the level of this toxin in cow's milk may exceed the tolerable daily intake of 5 ng/kg of BW per day for small children in some areas in Norway (Skaug, 1999).

The presence of mycotoxins in dairy products reflects the contamination of feedstuffs. Following ingestion of contaminated feeds, OTA is largely transformed by rumen microorganisms into the less toxic metabolite ochratoxin α (**OTα**; Kiessling et al., 1984). Ochratoxin A and OTα are mainly eliminated in the urine and feces, but they can also be found in milk. In contrast, AFB_1 is poorly degraded by rumen microorganisms (Kiessling et al., 1984). Absorbed AFB_1 is principally metabolized in the liver into AFM_1, a metabolite as toxic as the parent toxin, which appears in milk. The amount of AFM_1 found in milk represents normally 1 to 2% of the ingested AFB_1. However, it can be as high as 6% in high-producing cows (Veldman et al., 1992). For OTA, no information is available on the rate of transfer of this toxin into milk for dairy cows. In dairy sheep, the carryover is less than 1% (Boudra et al., 2005). Among the various feedstuffs susceptible to mycotoxin contamination, cereals and cereal by-products are the major source of AFB_1 and OTA. Aflatoxins are predominantly produced in hot climates. In temperate countries, the strict control of imported feeds has limited the cases of milk contamination above the maximum tolerable limits. However, the presence of aflatoxin was recently reported in feeds grown in Europe (EFSA, 2004). The emergence of aflatoxin in the European continent is concurrent with the increase in the annual average temperature registered in the past decade.

The presence of mycotoxins, aflatoxins in particular, is tightly controlled by feed manufactures and, conse-

Received August 30, 2006.
Accepted March 10, 2007.
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quently, contamination in commercial feeds is rare. A major risk of contamination, however, arises from cereals and forages, such as corn silage (Scudamore and Livesey, 1998; Garon et al., 2006), that are produced on the farm and are exposed to adverse climatic conditions or improperly conserved.

Investigations on milk AFM_1 contamination are regularly conducted in EEC countries (EFSA, 2004), but there is little information on the contamination of milk by other mycotoxins. Ochratoxin A is frequently found in Europe in feeds and foods of plant origin (Pittet, 1998; Scudamore and Livesey, 1998; Araguas et al., 2005). Occurrence of OTA in animal products has also been reported, particularly in meat and offal from pigs (Dragacci et al., 1999; Matrella et al., 2006). However, there is scarce information on the presence of OTA in cow's milk (Breitholtz-Emanuelsson et al., 1993; Valenta and Goll, 1996; Skaug, 1999), and no data are available in France concerning milk contamination by this mycotoxin.

The present study examined the presence of AFM_1 and OTA in bovine milk collected from French dairy herds receiving a diet of on-farm-produced cereals and corn silage, a feeding system that maximizes the risk of mycotoxin exposure.

MATERIALS AND METHODS

Farm Selection

The dairy farms studied were in 4 regions (Aquitaine, n = 34; Brittany, n = 33; Poitou-Charentes, n = 32; and Pays de Loire, n = 33) of western France, the main area in France for both corn and milk production (Sigwald and Dervishi, 2005). Farms were randomly selected from the national DHIA database according to the following criteria: Holstein breed, more than 20 cows, and corn silage constituting at least 50% of the dietary DM. The study did not involve any payment to the selected farmers, who were considered good managers and were advised regularly by competent technicians.

Data and Sample Collection

The DHIA technicians conducted 2 questionnaire surveys (February to March 2003 and September to October 2003) on the 132 selected farms. For each survey, the technicians collected data concerning farm characteristics, feeding practices, milk production, presence of molds in forages, and health problems (suspected or confirmed) due to mycotoxins. Moreover, the DHIA surveyors gave insight into the quality of the feeding management, and they collected raw bulk milk samples (n = 264). Milk samples were preserved by adding 1.5 μg/mL of NaN_3, kept on ice during transport from the farm to the laboratory, and stored at –20°C until analysis.

Mycotoxin Analysis

The mycotoxins AFM_1 and OTA were analyzed using the official French method (NF EN ISO 1451; Dragacci and Grosso, 2001) and the method of Boudra and Morgavi (2006), respectively. Briefly, for AFM_1, 50 mL of filtered milk was applied to an immunoaffinity column (**IAC**, Aflaprep, R-Biopharm, Lyon, France). The IAC was then washed with 20 mL of water, and AFM_1 was eluted with 4 mL of CH_3CN. For OTA, 10 mL of acidified (pH < 2) milk samples were extracted with 10 mL of $CHCl_3$. The toxin in the organic layer was back-extracted with 8 mL of PBS, pH 7.6, and the top aqueous layer was then loaded into an IAC (Ochraprep, R-Biopharm). Ochratoxins were slowly eluted with 3 mL of CH_3OH.

The purified extracts of AFM_1 and OTA were evaporated to dryness at 45°C under a stream of N gas, and the dried residues were redissolved in 200 μL of mobile phase by incubation in an ultrasonic bath for 3 min. A 50-μL volume of these extract solutions was injected into a HPLC system (Thermo Finnigan, Paris, France) equipped with an automatic sampler (Spectra-Physics, Paris, France) and a fluorescence detector (FL-3000, Thermo Finnigan). Separation was performed at room temperature on a Nucleodur C_{18} gravity column (125 × 4.6 mm, 5 μm, Macherey Nagel, Lyon, France), using an isocratic mobile phase pumped at a flow rate of 1 mL/min. The mobile phase was CH_3CN:distilled water (25 to 75 ratio) and CH_3CN:10% acetic acid (54 to 46 ratio) for AFM_1 and OTA, respectively. For fluorescence detection, excitation and emission were respectively set at 365 and 435 nm for AFM_1 and at 274 and 440 nm for OTA. Recovery and linearity were tested for both mycotoxins. In addition, the OTA analytical method was fully validated in terms of precision, accuracy, sensitivity, and stability as described previously (Boudra and Morgavi, 2006).

The calibration curve was determined daily, using a series of standard solutions in mobile phase containing different levels of each mycotoxin. The concentration of OTA and AFM_1 was calculated by using the following formula: $concentration\ (ng/mL) = \frac{M \times Vd}{V \times Vi}$, where M = the mass (ng) injected into the HPLC system; V = the volume of milk (mL) taken for analysis; Vd = the volume (mL) used to dissolve the dried extract; and Vi = the volume (mL) injected into the HPLC system. The limit of quantitation was calculated by using a signal-to-noise ratio of 3:1, and it was defined as the lowest con-