Absence of detectable fumonisins in the milk of cows fed *Fusarium proliferatum* (Matsushima) Nirenberg culture material

J.L. Richard$^1$, G. Meerdink$^2$, C.M. Maragos$^1$, M. Tumbleson$^2$, G. Bordson$^2$, L. G. Rice$^3$ & P.F. Ross$^3$

$^1$Mycotoxin Research, National Center for Agricultural Utilization Research, USDA/Agricultural Research Service, 1815 N. University Street, Peoria, IL 61604; $^2$Laboratories of Veterinary Diagnostic Medicine, University of Illinois, Urbana, IL 61801; $^3$National Veterinary Services Laboratories, APHIS, 13th & Dayton, Ames, IA 50010, USA

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Abstract

Fumonisins, a group of mycotoxins produced by the ubiquitous fungi *Fusarium moniliforme* and *F. proliferatum*, were first identified about eight years ago. They have been shown to cause a variety of health effects in animals, including epidemiological evidence of esophageal cancer in humans. Cattle are less sensitive to ill effects than horses and swine. Fumonisins are common contaminants of low quality grain fed to cattle. Culture material containing fumonisins (FB1, FB2, and FB3) was mixed into the total diet and fed for 14 days to two midlactation Jersey cows to determine if fumonisins are excreted in milk. The dietary equivalent of fumonisin was approximately 75 ppm and the two cows consumed an average of 3 mg fumonisin B1/kg body weight (bwt)/day. Fumonisins were not detected in any of the milk samples by two analytical laboratories using methods with a sensitivity of 5 ng/ml. Except for transient diarrhea at the beginning of the contaminant feeding period and an increase in serum cholesterol, clinical and hematologic changes were not observed in the animals. The appearance or carry over of fumonisins from feed to milk in dairy cows does not appear to be significant and likely not a hazard or food safety concern for humans.

Key words: fumonisins, milk, *Fusarium*, cows, analysis

Introduction

Since the discovery of the fumonisins, produced by *Fusarium moniliforme*, [1] and their ability to produce equine leukoencephalomalacia [2], porcine pulmonary edema [3], and liver cancer in the rat [4] there has been considerable concern for the presence of these mycotoxins in human foods. *F. moniliforme* and *F. proliferatum* [5] occur primarily in corn. A number of investigators found that corn meal and corn grits are frequently contaminated with fumonisins [6]. Another concern for the presence of fumonisins in a human food is their potential presence in milk from animals consuming a fumonisin-contaminated diet.

To detect the presence of fumonisins in milk, Maragos and Richard [7] developed a sensitive HPLC method and determined that the fumonisins were stable in spiked milk samples. Also, they conducted a limited survey of Wisconsin milk samples and found only one of 155 samples that had any detectable level (near the limit of detection) of fumonisins. Others have conducted experiments to determine if there was a carry over of fumonisins in milk in cows after a single IV or oral dose of purified fumonisin B1 [8]. From these studies, no detectable fumonisins were found in the milk during the three days after dosing even though the highest oral dose (5 mg FB1/kg bwt) was equivalent to 125 ppm in the food consumed by the cow during that day.

To determine the potential for the excretion of fumonisins into bovine milk, we added *F. proliferatum* culture material to the diet of two midlactation Jersey cows. The dietary equivalent of fumonisin was approximately 75 ppm and the two cows consumed an average of 3 mg fumonisin B1/kg body weight (bwt)/day. Fumonisins were not detected in any of the milk samples by two analytical laboratories using methods with a sensitivity of 5 ng/ml. Except for transient diarrhea at the beginning of the contaminant feeding period and an increase in serum cholesterol, clinical and hematologic changes were not observed in the animals. The appearance or carry over of fumonisins from feed to milk in dairy cows does not appear to be significant and likely not a hazard or food safety concern for humans.
tum culture material to the total mixed diet to provide a dietary concentration of 75 ppm fumonisin B1 for 14 days to two dairy cows during midlactation. Milk samples were collected at scheduled intervals throughout the study period. This experimental design was selected to provide an optimum opportunity for the fumonisins to occur in the milk of cows when exposed by a natural route.

**Material and methods**

**Animals.** Two gravid Jersey cows which were 130 days into their second and third lactation periods were used. They averaged 400 and 413 kg body weight (bwt) through the 28-day study period. The cows were housed in individual stanchion stalls and given water ad libitum. The cows were milked twice daily in a parlor system and otherwise handled as routine in the 400 cow dairy. The animals were fed a corn silage-based total mixed diet, ad libitum, with a daily weigh-back of the amount not consumed.

**Culture material.** The culture material used in this study was prepared as previously described [9]. Briefly, batches of 500 g of yellow dent corn were inoculated with F. proliferatum (M-5991) and incubated in the dark at 20–22 °C for 4 weeks. After incubation, cultures were soaked in chloroform-acetone (50 : 50, v/v) overnight, then filtered and air dried for 24–48 h. The dried culture material was ground to a uniform consistency and analyzed by HPLC for fumonisin content [10]. The culture material used for this experiment contained 5018 ppm fumonisin B1 (FB1), 1066 ppm FB2 and 320 ppm FB3.

**Experimental design.** Prior to the study the cows were consuming approximately 23 kg food/cow/day. To provide a dietary concentration of 75 ppm of FB1, 340 g of culture material (1.7 g FB1) was mixed with 1.3 kg of cracked corn to formulate a premix. The corn-culture material was preweighed and bagged by individual daily dose of 1.7 g FB1 for daily addition to the total mixed diet. This daily dose of culture material was added for 14 days (day 0 through 13).

Milk samples were collected from the AM milking two days prior to the culture material addition, from the AM and PM milking for day 0 (1st day of culture material feeding) through day 2 and from the AM milking of day 3 through day 22 of the experiment. Three milk samples were collected into wide mouth plastic jars from the weigh jar from each cow at each sampling time and were immediately refrigerated. One sample each went to the two analytical laboratories for fumonisin analysis and the third was frozen for potential future need.

Feed consumption and milk production data were recorded daily; cows were weighed weekly. The cows were observed daily and examined clinically (including temperature, pulse, respiratory rate and rumen motility). Blood and urine samples were collected at scheduled intervals during the feeding and observation periods.

**Fumonisin analyses.** Samples of milk were analyzed for fumonisins B1 and B2 using a previously described procedure with a sensitivity of 5 ng/ml [7]. Briefly, 5 ml samples of milk were extracted with a mixture of methanol-acetone, and then fumonisins B1 and B2 were isolated from the organic extract by using solid phase extraction columns with a strong anion exchange bonded phase. The fumonisins were derivatized with naphthalene-2,3-dicarboxaldehyde and separated by reverse phase HPLC. The fluorescent products were quantitated relative to fumonisin B1 and B2 standards derivatized concurrently with the samples.

**Results and discussion**

Both cows evidently detected the change in food because consumption decreased significantly. Based on the weight of food consumed each day, the two cows averaged an FB1 intake of 1.1 and 1.2 g/head/day (of the 1.7 g FB1 available). During the first 10 days of the culture material feeding period, intake was erratic; the daily dose of FB1 ranged from 1.8 to 4.3 mg/kg bwt with averages of 2.9 and 3.1 mg/kg bwt for the two cows. (If each had been consuming her usual 23 kg/day, the intake would have been equivalent to 50 ppm dietary concentration of FB1.) Although the amount of intake was less than intended, 50 ppm FB1 in the total diet is very high and in a 14-day feeding trial adequate for the determination of the potential presence of fumonisin in milk. Like food consumption, milk production of both cows decreased by about 20% during the first week of the feeding trial. This decrease was likely due to the erratic and decreased food consumption and both animals were back to their pretrial level of production before the end of the 14-day contaminate food period.