Excretion of Deoxynivalenol and Its Metabolite, DOM-1, in Milk, Urine, and Feces of Lactating Dairy Cows

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Summary

Corn contaminated with deoxynivalenol (DON) was added to the diets of 3 dairy cows for 5 days. Total milk, urine and feces were collected for 4 days prior to, during, and 3 days following the feeding of DON-contaminated diets. DON was added to the diets twice a day at feeding time. The dietary concentrations of DON averaged 66 ppm. Following exposure to DON-contaminated diets, DOM-1, a metabolite of DON, was present in milk at concentrations up to 26 ppb. DON was not detected in the milk. Approximately 20% of the DON fed was recovered in the urine and feces in the unconjugated forms as DOM-1 (96%) and DON (4%). After incubating urine with B-glucuronidase, the concentration of unconjugated DOM-1 increased by 7-15 fold while unconjugated DON increased 1.6-3 fold.

Detectable concentrations of DOM-1 were found in urine and feces up to 72 hours after the last oral exposure. Thus, excreta are the diagnostic specimens of choice for the determination of DON exposure in cows.

Feeding DON-contaminated diets for 5 days did not alter feed intake or milk production, nor were the milk concentrations of calcium, phosphorous, sodium, potassium, magnesium or nitrogen altered.

Introduction

Deoxynivalenol (DON, vomitoxin) is a trichothecene mycotoxin that frequently contaminates corn, wheat and barley in temperate climates. It is produced by many species of *Fusarium* especially *F. graminearum* during those years when the harvest season is wet and cold. Large scale outbreaks have occurred in corn crops of North America in 1966, 1972, 1975, 1977, and 1981 (6, 16, 19, 20, 21). Most of the detrimental effects from the feeding of DON-contaminated grain to livestock have been reported in swine and include feed refusal, decreased feed efficiency, reduced weight gain, emesis, depletion of hepatic glycogen and hypoglycemia (4, 6, 13, 20). It has been suggested that both dairy and beef cattle are relatively insensitive to DON-contaminated diets (9, 17). A recent survey in North Carolina, however, revealed a close correlation between DON in the diet of dairy cattle and a drop in milk production, as well as a reduced conception rate (2).

Because swine are sensitive to DON, livestock producers have been encouraged to feed DON-contaminated grains to more tolerant species such as cattle. This practice raises a question as to whether this mycotoxin or its metabolite can be transmitted to the milk. Recently Prelusky et al. (11) reported that DON was not transmitted to the milk of 2 lactating dairy cows given single bolus oral doses of 920 mg DON (as an extract of DON-contaminated corn). This dose was equivalent to about 2 mg/kg body weight.

The study reported herein was conducted to determine the excretion patterns of DON and its metabolite, DOM-1, in milk, urine and feces of lactating dairy cows fed DON-contaminated diets.

Materials and Methods

Experimental Design

A group of three dairy cows in the same stage of lactation (2 Holsteins, 1 Ayrshire), each weighing about 450 kg were fed a commercial dairy concentrate and mixed fescue-alfalfa hay in a ratio of 1:2 during a 10-day acclimation period. The animals were fed twice daily at milking time (8:00 and 16:00).

On day 1 of the experiment each cow was stanchioned and equipped with a 3 meter, 7.5 cm i.d. rubberized flexible hose for urine collection. One end of the hose was fitted over the vulva and the other end was attached to a 12 L plastic container in a fashion similar to that described by Vinet (22). This way, urine was collected without cross-contamination with feces. The feces were allowed to be voided on the concrete floor from which they were shovelled into a galvanized tub and mixed. Beginning at the 8:00 milking time on day 1 and continuing through day 4, control urine and feces voided prior to the respective milking times were mixed, weighed and an aliquot (750 ml urine, 500 g feces) of each was taken for analysis. Aliquots of milk (450 ml) from each cow were also taken for analysis.

At the 8:00 milking time on day 5 and continuing for 5 days *F. graminearum* innoculated corn (Supplied by H. L. Trenholm, Animal Research Center, Agriculture Canada, Ottawa, ONT.) containing approximately 1300 ppm DON was added to the commercial ration to provide a dietary concentration averaging 66+ 11 (S.E.) ppm. The DON-contaminated corn was kept frozen because of its high moisture content (60% dry matter). At each feeding time the total ration for all 3 cows was weighed and the frozen DON-contaminated corn was added to the grain concentrate and mixed. The concentrate containing the DON was then apportioned to each cow according to her milk production. Milk, urine, and feces were weighed, mixed and sampled as during the control period.

Feed, Milk, Urine and Feces Analyses

Three representative samples of the *Fusarium* innoculated corn were analyzed for DON and DOM-1 as described below. These 3 corn samples plus a representative sample of the grain concentrate used in the 12-day feeding study were also analyzed for T-2 toxin, diacetoxyscirpenol, zearalenone, and aflatoxin B1, B2, G1, and G2 using thin layer screening procedures (12).

Representative samples of the grain mixture fed the cows at each milking time were taken on days 1 through 12, ground in a Wiley mill and analyzed for DON using a modification of the method described by Chang et al., 1984.

Representative samples of milk from each cow at each milking time were analyzed for calcium, magnesium and sodium using atomic absorption spectroscopy (10). Phosphorus was determined by UV spectrophotometry (14) and nitrogen content was determined using a Kjeldahl method (1).

All milk samples were frozen (-20° C) and subsequently analyzed for DON and DOM-1. Special effort was made to thaw the samples and mix the particulate materials by placing the milk container in a shaking waterbath at 37° C. A gas chromatographic (GC) procedure for analysis of DON and DOM-1 was developed in our laboratory (15).

Gas chromatographic procedures for the analysis of DON and DOM-1 in urine and feces were performed according to the method of Dahlem et al. (7).

Conjugation Study

Representative urine samples from Cow #1 taken during and following the DON exposure period were incubated with B-glucuronidase according to the method of Corley et al. (5).

Results

Feed Analysis

The inoculated corn contained approximately 1300 ppm DON, but no detectable DOM-1. No T-2 toxin, diacetoxyscirpenol, zearalenone, nor aflatoxin B1, B2, G1, and G2 were detected in the samples of concentrate after addition of the DON-contaminated corn.

Animal Performance

There were no differences ($P<0.05$) in feed consumption and milk production by the cow fed DON-free diets when compared to the 5 days they were fed DON-contaminated diets. The daily average weights of milk produced during the 12-day trial were: 19.2±2.1, 14.5±0.9 and 11.7±1.0 kg for cows 1, 2 and 3, respectively. The composition of the milk in terms of nitrogen content, calcium, phosphorus, sodium potassium and magnesium remained the same before, during and after exposure to DON. There were no signs of adverse effects observed in the cows at any time during the experiment. Their body weights remained the same at the end of the 12-day experiment as they were at the beginning.

Residues in Milk, Urine and Feces

No DON was detected in any of the milk samples during the 12-day feeding trial (detection limit, 1ppb). DOM-1, however, was detected in milk of all 3 cows during the 5-day period of feeding DON-contaminated diets and was confirmed by GC-MS (23). The concentrations of DON-1 tended to be greater in the PM milk, ranging up to 26 ppb. Higher concentrations of DOM-1 were consistently found in the milk of cow #1 than in the milk of the other two cows. DOM-1 was still detectable in the milk 16 hours after the last feeding of DON, but not after 24 hours.

Unconjugated DON and DOM-1 were detected in the urine and the feces of all 3 cows during the 5-day DON-feeding period. The concentration and total excretion of DON was negligible, however, compared to that of DOM-1 (4% DON, 96% DOM-1; 59 mg DON, 1697 mg DOM-1; see Table). Thus, approximately 20% of the DON fed over the 5 day period was excreted as unconjugated DON and DOM-1 via these routes. Detectable concentrations of unconjugated DOM-1 were recovered in both the urine and feces from cow 1 for 3 days after the last feeding of DON, and for 40 hours in the case of cows 2 and 3. Since the total amount of DON fed varied with each feeding during the 5-day period, the concentrations of DON and DOM-1 in the urine and feces had large variation.

Glucuronied Conjugation of DON and DOM-1 in Urine

Representative samples of all of the urine collections from cow 1 were analyzed for both unconjugated and total DON and DOM-1 after incubation with and without B-glucuronidase. After enzyme incubation, the concentration of DON increased by factors ranging from 1.6 to 3, while DOM-1 increased by 7-15 fold.

Discussion

This is the first report of DOM-1 transmission to milk.

Prelusky et al. (11) reported the non-transmission of DON in milk of 2 cows given single oral boluses of 920 mg DON (approximately 2 mg/kg). In our study, 3 cows were fed a calculated total amount of 168 to 950 mg DON twice a day for 5 days, which is equivalent to 56-317 mg per cow (approximately 0.1-0.7 mg/kg). Although the cows in our study were fed considerably less DON at each feeding than those in the study by Prelusky et al. (11), they were fed multiple rather than single doses. The results reported herein confirm their findings with regard to DON. However, in our study the milk was also analyzed for DOM-1.

Since our studies with B-glucuronidase indicate that the concentration of unconjugated DOM-1 in urine increased by 7- to 15-fold following enzyme incubation, there may be more conjugated than unconjugated DOM-1 in milk of animals that have consumed DON-contaminated diets.

The public health significance of finding up to 26 ppb unconjugated DOM-1 in milk of cows fed DON-contaminated diets is undetermined. In view of the reduced survival and weight gain in pups of DON-exposed pregnant and lactating mice (8) together with the detrimental effects on the immune response systems in mice given DON(18), even the low concentrations of DOM-1 transmitted in milk suggest that additional studies should be undertaken to determine the public health implications of DON and DOM-1.

In this study most of the DON consumed was excreted in the urine and feces as DOM-1. Approximately 20% was excreted as the unconjugated metabolite. The remaining 80% may have been excreted as a glucuronide conjugate (at least in urine). This has significant diagnostic value, since unconjugated and conjugated DOM-1 were found in urine, and unconjugated DOM-1 was found in feces as long as 3 days after DON-contaminated feed was discontinued. Thus, to estimate the retrospective consumption of DON by dairy cows one should test both urine and feces for the unconjugated and conjugated DOM-1 metabolite as well as the parent compound.

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