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Bioavailability of aflatoxin B_1 and ochratoxin A, but not fumonisin B_1 or deoxynivalenol, is increased in starch-induced low ruminal pH in nonlactating dairy cows

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ABSTRACT

High-production dairy and beef systems require diets rich in starch. This practice may induce ruminal acidosis and also increase exposure to mycotoxins because starches in starch-rich diets are the main vehicles of mycotoxin contamination. The aim of this study was to investigate the effects of low ruminal pH on the bioavailability of 4 major mycotoxins [i.e., aflatoxin B_1 (AFB1), ochratoxin A (OTA), deoxynivalenol (DON), and fumonisin B_1 (FB1)]. Eight nonlactating dairy cows fitted with rumen cannulas were used in a double crossover experiment. The trial was divided into 4 periods with 2 periods per crossover. Cows were divided into 2 groups receiving a low (15% dry matter basis) and high-starch diet (30.8%) with and without live yeast supplementation (1×10^{10} cfu per cow) in the first and second crossover, respectively. At the end of each period, cows received a single dose of mycotoxin-contaminated feed containing 0.05, 0.2, 0.24, and 0.56 mg of AFB1, OTA, DON, and FB1 per kg of feed, respectively. The fecal and urinary excretion of mycotoxins and their metabolites was monitored for up to 48 h postdosing. As expected, ruminal pH decreased in cows fed the high-starch diet. The high-starch diet increased the bioavailability of OTA and AFB1. Urinary excretion of OTA 24 h after mycotoxin administration increased 3-fold in the high-starch diet, correlated with lower fecal excretion. Similarly, a decrease in fecal excretion of AFB1 was accompanied by an increase in urinary excretion of its major metabolite, aflatoxin M_1, 48 h after mycotoxin administration. In contrast to AFB1 and OTA, the bioavailability of DON and FB1 remained unchanged. Yeast supplementation had no effect on the excretion balance of these 2 mycotoxins. In conclusion, these results show that high-starch diets increased the bioavailability of OTA and AFB1, most probably through the lowering effect on ruminal pH. This greater bioavailability potentially increases the toxic effects of these mycotoxins.
Key words: mycotoxin, low ruminal pH, dairy cow, live yeast supplementation

INTRODUCTION

High-producing dairy and beef cattle need diets rich in energy and protein to meet their requirements and maximize performance levels. However, the use of large amounts of cereal in the diet may induce SARA, which is often characterized by a decreased ruminal pH (Kleen and Cannizzo, 2012). A starch-rich diet may also increase animal exposure to mycotoxins as starch in these diets are the main vehicles of mycotoxin contamination in feeds. Many reports have indicated the separate negative effects of mycotoxins and SARA in ruminants, but no information is available on any interaction that may exist between a pH-modified ruminal environment as encountered in SARA and the toxicokinetics of mycotoxins. For instance, it has been established that SARA is associated with major changes in the composition of the rumen microbiota (Martin et al., 2006; Fernando et al., 2010; Mao et al., 2013), but it is not known if these changes affect the resistance of ruminants against mycotoxins. Another consequence of SARA is the potential modification of ruminal absorption of mycotoxins. Some authors have reported greater availability of ochratoxin A (**OTA**) in ruminants fed diets rich in starch (Xiao et al., 1991; Blank et al., 2003).

Live yeast used as a direct-fed microbial (**DFM**) exerts a positive effect on the ruminal environment, increasing pH and the numbers of total and cellulolytic

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bacteria as well as protozoa (Jouany and Morgavi, 2007; Chaucheyras-Durand et al., 2008). The effects of yeast DFM on ruminal pH and on some ruminal microbes [e.g., protozoa are active degraders of some mycotoxins (Galtier and Alvinerie, 1976)] may indirectly reduce mycotoxin absorption and toxicity. Yeast cell walls also have the capacity to bind to mycotoxins (Shetty and Jespersen, 2006) and thus could reduce gastrointestinal absorption. This property is strain dependent, with reduction of mycotoxin adsorption reported with cell extract preparations (Firmin et al., 2011) but not with a yeast strain tested on OTA in sheep (Blank and Wolffram, 2009).

Mycotoxins such as aflatoxin B_1 (**AFB1**), OTA, deoxynivalenol (**DON**), and fumonisin B_1 (**FB1**) are commonly found in cattle feeds (Rodrigues and Naehrer, 2012) and can cause a variety of toxic responses. Due to their importance on animal health and production, and possible health consequences on humans, the maximum concentration of these mycotoxins in feeds is regulated by the European legislation. The aim of this study was to evaluate the effects of low ruminal pH with and without yeast DFM supplementation on the bioavailability of these 4 mycotoxins.

MATERIALS AND METHODS

Preparation of Mycotoxin-Contaminated Feeds

Aflatoxins and ochratoxins were produced by culture of *Aspergillus flavus* and *Aspergillus ochraceus*, respectively, on wheat as previously described (Boudra et al., 2013). Corn naturally contaminated with fumonisins or DON was obtained locally from an experimental field (Limagrain, Clermont-Ferrand, France). The 4 contaminated batches of wheat and corn were ground to pass through a 1-mm screen and mixed in different proportions to obtain a contaminated cereal mixture containing 3, 12, 15, and 35 mg/kg of AFB1, OTA, DON, and FB1, respectively. Aflatoxins are carcinogenic to humans (group 1) and OTA and FB1 are classified by the World Health Organization International Agency for Research on Cancer as possibly carcinogenic to humans (group 2B). All contaminated batches of wheat and corn were manipulated under a dedicated safety hood for grinding and dose preparation. All personnel wore coats, disposable gloves, protective masks, and goggles.

Animals and Experimental Procedures

The experiment was conducted at the experimental animal facilities of INRA Auvergne-Rhône-Alpes (Saint-Genès Champanelle, France). Animals were cared for in accordance with the guidelines for animal research of the French Ministry of Agriculture and applicable European guidelines and regulations for experimentation with animals (http://www2.vet-lyon.fr/ens/expa/acc_regl.html). The experimental protocol was approved by the Regional Ethics Committee on animal experimentation (CE N° 22612).

Eight nonlactating Holstein cows fitted with a rumen cannula were used. The cows were housed in a tie-stall barn and had free access to water and mineral salt blocks. At the start of the experiment, BW was 650 ± 115 kg. The experiment was a double crossover design with a 2-wk washout period between each crossover to minimize carryover effects. The cows were divided into 2 groups of equivalent BW and age. In the first crossover trial, the cows received a low-starch diet (15.1% DM basis) with or without yeast DFM. In the second crossover, cows received a high-starch diet (30.8% DM basis) with or without yeast DFM. To avoid a possible yeast carryover effect, cows were regrouped for the second crossover with each group having 2 cows that received yeast treatment in the first period and 2 that received yeast in the second period. Each crossover period lasted 3 wk, with a 2-wk adaptation to diet and the last week for measurements. The ingredients and chemical composition of the experimental diets are presented in Table 1. All feed ingredients were tested for presence of mycotoxins. Wheat in the form of pellets was fed at 0700 h, and hay was fed twice daily at 0800 (60%) and 1400 (40%) h. The yeast DFM (*Saccharomyces cerevisiae* CNCM I-1077, Lallemand Animal Nutrition, Blagnac, France) was administered daily before the morning feeding through the ruminal cannula at a dose of 1×10^{10} cfu per cow. Feed offer was limited to 90% of the hay ad libitum intake measured before the start of the trial (DMI is reported in Table 1). At the end of each adaptation period, the cows received a single dose of the contaminated cereal mixture through the ruminal cannula 4 h after the morning feeding, corresponding to the expected nadir in pH. The doses used were 0.05, 0.2, 0.24, and 0.56 mg of AFB1, OTA, DON, and FB1 per kg of feed, respectively. Doses were based on the detection limits of the liquid chromatography-tandem mass spectrometry (**LC-MS/MS**) method used (see below) and all except for AFB1 were below the European Union regulation limits. Each dose of the contaminated cereal mixture (400 g) was divided into 4 equal portions of 100 g and administered through the ruminal cannula in different locations in the rumen to ensure complete ingestion and to facilitate homogenization of mycotoxins with ruminal contents. Total (24 h) and separate collection of feces and urine was performed for 6 d after mycotoxin administration for determination of total-tract digestibility as previously