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Effects of clay after an aflatoxin challenge on aflatoxin clearance, milk production, and metabolism of Holstein cows

S. A. Sulzberger,* S. Melnichenko,† and F. C. Cardoso*[1]
*Department of Animal Sciences, University of Illinois, Urbana 61801
†United Minerals Group, Kyiv 04053, Ukraine

ABSTRACT

Oral supplementation of clay to dairy cattle has been reported to reduce toxicity of aflatoxin (AF) in contaminated feed. The objective of this study was to determine the effects of 3 concentrations of dietary clay supplementation in response to an AF challenge. Ten multiparous rumen-cannulated Holstein cows [body weight (mean ± SD) = 669 ± 20 kg and 146 ± 69 d in milk], were assigned to 1 of 5 treatments in a randomized replicated 5 × 5 Latin square design balanced to measure carryover effects. Periods (21 d) were divided in an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). From d 15 to 17, cows received an AF challenge. The challenge consisted of 100 μg of aflatoxin B_1 (AFB_1)/kg of dietary dry matter intake (DMI). The material was fitted into 10-mL gelatin capsules and administered into the rumen through a rumen-cannula based on the average DMI obtained on d 12 to 14. Treatments were no clay plus an AF challenge (POS); 3 different concentrations of clay (0.5, 1, or 2% of dietary DMI) plus an AF challenge; and a control consisting of no clay and no AF challenge (C). Statistical analysis was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Two contrasts, CONT1 (POS vs. C) and CONT2 (POS vs. the average of 0.5, 1, and 2% clay), were compared along with the linear and quadratic treatment effects (POS, 0.5%, 1%, 2%). Cows supplemented with clay had lower AF excretion in milk as aflatoxin M_1 (AFM_1; 0.5% = 20.83 μg/d, 1% = 22.82 μg/d, and 2% = 16.51 μg/d) and AF transfer from rumen fluid to milk (AFM_1; 0.5% = 1.01%, 1% = 0.98%, and 2% = 0.74%) compared with cows in POS (AFM_1 = 27.81 μg/d and AF transfer = 1.37%, CONT2). Similarly, concentrations of AFM_1 in milk (0.5% = 0.35 μg/kg, 1% = 0.30 μg/kg, 2% = 0.25 μg/kg), AFB_1 in feces (0.5% = 1.79 μg/g, 1% = 1.52 μg/kg, 2% = 1.48 μg/kg), and AFB_1 in rumen fluid (0.5% = 0.05 μg/kg, 1% = 0.02 μg/kg, 2% = 0.02 μg/kg) were reduced in cows fed clay compared with POS (0.43 μg/kg, 2.78 μg/kg, and 0.10 μg/kg, respectively, CONT2). Cows supplemented with clay tended to have lower 3.5% fat-corrected milk [0.5% = 38.2 kg, 1% = 39.3 kg, 2% = 38.4 kg, standard error of the mean (SEM) = 1.8] than cows in POS (41.3 kg; SEM = 1.8; CONT2). Plasma superoxide dismutase (SOD) concentration tended to be lower for cows fed clay in the diet (0.5% = 2.16 U/mL, 1% = 1.90 U/mL, 2% = 2.3 U/mL; SEM = 0.3) than for cows in POS (2.72 U/mL; CONT2). Additionally, when cows were exposed to AF without clay in the diet, plasma concentrations of aspartate aminotransferase (AST) decreased from 84.23 (C) to 79.17 (POS) and glutamate dehydrogenase (GLDH) decreased from 91.02 (C) to 75.81 (POS). In conclusion, oral supplementation of clay reduced the transfer of AF from the rumen to milk and feces.
Key words: clay, aflatoxin, milk, urine

INTRODUCTION

Each year, the USDA, Cooperative State Research, Education, and Extension Service (CSREES), and the Food and Drug Administration (**FDA**) spend approximately US$31.1 million in combined efforts for mycotoxin research (Robens and Cardwell, 2005). Aflatoxins (**AF**) are most commonly found on corn, peanuts, and cottonseed. This mycotoxin is produced by *Aspergillus flavus* and *Aspergillus parasiticus* and, when ingested, one of the AF derivatives ($\mathbf{AFB_1}$) is bio-transformed into a toxic secondary metabolite (aflatoxin M_1; $\mathbf{AFM_1}$). Both AFB_1 and AFM_1 are carcinogenic; however, AFM_1 is the most toxic secondary metabolite that is secreted into milk and is classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2002). As such, AFM_1 represents a food safety risk to humans (Plasencia, 2005; Whitlow and Hagler, 2005; Gallo et al., 2015). The production of AF is influenced by several environmental factors, including temperature and humidity.

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[1]Corresponding author: cardoso2@illinois.edu

Aflatoxins are estimated to affect 25% of all agricultural commodity crops (FAO, 2004). Governmental research efforts have found a multitude of preventative measures that include preharvest and postharvest strategies. Before harvesting, studies have focused on selecting seed varieties for *Aspergillus* spp. resistance or applying crop rotations with plants that are not susceptible to *Aspergillus* spp. Crop rotations work to diminish the infectious spores left in the soil (Betrán et al., 2005; Kabak et al., 2006). It has been well documented that applying chemical agents to harvested feed before ensiling can also prevent fungal growth (Guo et al., 2005; Kabak et al., 2006). Whichever method is applied, the FDA has regulated AF concentrations in milk and in feed to be a maximum of 0.5 and 20 ppb in the United States, respectively (Peraica et al., 1999). However, AF is resilient and continues to be problematic for cattle producers. Acute exposure to aflatoxins causes health problems, including inappetance, lethargy, and reproductive disorders. Chronic exposure reduces feed efficiency and milk production, can cause jaundice, and interferes with vaccine-induced immunity. Overall, aflatoxins are responsible for immunosuppression as well as carcinogenic effects on the liver (Whitlow and Hagler, 2005; Shrestha and Mridha, 2015). According to Abrar et al. (2013), the toxicity of aflatoxins originates from the generation of enzymatic intracellular reactive oxygen species, CYP450, that ultimately biotransforms AFB_1 to the aflatoxin product binding to DNA, RNA, and proteins.

Various types of adsorbents are described by Kolosova and Stroka (2012). The main characteristic of these materials is the capacity they have to exchange ions and reduce the mycotoxin's bioavailability to the cow (Carson and Smith, 1983; Trckova et al., 2004; Kabak et al., 2006; Karnland et al., 2006). Kaolinite, smectite, chlorites, and micas are groups of silicates or clay-based materials. Montmorillonites are a class of the smectite clay group, which has 3-layer structures that allow for internal adsorption of mono- and divalent ions into each interlayer sheet. Smectites have a wide range of commercial uses and have been reported to adsorb heavy metals, bacteria, and toxic antinutritive agents, such as AF (Trckova et al., 2004).

Different types of adsorbents have been studied to act as potential binding agents for aflatoxins. Kutz et al. (2009) and Queiroz et al. (2012) fed cows a TMR mixture with aflatoxin and used hydrated sodium calcium aluminosilicates (HSCAS), a clay material, and a modified yeast cell culture (MTB-100, Alltech Inc., Nicholasville, KY). The HSCAS reduced AFM_1 concentrations by 45 to 48%; however, the MTB-100 only reduced AFM_1 by 4% in both studies. Maki et al. (2016a) performed a similar study using an improved product that was calcium montmorillonite clay. This product reduced AFM_1 transfer into milk from 1.07 to 0.52%. Xiong et al. (2015) reported that the transfer of AFM_1 from the TMR to milk was reduced when Solis Mos (Novus International Inc., St. Charles, MO) was added to a TMR contaminated with aflatoxin B_1 compared with the control (0.46 vs. 0.56%, respectively).

Understanding how rumen, milk, feces, and urine are affected by clay after an aflatoxin challenge in Holstein cows and the clay's effect on production parameters deserves attention. Therefore, the objectives of this experiment were (1) to determine the effects of a commercially available clay product in response to an aflatoxin challenge on blood chemistry, ruminal, milk, and feces aflatoxin concentrations (i.e., transfer), and milk composition of mid-lactation Holstein cows; and (2) to determine the most appropriate clay concentration to be used in the diet of lactating dairy cows.

MATERIALS AND METHODS

Animal Care and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee. The experimental period occurred from October 2014 to January 2015. Cows were housed in tiestalls with sand bedding and ad libitum feed and water access. The diet (a TMR) was formulated according to NRC (2001) recommendations.

Experimental Design and Aflatoxin Challenge Procedure

Ten multiparous rumen-cannulated Holstein cows (BW = 669 ± 20 kg; DIM = 146 ± 69; mean ± SD) were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Therefore, treatments were arranged so that the carryover effects could be evaluated. Periods (21 d) were divided in an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). From d 15 to 17, cows received an AF challenge. The aflatoxin challenge was similar to that proposed by Kutz et al. (2009). Dietary aflatoxin was obtained from the Veterinary Medical Diagnostic Laboratory, College Veterinary Medicine, University of Missouri (Columbia) and consisted of *Aspergillus parasiticus* (NRRL-2999) culture material containing 102 mg/kg of AFB_1, 3.5 mg/kg of AFB_2, 35 mg/kg of AFG_1, and 0.9 mg/kg of AFG_2. The challenge consisted of 100 μg of AFB_1/kg of dietary DMI via 10-mL gelatin capsules (Torpac, Fairfield, NJ) admin-