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Physiological responses to a yeast and clay-based adsorbent during an aflatoxin challenge in Holstein cows

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ABSTRACT

The objective of this study was to determine the effects of 2 different adsorbents (**AD**) composed of yeast fractions and bentonite (**AD1 and AD2**) during an aflatoxin (**AF**) challenge on the health and performance of lactating dairy cows. Lactating Holstein cows [(n = 76); BW (mean ± SD) = 698 ± 72 kg; DIM = 153 ± 83 d] were assigned to 1 of 5 treatments in a randomized complete block design. The trial lasted 28 d and measurements were made from d 22 to 28. From d 22 to 24 cows received an AF challenge (100 μg of AFB_1/kg of diet DM administered orally). Treatments were: control (**CON**), no AD or AF; positive control **(POS)**, no AD plus AF challenge; 30 g per cow per day of AD1 and AF challenge (**P30**); 60 g per cow per day of AD1 and AF challenge (**P60**); and 60 g per cow per day of AD2 and AF challenge (**PROT**). The appearance and disappearance of AF excreted in milk was tested at each milking from d 22 to 28 using SNAP tests (**SNP**; IDEXX, Inc.). Blood was sampled on d 22 and 26 (n = 2 per cow), and analyzed for superoxide dismutase (**SOD**) concentration. Milk samples from d 22 to 26 were analyzed for AFM_1 concentrations by HPLC. Fecal samples collected from the rectum on d 22 and 24 were analyzed for AFB_1 concentrations via HPLC. Statistical analysis was performed using the MIXED procedure of SAS. A quadratic treatment effect ($P < 0.001$) was observed for plasma SOD concentrations at 2.77, 1.99, and 1.97 ± 0.05 U/mL for POS, AD30, and AD60 treatments, respectively. Aflatoxin M_1 transfer (11.4 and 0.00 ± 1.60 g/kg), excretion (29.52 and 0.00 ± 4.58 μg/d), and concentrations in milk (0.76 and 0.00 ± 0.16 μg/kg) were greater for POS than CON, respectively ($P < 0.001$) but no differences were observed among other treatments. A tendency for a quadratic treatment effect ($P = 0.08$) was observed for fecal AFB_1 concentrations at 6.78, 8.55, and 5.07 ± 1.17 μg/kg for the POS, P30, and P60 treatments, respectively. Oral supplementation of yeast and bentonite clay-based adsorbents during an AF challenge resulted in quadratic changes in plasma SOD and fecal AFB_1 concentrations; however, no differences were observed for DMI or milk yield. In conclusion, yeast cell wall and bentonite-based adsorbent may be beneficial in reducing inflammation during an AF challenge but there were no treatment differences regarding its adsorbent capacity.

Abbreviations: AD, adsorbent; AD1, adsorbent 1; AD2, adsorbent 2; ADF, acid detergent fiber; AF, aflatoxin; AFM_1, aflatoxin M_1; AFB_1, aflatoxin B_1; ALP, alkaline phosphatase; BCS, body condition score; BW, body weight; CON, control; CONT1, contrast 1; CONT2, contrast 2; CP, crude protein; DIM, days in milk; DM, dry matter; DMI, dry matter intake; ECM, energy-corrected milk; FCM, fat-corrected milk; LPS, lipopolysaccharide; MUN, milk urea nitrogen; NEFA, nonesterified fatty acids; NDF, neutral detergent fiber; NRC, National Research Council; SCC, somatic cell count; SNP, snap test; SOD, superoxide dismutase; TMR, total mixed ration

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1. Introduction

Aflatoxins (**AF**) are a class of mycotoxin produced as secondary metabolites by several species from the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* that are commonly found in dairy cattle feedstuffs (Xiong et al., 2015; Kutz et al., 2009). When consumed by dairy cows, an AF derivative (**AFB_1**) is converted to aflatoxin M_1 (**AFM_1**) and is commonly considered a toxic carcinogen (IARC, 2002). Aflatoxin M_1 can be detected in plasma and milk in as little as 5 min and 1 h after ingestion, respectively (Moschini et al., 2008; Battacone et al., 2012). Due to its rapid secretion into milk, AF can be considered a food safety risk (Kutz et al., 2009; Sulzberger et al., 2017). Transfer percentages from feed to milk vary among individual animals but values as high as 6.2% have been reported for high producing dairy cows (Veldman et al., 1992).

In the United States, the food and drug administration (FDA) has set a maximum threshold limit of 0.5 μg/kg AF in milk while the European Union adheres to a 0.05 μg/kg maximum limit (Campbell et al., 2003). Not only are there risks associated with even small amounts of AF consumed by humans, but there are immunotoxic effects on cows as well. Alterations in blood metabolites when cows are exposed to AF are commonly reported, indicating some degree of immune response to the toxin (Marin et al., 2002; Fink-Gremmels and Malekinejad, 2007; Xiong et al., 2015; Sulzberger et al., 2017). Specifically, changes in SOD and ALP have been found in cows receiving an AF challenge (Sulzberger et al., 2017). Others have used additional blood constituents (e.g.; gamma-glutamyl transferase) to monitor liver function with AF intoxication in dairy cows (Applebaum and Marth, 1983; Arthington et al., 2003) and dairy sheep (Battacone et al., 2005). The short time between AF ingestion and secretion, and the seriousness of its consequences, warrant close attention.

Corn silage is one of the primary feedstuffs in dairy cow diets and may be contaminated by mycotoxins such as deoxynivalenol, zearalenone, fumonisin, and AF, either when plants are growing in the field or when they are stored under inadequate conditions (Miller, 2008; Richard et al., 2009). Drought conditions may also increase risk for pre-harvest AF contamination in crops commonly fed to dairy cows (Cotty and Jaime-Garcia, 2007; Guo et al., 2008). Mycotoxin production is influenced by temperature and humidity, and is estimated to affect 250 g/kg of all agriculture commodity crops (FAO, 2004). Because of the wide range of conditions under which fungi may thrive and the high incidence of the contaminant, it is important to develop strategies to mitigate the negative effects of AF when fed to dairy cows.

The use of different types of sequestering agents in alleviating AF fed to cows and other livestock has been widely examined. The major benefit of AF sequestering agents is that they are able to adsorb AF during digestion allowing the toxin to pass through the animal without causing harm (Davidson et al., 1987; Phillips et al., 1989; Kutz et al., 2009). Advantages of using adsorbents when pursuing decontamination of milk are that they are safe, inexpensive, and easy to feed (Kutz et al., 2009). Common types of adsorbents studied include activated carbons or charcoals, yeast cell cultures, and clay-based products such as bentonite and hydrated sodium calcium aluminosilicates (Diaz et al., 2004; Moschini et al., 2008; Kutz et al., 2009). The degree of efficacy of these sequestering agents differs due to the variable nature of the mechanisms behind which the adsorbents target AF in the body (Stroud, 2006). Ultimately, addition of these sequestering agents to the diet of cows consuming AF should decrease the bioavailability of free toxins to the cow and allow the AF to pass safely through the animal (Phillips et al., 1990).

Of particular interest for the present study is the effect of sequestering agents containing bentonite in combination with yeast cell wall extracts on the efficacy of AF adsorbance in lactating dairy cows. The objectives of the present study were to determine the effects of a yeast cell wall and bentonite clay-based adsorbent on excretion of AF, milk composition, health and blood metabolites of mid-lactation Holstein cows under an AF challenge.

2. Materials and methods

2.1. Animal care and housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee (#15204). The experiment occurred from January 25 to April 18, 2016. Cows were housed in tie stalls with sand bedding and ad libitum feed and water access. A TMR was formulated according to NRC (2001) recommendations (Table 1). Diets were formulated based on cows in their 3rd lactation, at 85 days in milk (DIM), 680 kg of BW, producing 36 kg of milk/d with a target 38 g/kg of milk fat, 32 g/kg of milk protein, and a predicted DMI of 25 kg/d.

2.2. Experimental design and aflatoxin challenge procedure

A total of 80 multiparous Holstein cows [BW (mean ± SD) = 698 ± 72 kg; DIM = 153 ± 83] were initially assigned to 1 of 5 treatments in a randomized complete block design consisting of 16 blocks. Data for 4 cows were excluded because of mastitis or very low milk yield. Therefore, 76 cows completed the experiment. Cows were distributed into blocks with regard to lactation number, DIM, previous lactation 305-d milk yield, and BCS to ensure that these variables had minimal chance of influencing the measured variables of the study.

One week before the experiment, the covariate period (week – 1), baseline levels for milk yield, BW, BCS, and DMI were measured. The experimental period (28 d) was divided into an adaptation phase (d 1–21) and a measurement phase (d 22–28). From d 22–24 cows received an AF challenge similar to the one proposed by Kutz et al. (2009). Dietary AF 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 (University of Missouri, Columbia, MO). The challenge consisted of 100 μg of AFB_1/kg of dietary DMI measured into 28-mL gelatin