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Research paper

Assessment of aflatoxin B_1 adsorption efficacy of natural and processed bentonites: In vitro and in vivo assays

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ABSTRACT

The presence of aflatoxin B_1 in livestock's feed results in contamination of milk and its products with aflatoxin M_1. Different sequestering agents have been added to cows' ration to adsorb these toxins, although influence of processed bentonites on aflatoxins adsorption has not been evaluated yet. This experiment was carried out to assess the effects of incorporating natural and processed bentonite (local or commercially available), to the diet of Holstein dairy cows subjected to an aflatoxin B_1 diet, and the transfer of aflatoxin metabolites (AFM_1) to milk. Aflatoxin sequestering capacity, pH, CEC, XRD and XRF of natural and processed bentonites were measured. Then, twelve Holstein dairy cows were assigned to 3 treatments as the following: 1) local processed bentonite (G.Bind™), 2) local unprocessed bentonite (F), and 3) commercially available bentonite (M). Aflatoxin content in feed and milk was evaluated and transfer rate was measured. Results of the present study showed that the aflatoxin contents of milk were remained unchanged except for treatment G.Bind™ that considerably decreased aflatoxin M_1 in milk after the second and third weeks of the experiment. G.Bind™ lowered the transfer rate of aflatoxin B_1 from 1.17% at the beginning of the experiment to 0.43% and 0.39% after the first and second weeks, respectively. Processing of bentonites (basic processing in present study) can considerably help to adsorb aflatoxin from feed and also to decrease aflatoxin transfer to milk.

1. Introduction

A group of fungi can produce toxic secondary metabolites known as mycotoxins, which have detrimental effects on human health and animal performance (Bryden, 2012; Jard et al., 2011; Robens and Richard, 1992). The Food and Agriculture Organization (FAO) reported that about 25% of crops produced all over the world are contaminated with mycotoxins (Fokunang et al., 2006). Aflatoxins are the most common and harmful metabolites, which are produced by different strains of *Aspergillus* such as *Aspergillus flavus* and *Aspergillus parasiticus* (Heathcote and Hibbert, 1978). Aflatoxins detrimentally affect hepatocytes (Schlemper et al., 1991), decrease milk production (Malka et al., 2013), decline egg production (Hamilton and Garlich, 1971), attenuate immune response and resistance against pathogens (Ghosh et al., 1990; Bondy and Pestka, 2000), and dramatically depress performance (Kermanshahi et al., 2009; Dersjant-Li et al., 2003) in farm animals. Among different members of the aflatoxin family, aflatoxin B_1 (AFB_1) possesses the most toxic and carcinogenic characteristics to animals and humans (McLean and Dutton, 1995). About 5 billion humans in different countries are at risk of AFB_1 through different contaminated food and livestock products (Liu and Wu, 2010).

AFB_1 can be partially destroyed in the rumen if ruminants are fed with the contaminated feedstuffs, although AFB_1 may absorb and undergo different metabolic processes to transform to other metabolites in the liver (Kuilman et al., 1998). The most common metabolite of AFB_1 is aflatoxin M1 (AFM_1) which can be excreted in the urine and milk in different amounts based on the AFB_1 existing in feedstuffs (Prandini et al., 2007). AFM_1 is stable and remains unaffected by common milk processing procedures, such as pasteurization and sterilization. This metabolite can be transported to different dairy products and increase the aflatoxin exposure. The International Agency for Research on Cancer (IARC, 2002) classified AFB_1 as a type I carcinogenic agent and AFM_1 as type II. Health organizations in different parts of world have established minimum levels of AFM_1 in dairy products; for example, the European Union (EU, 2006) applies 0.05 μg AFM_1 per kg in ruminant milk as the maximum residual level, while in the USA, some Asian and South American counties accept a higher level (0.5 μg AFM_1/kg ruminant milk) (Binder et al., 2007).

About 1% to 3% of AFB_1 existing in feedstuffs appear in milk as AFM_1 (Diaz et al., 2004). So, different methods have been applied to sequester AFB_1 in feed and subsequently prevent transformation to AFM_1 and presence in milk. Some of these methods comprise irradiation,

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biological detoxification, chemical detoxification, and deactivation by heat. Although these methods are successful in decreasing AFB_1 in feedstuffs, they might also depress the nutritional value of dairy products (Phillips et al., 1994). Clay based adsorbents are the most efficient ones in adsorbing AFB_1 (Phillips, 1999). Among different clay adsorbents, special attention is paid to bentonite and its main mineral, montmorillonite (Mt). The layer structure of Mt allows swelling in aqueous environment, promoting an AFB_1 adsorption between the layers and prevents the absorbance of these toxic molecules by gut cells (Diaz et al., 2004: Eckhardt et al., 2014). Deng et al. (2010) reviewed different possible mechanisms by which clays may adsorb AFB_1 molecules; these mechanisms mainly contain chemical bonds between active sites in AFB_1 and clays. Based on the in vitro results (Diaz et al., 2002), which showed the beneficial effects of clays on sequestering AFB_1, animal nutritionists have tended to incorporate clays to livestock's diet contaminated with AFB_1 to examine the effects of such additives on AFB_1 decontamination and animals' performance. Mt successfully adsorbed AFB_1 in the gastrointestinal tract (Eckhardt et al., 2014), reduced hepatic lesions and mortality (Pasha et al., 2007), reduced AFM_1 in milk (Diaz et al., 2004), and improved performance (Shi et al., 2006). It is also well-documented that natural Mt, without processing markedly adsorbed AFB_1 under in vitro and in vivo conditions (Dakovic et al., 2008; Phillips et al., 1988). Although several studies were conducted to assess the sequestering effects of natural Mt on AFB_1, there are few reported studies that compare natural vs. activated Mt on AFB_1 decontamination. The objective of present study was to evaluate the effects of activated clay in comparison to both non-activated and commercially available clay binders on AFB_1 adsorption in dairy cows' ration and transport rate from feed to milk.

2. Materials and methods

2.1. Animals, treatments, experimental conditions and sampling

Twelve Holstein dairy cows with similar physiological and productive attributes (average body weight of 480 ± 55 kg; average age of 60 ± 8 months; average milk production of 45 ± 6 kg) were used for two weeks in this experiment. All dairy cows were assigned to three groups and fed the same total mixed ration (TMR) for a week before the beginning of the experiment in order to bear the adaptation period. The experimental diets were: 1) unprocessed Mt (F), 2) imported commercially available clay (M), and 3) local processed Mt (G.Bind™,[1]). A source of cottonseed meal, which had been naturally contaminated with AFB_1, was added to the diets, completely mixed and samples were collected and tested to confirm that all cows received the same dose of AFB_1 during the two weeks of the experiment. Then, all experimental clay binders were added to the diets according to recommendations of producing companies (i.e. 0.6%), at the expense of corn and thoroughly mixed. The diet was formulated according to the recommendation of NRC, 2001 (Table 1) for an average cow weighing 450 kg, 160 days in lactation and with a 40 kg milk yield (3.8% fat, 3.35% protein). The TMR was fed ad libitum twice a day at 10:00 h and 16:00 h with checking that no empty trough was observed. Cows were milked 3 times a day at 3:30, 11:30 and 19:30. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

2.2. Bentonite characteristics

Clay samples were characterized by X-ray Fluorescence (XRF) using an XMF104 Unisantis Electronics spectrophotometer operating at a power of 1 kW and equipped with an Rh X-ray source. The clay samples were prepared as random powders to record the X-ray diffraction patterns on a XMD300, Unisantis Electronics diffractometer employing Cu Kα radiation (λ = 1.54 Å) with a rotating sample stage and a fixed divergence slit size of 0.5°. The samples were scanned between 3 and 65° 2θ. The step size was 0.02° 2θ and counting time was 80 s per step. Physical attributes of bentonites such as cation exchange capacity (CEC: according to ASTM[2] C837), swelling (according to ASTM D5890), water absorption (according to ASTM E946-92), methylene blue index (according to ASTM C837-09), pH (according to ASTM D4972-13) and percentage of <2 μ particles with particle size analyzer (Sedigraph 5100, Micromeritics, GA, USA) were analyzed.

Table 1
Ingredient and nutrient composition of the TMR fed to lactating dairy cows based on NRC (2001)[a].

Ingredients	(%)
Alfalfa Hay	10.0
Corn silage	59.0
Ground corn	6.0
Barley	8.0
Cottonseed	2.0
Soybean meal	6.5
Cottonseed meal	1.0
Sunflower meal	1.5
Meat meal	3.0
Fat meal	1.5
Sodium bicarbonate	0.2
DCP	0.6
Mineral and vitamin supplements[b]	0.5
Salt	0.2
Nutrients	
NE_L (Mcal/kg)	1.63
CP (%)	18.40
RUP (%CP)	6.60
RDP (%CP)	11.80
NFC (%)	38.10
ADF (%)	20.00
NDF (%)	21.00
Forage NDF (%)	21.30
Ca (%)	1.00
P (%)	0.70
DCAD (meq/kg)	188.00

[a] Clay samples were added to the diets in the expense of corn according to the recommendation of producing factory.
[b] Each kilogram of mineral and vitamin supplement contains: 190,000 mg Ca, 90,000 mg P, 50,000 mg Na, 19,000 mg Mg, 3000 mg Fe, 300 mg Cu, 3000 mg Zn, 100 mg Co, 100 mg I, 1 mg Se, 3000 antioxidant, 50,000 IU vit. A, 100,000 IU vit. D_3, and 1000 mg vit. E.

2.3. Sample preparation and AFB_1 and AFM_1 measurement

At the beginning of the experiment, a sample of the TMR was collected, oven dried at 60 °C until constant weight, ground and frozen until AFB_1 analysis. Three samples of milk obtained from each milking time for each group were collected in sterile 15 ml propylene falcon at the first day of experiment and also at the end of each week (two weeks). Collected samples were stored at 4 °C and frozen at −20 °C for later AFM_1 analysis.

For measuring AFB_1 in the feed samples, 6 replicates of 5 g (total of 30 g) of the TMR sample were thawed, passed through 1 mm sieve, oven-dried and sonicated in 25 ml methanol: water (70:30 v/v) solution in an ultrasonic bath for 10 min. The obtained solution was passed through a Whatman No. 1 filter paper, transferred to enzyme-linked immunosorbent assay (ELISA) kit, following the instructions of the kit producer (Neogen, USA) and finally the prepared kit was placed in ELISA (BioTek, ELX 808, Winooski, VT, USA). Ten wells were assigned for each replicate of the TMR sample (total of 60 wells) and average data

[1] G.Bind™ is treated with a basic solution to activate clay through sodium entrance to the interlayer of montmorillonite to improve physical characteristics of clay. G.Bind™ is a product of PayaFarayand Hezareh Novin micronized mineral manufacturing, Mashhad, Razavi Khorasan, Iran.

[2] American Society for Testing and Materials, West Conshohocken, Pennsylvania, United States.