Animal Feed Science and Technology 214 (2016) 130–135

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Calcium montmorillonite clay in dairy feed reduces aflatoxin concentrations in milk without interfering with milk quality, composition or yield

C.R. Maki[a], A.P.A. Monteiro[b], S.E. Elmore[a], S. Tao[b], J.K. Bernard[b], R.B. Harvey[c,d], A.A. Romoser[a], T.D. Phillips[a,d,*]

[a] College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, United States
[b] College of Agricultural and Environmental Sciences, University of Georgia, Tifton, GA 31793, United States
[c] United States Department of Agriculture, Agricultural Research Service, Food and Feed Safety Research, College Station, TX 77845, United States
[d] Texas A&M AgriLife Research, College Station, TX 77843, United States

ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form 15 February 2016
Accepted 16 February 2016

Keywords:
Aflatoxin
Calcium montmorillonite clay
Milk vitamins
Milk composition
Dairy cow

ABSTRACT

This study was designed to determine if a calcium montmorillonite clay (Novasil Plus, NSP), can significantly reduce aflatoxin M_1 (AFM_1) concentrations in milk without affecting dry matter intake (DMI), milk yield, milk composition, vitamin A, or riboflavin concentrations. The study was designed using 15 lactating dairy cows randomly assigned to one of 5 treatment groups in a 5×5 Latin square experimental design. The cows were treated with diets containing AFB_1 at 0 or 121 ppb and three different levels of NSP (0.0, 6.0 and 12.1 g/kg) of the diet. Milk samples were collected and analyzed for AFM_1, milk composition, vitamin A, and riboflavin. In this study, AFM_1 concentrations in milk were significantly reduced by both 6.0 g/kg and 12.1 g/kg NSP treatment levels. The low dose of NSP (6.0 g/kg) resulted in a decrease in the level of AFM_1 in the milk equal to 55%; whereas, the high dose (12.1 g/kg) reduced the level of AFM_1 by 68%; milk composition, vitamin A, and riboflavin concentrations were unaffected by any of the dietary treatments. Additionally, dry matter intake (DMI) and milk yield were not different among treatments.

1. Introduction

Aflatoxin (AF) is a hazardous chemical produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. It has been shown to be toxic and carcinogenic in humans and animals. The four naturally occurring aflatoxins are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). Of these, AFB_1 is the most toxic and has been classified as a carcinogen by the International Agency for Research on Cancer (IARC 2002). Contamination of food with AF occurs frequently between the latitudes of 40°N and 40°S in regions that experience high temperatures and drought and typically include Sub-Saharan Africa, Southeast Asia, Central America and the southern United States (Williams et al., 2004).

Abbreviations: AF, aflatoxin; AFM_1, aflatoxin M_1; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2; DM, dry matter; DMI, dry matter intake; NS, NovaSil; NSP, NovaSil Plus; BCS, body condition score; TMR, total mix ration; MUN, milk urea nitrogen.

* Corresponding author at: VMA, Building 107, MS 4458, College Station, TX 77843-4458, United States. Fax: +1 9798624929.
E-mail address: tphillips@cvm.tamu.edu (T.D. Phillips).

http://dx.doi.org/10.1016/j.anifeedsci.2016.02.014
0377-8401/

Other than dietary exposure to AF, humans and animals are also exposed to toxic metabolites of AF. When lactating animals consume AFB_1-contaminated diets, the toxin is metabolized and excreted into the milk as a hydroxylated derivative known as aflatoxin M_1 (AFM_1) (Van Egmond, 1989). Although AFM_1 is less carcinogenic than the parent AFB_1 molecule, it is still toxic and is considered to be a risk factor for aflatoxicosis in vulnerable populations. The young of all species are more susceptible to the effects of AF, and milk is a major nutrient source for the young. Therefore AFM_1 is strictly regulated in milk and milk products by the USFDA. Moreover, studies have shown that AFM_1 is stable in milk and resistant to pasteurization and processing (Stoloff et al., 1975; Stoloff and Trucksess, 1981; Yousef et al., 1989). Thus, after AF has been transferred to milk, mitigation is difficult.

Because of this problem, a field practical and inexpensive approach to reduce the contamination of AF in milk, without affecting milk production or nutrition, is warranted. A common strategy to mitigate exposures is the inclusion of high affinity AF enterosorbents in the diet. Montmorillonite clays, including NovaSil (NS) and NovaSil Plus (NSP) have been widely studied and reported to be effective in reducing AF exposure in animals and elsewhere: Harvey et al., 1994; Kutz et al., 2009; Smith et al., 1994. NSP clay tightly binds AF with high affinity and capacity in the gastrointestinal tract, thus reducing AF bioavailability and distribution to the blood, liver and other target organs. (Phillips et al., 1988, 2002; Phillips, 1999) In preliminary studies, NS clays have been shown to significantly reduce AF excretion and transfer into the milk of dairy cows when included in the diet and elsewhere: Harvey et al., 1991; Kutz et al., 2009. In all preliminary studies, NS did not interfere with vitamin or nutrient uptake (Afriyie-Gyawu et al., 2005, 2008; Phillips et al., 2008).

Considering that milk is frequently consumed, NSP inclusion in the diet may be a practical strategy to reduce AFM_1 transfer into milk and AF exposures in susceptible humans and animals. This study was designed to investigate the effects of NSP on dry matter intake (DMI), milk yield, milk composition, vitamin A, riboflavin, and AFM_1 in milk.

2. Materials and methods

2.1. Chemicals and reagents

Standards of AFB_1 and AFM_1 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents were purchased from Fisher Scientific (Waltham, MA, USA). Immunoaffinity columns were purchased from VICAM (Watertown, MA). NovaSil Plus, lot: 0201581141, (NSP, calcium-montmorillonite clay) was supplied by BASF Chemical Co. (Ludwigshafen, Germany). Rice Powder containing 758 ppm AFB_1 was obtained from the Food and Feed Safety Research Facility (USDA/ARS, College Station, TX).

2.2. Study design

Fifteen lactating dairy cows averaging 683.0 ± 24 kg body weight (BW) and 227 ± 12 days in milk (DIM) were selected from the University of Georgia Dairy Research Center (Tifton, GA) for use in this study. Prior to beginning the study, all cows were trained to eat behind Calan gates (American Calan, Northwood, NH). The experimental design consisted of a triplicate 5×5 Latin square. Cows were blocked into 3 replicates (5/replicate) by their milk production in the last 30 days, BW, and parity. In each replicate, cows were considered as rows and experimental periods were considered as columns. After randomization of the columns and rows, treatments were randomly assigned into each square. Each experimental period included a treatment period of 7 days, followed by a 5 day washout period. Treatments consisted of: control (no AF or NSP), AF control (121 ppb), NSP control (12.1 g/kg), high dose NSP (AF + 12.1 g/kg NSP) and low dose NSP (AF + 6.0 g/kg NSP). Diets were fed as a total mix ration (TMR) once daily using a Data Ranger (American Calan) and consisted of 275 g/kg dry matter (DM) corn silage, 59 g/kg DM ryegrass silage, 98 g/kg DM sorghum silage, 177 g/kg DM ground corn, 128 g/kg DM brewers grain, and 263 g/kg DM concentrate. Cows were housed in a 4 row free stall barn and milked at 0500, 1400, and 2200 h each day. Milk weights were recorded electronically at each milking (Alpro, DeLaval, Kansas City, MO) and totaled for each day. Milk samples were collected at each milking on days-6 and 7 of each period for analysis of milk composition, AFM_1, vitamin A, and riboflavin. BW was recorded immediately after afternoon milking before feeding on days 6 and 7 of each period and body condition score (BCS) was evaluated at day 7 of each period.

Daily dosing occurred immediately following the afternoon milking. At each feeding period cows were fed a preloaded mixture containing treatments along with a small amount of ground corn and molasses to encourage consumption. After the treatment mixture was consumed, the TMR was offered and cows were fed *ad libitum* intake. The amount of TMR offered and refused was recorded daily. A divider was included between each Calan gate to prevent AFB_1 contamination of adjacent feed rations.

2.3. Isothermal analysis

Isotherms were performed to characterize capacity and affinity of clay surfaces for aflatoxin using methods previously developed and validated by Grant and Phillips (1998). The sorption of AFB_1 to surfaces on NSP was analyzed in purified deionized water. The samples were then measured using a UV–vis spectrophotometer at 362 nm. Sorption data were analyzed using TableCurve 2D software (Systat, Chicago, IL).