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Anti-mycotoxin feed additives: Effects on metabolism, mycotoxin excretion, performance, and total-tract digestibility of dairy cows fed artificially multi-mycotoxin-contaminated diets

Daniel J. C. Vieira,[1]* Luzianna M. Fonseca,[2]* Guilherme Poletti,[1] Natalia P. Martins,[1] Nathália T. S. Grigoletto,[1] Rodrigo G. Chesini,[1] Fernando G. Tonin,[3] Cristina S. Cortinhas,[4] Tiago S. Acedo,[4] Ignácio Artavia,[5] Johannes Faas,[5] Caio S. Takiya,[6] Carlos H. Corassin,[3]† and Francisco P. Rennó[1]†
[1]School of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, Brazil 13635-900
[2]Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, Brazil 13418-900
[3]Faculty of Animal Science and Food Engineering, University of Sao Paulo, Pirassununga, Brazil 13635-900
[4]dsm-firmenich, Nutritional Products, São Paulo, SP, Brazil 03178-200
[5]dsm-firmenich, ANH R&D Center Tulln, Tulln, Austria 3430
[6]Federal University of Technology–Paraná (UTFPR), Pato Branco, Brazil 85503-390

ABSTRACT

The aim of this study was to evaluate the effects of different anti-mycotoxin feed additives on the concentration of mycotoxins in milk, urine, and blood plasma of dairy cows fed diets artificially contaminated with mycotoxins. Secondarily, performance, total-tract apparent digestibility of nutrients, and blood parameters were evaluated. Twelve multiparous cows (165 ± 45 DIM, 557 ± 49 kg BW, and 32.1 ± 4.57 kg/d milk yield at the start of the experiment) were blocked according to parity, milk yield, and DIM and used in a 4 × 4 Latin square design experiment with 21-d periods, where the last 7 d were used for sampling and data analysis. Treatments were (1) mycotoxin group (MTX), basal diet (BD) without anti-mycotoxin feed additives; (2) hydrated sodium calcium aluminosilicate (HSCA), HSCA added to the BD at 25 g/cow per day; (3) mycotoxin deactivator (MD; Mycofix Plus, dsm-firmenich) added to the BD at 15 g/cow per day (MD15); and (4) MD added to the BD at 30 g/cow per day (MD30). Cows from all treatments were challenged with a blend of mycotoxins containing 404 μg of aflatoxin B_1, 5,025 μg of deoxynivalenol (DON), 8,046 μg of fumonisins (FUM), 195 μg of T2 toxin (T2), and 2,034 μg of zearalenone (ZEN) added daily to the BD during the last 7 d of each period. Neither performance (milk yield and composition) nor nutrient digestibility was affected by treatments. All additives reduced aflatoxin M_1 (AFM1) concentration in milk, whereas MD15 and MD30 group had lower excretion of AFM1 in milk than HSCA. Deoxynivalenol, FUM, T2, or ZEN were not detected in milk of MD15 and MD30. Concentrations in milk of DON, FUM, T2, and ZEN were similar between MTX and HSCA. Except for AFM1, none of the analyzed mycotoxins were detected in urine of MD30 group. Comparing HSCA to MD treatments, the concentration of AFM1 was greater for HSCA, whereas MD30 was more efficient at reducing AFM1 in urine than MD15. Aflatoxin M1, DON, FUM, and ZEN were not detected in the plasma of cows fed MD30, and DON was also not detected in MD15 group. Plasma concentration of FUM was lower for MD15, similar plasma FUM concentration was reported for HSCA and MTX. Plasma concentration of ZEN was lower for MD15 than MTX and HSCA. Serum concentrations of haptoglobin and hepatic enzymes were not affected by treatments. Blood concentration of sodium was lower in HSCA compared with MD15 and MD30 groups. In conclusion, the mycotoxin deactivator proved to be effective in reducing the secretion of mycotoxins in milk, urine, and blood plasma, regardless of the dosage. This reduction was achieved without adverse effects on milk production or total-tract digestibility in cows fed multi-mycotoxin-contaminated diets over a short-term period. Greater reductions in mycotoxin secretion were observed with full dose of MD.
Key words: adsorbents, decontamination, mycotoxin

INTRODUCTION

Mycotoxins are low-molecular-weight secondary metabolites produced by filamentous fungi (Jarvis and Miller, 2005), which are widespread toxic contaminants and endanger both animal and human health (Bryden, 2012). The paramount fungal genera responsible for mycotoxin production include *Aspergillus*, *Penicillium*,

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*These authors contributed equally to this work.
†Corresponding authors: carloscorassin@usp.br and francisco.renno@usp.br

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and *Fusarium*. Within these genera, prominent classes of mycotoxins are aflatoxins (**AF**), ochratoxin A (**OTA**), fumonisins (**FUM**), deoxynivalenol (**DON**), and zearalenone (**ZEN**; Sengling Cebin Coppa et al., 2019). The production of mycotoxins is linked to environmental conditions, plant stress, and damage caused by rodents and pests to grains, as well as abiotic factors such as food pH and moisture content (Bhat et al., 2010; Magan et al., 2011; Makau et al., 2016). Because mycotoxins exhibit remarkable stability due to their physical and chemical properties, they persist over extended periods throughout the grain harvesting, transportation, and storage processes (Qin et al., 2020).

Upon consuming feed tainted with mycotoxins, animals may manifest an array of symptoms, including gastrointestinal dysregulation, diarrhea, soft stools, immunosuppression, and a general decline in performance (EFSA, 2004; Pestka et al., 2004). Although the precise biological mechanisms driving these responses remain have not been fully elucidated, hallmark indicators often include ruminal or gut dysbiosis, heightened permeability of the rumen or gut epithelia, and damage of gut epithelium (Antonissen et al., 2014). In addition, when dairy cows ingest mycotoxins, their metabolites or unmetabolized compounds may be transferred to milk providing an additional source of dietary mycotoxins exposure for humans (Campagnollo et al., 2016).

Among the postharvest mitigation strategies to mycotoxin-contaminated diets, feeding hydrated sodium calcium aluminosilicate (**HSCA**)—a clay-based sequestering agent—has been effectively used to reduce gastrointestinal absorption of AF and its secretion in milk (Jiang et al., 2021). However, clay-based sequestering agents have shown low efficacy against other type of mycotoxins that impairs animal performance, especially against *Fusarium* toxins (Döll et al., 2005; Van Le Thanh et al., 2015; Liu et al., 2022). As an alternative to clay-based sequestering agents, studies have investigated the effects of mycotoxin-deactivating products (**MDP**) composed of a blend of inorganic components, biological components, enzymes, and phycophytic compounds in diets of dairy cows. The effectiveness of MDP was supported by positive responses in performance, immunity, and metabolism parameters in animals exposed to different types of mycotoxins (Pietri et al., 2009; Kiyothong et al., 2012; Gallo et al., 2020). Despite studies revealing positive effects of MDP on several health and performance attributes, they have failed to report the effects of MDP on toxins excretion in milk.

The aim of this study was to evaluate the effects of different anti-mycotoxin feed additives on the concentration of mycotoxin in milk, urine, and blood plasma of dairy cows fed artificially multi-mycotoxin-contaminated diets over a short-term period. We hypothesized that the contaminated diet, even provided for a short-term period, would reduce total-tract digestibility, hence reduce the performance of cows, as well as the MDP in the diets would inhibit these effects and reduce the concentration of mycotoxins in the blood plasma, urine, and milk.

MATERIALS AND METHODS

This study was carried out between April and July 2021 at the Laboratory on Dairy Cattle Research (Laboratório de Pesquisa em Bovinos de Leite, Pirassununga, Brazil) under the approval of the Ethics Committee on Animal Use from the School of Veterinary Medicine and Animal Sciences, University of Sao Paulo (protocol 6324160123).

Treatments and Experimental Design

Twelve Holstein cows (165 ± 45 DIM, 557 ± 49 kg BW, and 32.1 ± 4.57 kg/d milk yield at the start of the experiment; mean ± SD) blocked according to parity, milk yield, and DIM were enrolled into a replicated 4 × 4 Latin square design experiment and cows within each block (Latin square) were randomly assigned to one of the treatment sequences. Experimental periods lasted 21 d of which the first 14 d were allowed for treatment adaptation and 7 d were used to for mycotoxin challenge and data collection. Treatments were (1) mycotoxin group (**MTX**), basal diet (**BD**) without anti-mycotoxin feed additives; (2) HSCA added to the BD at 25 g/cow per day; (3) mycotoxin deactivator (**MD**; Mycofix Plus, dsm-firmenich) added to the BD at 15 g/cow per day (**MD15**); and (4) MD added to the BD at 30 g/cow per day (**MD30**; Table 1). The doses used were those recommended by the product manufacturers. According to the manufacturer, the MD mechanism of action is associated with 3 fundamental properties (Pietri et al., 2009; Grenier et al., 2013; Murugesan et al., 2015): (1) An inorganic component, such as bentonite, can adsorb polar and planar mycotoxins such as aflatoxin B_1 (**AFB1**); (2) the genus nov. sp. nov. of the family *Coriobacteriaceae* strain Biomin BBSH797 deactivates trichothecenes, and a biological component derived from inactivated yeast, which deactivates ZEN, as well as a purified enzyme called FUMzyme (dsm-firmenich) at a concentration of 30,000 U/kg of Mycofix transforms FUM into nontoxic metabolites; and (3) phycophytic substances, typically a blend of plant and algae extracts, offer "bio-protection" that protects vulnerable organs such as the liver and strengthen the immune system of animals. Mycotoxins culture material pool (AFB1, FUM, ZEN, DON, and T2 toxin [**T2**]) and the HCSA and MDP were administered on TMR top-dressed and hand mixed into the first third top of TMR to guarantee total intake by animals. The

Table 1. Description of treatments

Item	Treatment[1]			
	MTX	HSCA	MD15	MD30
Basal diet	✓	✓	✓	✓
Mycotoxin pool[2]	✓	✓	✓	✓
Hydrated sodium calcium aluminosilicate, g/d	0	25	0	0
Mycotoxin deactivator, g/d	0	0	15	30

[1]MTX = basal diet + mycotoxin pool (the checkmark means that this diet was included for all treatments). HSCA = basal diet + mycotoxin pool + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + mycotoxin pool + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + mycotoxin pool +30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN.

HSCA and MDP were administered during 21 d of each experimental period once a day at 0700 h, whereas the mycotoxins pool was administered during the last 7 d of each experimental period once a day at 0700 h. Cows received the multi-mycotoxin culture material with 404 µg of AFB1 + 5,025 µg of DON + 2,034 µg of ZEN + 8,046 µg of FUM + 195 µg of T2 per day during the 7 d of contamination period.

Cows were housed in a barn with individual pens (17.5 m^2 of area), with concrete floor, sanded beds, individual feed bunks, fans, and free access to water. Diets were provided twice a day (0700 and 1300 h). The BD (Table 2) was formulated according to the NRC (2001) nutrient requirements estimates of cows with 600 kg BW, 32.0 kg/d milk yield, and 3.5% fat. Diets had a forage concentrate ratio of 48:52. Refusals of each cow were weighted daily to maintain orts between 5% and 10% (on an as-fed basis) of feed supplied on the previous day.

Mycotoxin Production and Evaluation in the Diets

The implemented doses of mycotoxins in this study were based on an initial survey that aimed to determine the types and levels of mycotoxins occurring in the dairy farms from Brazil (Gruber-Doerninguer et al., 2019). The production of aflatoxins (B_1, B_2, G_1, and G_2), fumonisins (B_1 and B_2), T2, deoxynivalenol, and zearalenone was carried out at the University of São Paulo (Pirassununga, Brazil) following the protocol described by Müller et al. (2017). The concentration of each mycotoxin in the diet was standardized throughout the entire experimental period. After weighing, these mycotoxins were added and mixed into the diets in the last 7 d with each experimental period. A mixture of 400 µg of AFB, 5,000 µg of DON, 2,000 µg of ZEN, 8,000 µg of FUM, and 200 µg of T2 was administered daily for 7 d during the contamination period.

Once a week, 3 samples of TMR were collected and analyzed for mycotoxin evaluation, the samples were immediately sent to the Laboratory of Food Microbiology and Mycotoxicology at the University of São Paulo. The quantification of mycotoxins (AFB1, AFB2, AFG1, AFG2, DON, FUM1, FUM2, T2, and ZEN) was made by ultra-performance liquid chromatography (**UPLC**) electrospray ionization tandem MS (Waters Acquity, Waters Corp.), involving the isotopic dilution step and a data normalization process, as described by Franco et al. (2019).

Multi-Mycotoxin Evaluation in Milk, Urine, and Plasma Samples

Multi-mycotoxins were evaluated in milk, urine, and blood plasma samples from the dairy cows. Before deter-

Table 2. Ingredients and chemical composition of the basal diet used in the experiment

Item	Value
Ingredient, %	
Corn silage	48.0
Ground corn	18.5
Soybean meal	12.7
Whole raw soybean	6.40
Citrus pulp	8.20
Dried distillers grains with solubles	3.30
Urea	0.20
Sodium bicarbonate	0.80
Limestone	0.20
Salt	0.20
Minerals and vitamins[1]	1.50
Chemical composition, %	
DM	49.5
OM	92.1
Starch	29.1
CP	17.6
Ether extract	3.74
Forage NDF	26.8
NDF	36.5
ADF	21.7
Lignin	4.25
Undigested NDF	10.6

[1]Contained per kilogram of product: 160–235 g of Ca, 60 g of P, 13.3 g of Mg, 114 g of Na, 20 g of S, 666.7 mg of Cu, 2,666.7 mg of Mn, 2,666.7 mg of Zn, 33.3 mg of Co, 50 mg of I, 20 mg of Se, 266,700 IU of vitamin A, 66,700 IU of vitamin D_3, and 1,667 IU of vitamin E.

mining multi-mycotoxins in milk, milk production was measured daily, and the data from the last 7 d in each experimental period were recorded. For the evaluation of mycotoxins in milk and urine, samples were collected on the day before supplying a pool of the mycotoxins and on the last day of the contamination period. Blood was collected on the final day of each multi-mycotoxin contamination period without the addition of preservatives and was stored under refrigeration (−80°C) before the analytical testing.

The analysis of multiple mycotoxins in milk, urine, and blood plasma was performed as the methodology proposed by Solfrizzo et al. (2011) and Flores-Flores and Gonzalez-Penas (2017), with minor modifications. Before the extraction of mycotoxins in urine and blood plasma, samples were centrifuged to remove particulate matter and supernatants. After this procedure, glucuronidase/sulfatase was added to the sample for the enzymatic deconjugation of mycotoxins. Then, samples were incubated under static conditions at 37°C overnight. The determination of multi-mycotoxins milk, urine, and blood plasma samples were conducted using a Waters Acquity I-Class UPLC system (Waters Corp.) equipped with a BEH Column C18 (2.1 × 50 mm, 1.7 µm) and coupled to a Xevo TQ-S mass spectrometer (Waters Corp.). Mass spectrometry analyses were carried out in a multiple reaction monitoring (**MRM**) mode by using electrospray ionization in a positive ion mode. The chromatographic procedure, MS parameters, and the MRM transitions (Supplemental Tables S1, S2, and S3, see Notes) were the same adopted by Franco et al. (2019) and Frey et al. (2021).

Nutrient Intake, Feed Sorting Index, and Apparent Digestibility

Feed offered and refusals were recorded daily to determine feed intake. Samples of silage and refusals were collected daily during the last 7 d of each experimental period, the concentrate ingredients were sampled during the concentrate manufacturing before starting the collection period. Samples of daily refusals represented approximately 10% of total refusals. The refusal and feed samples were analyzed for contents of DM (method 930.15; AOAC International, 2000), ash (method 942.05; AOAC International, 2000), CP (N × 6.25; Kjeldahl method 984.13; AOAC International, 2000), and ether extract (method 920.39; AOAC International, 2000). Neutral detergent fiber (Van Soest et al., 1991) was analyzed using α-amylase (TE-149 fiber analyzer; Tecnal Equipamentos para Laboratório Inc.), and ADF and lignin (method 973.18) were analyzed according to AOAC International (2000). Feed ingredients were analyzed for contents of starch using an enzymatic degradation method (Amyloglicosidase, Novozymes Latin America Ltda.) and absorbances measured on a spectrophotometer (SBA-200, Celm) according to Hendrix (1993). The feed sorting index was calculated based on the predicted intake of particle size distribution of TMR and refusals as described by Silveira et al. (2007).

Undigested NDF (**uNDF**) content in feeds, refusals, and feces were used to estimate fecal excretion of DM. Fecal samples (n = 8) were collected directly from the rectum of cows every 9 h during 3 consecutive days (d 15, 0600, 1500, and 0000 h; d 16, 0900 and 1800 h; d 17, 0300, 1200, and 2100 h) and pooled for further analyses. For the uNDF analysis, ground samples (2 mm) of feeds, refusals, and feces were placed in nonwoven fabric bags (12-µm pore size, 5 × 5 cm at 20 mg DM/cm^2) and incubated in the rumen of 2 cannulated dry cows during 288 h (Huhtanen et al., 1994; Casali et al., 2008). After removal from the rumen, bags were washed in running tap water, dried, and NDF content of residue was determined. Digestibility of DM and nutrients were calculated using following equations:

$$\text{DM digestibility (\%)} = 100 - \left[100 \times \left(\frac{\text{\% uNDF intake}}{\text{\% uNDF in feces}}\right)\right],$$

$$\text{Nutrient digestibility (\%)} = 100 - \left[100 \times \left(\frac{\text{\% uNDF intake}}{\text{\% uNDF in feces}}\right) \times \left(\frac{\text{\% nutrient in feces}}{\text{\% nutrient intake}}\right)\right].$$

Milk Yield, Composition, and Serum Metabolites

Cows were milked twice daily (0600 and 1700 h), and milk production was recorded electronically (Alpro, DeLaval). Milk samples (300 mL) were collected from morning and evening milkings for 3 consecutive days during each experimental period to assess concentrations of protein, fat, and lactose using mid-infrared method (Lactoscan, Entelbra). Fat-corrected milk was calculated according to NRC (2001), where 3.5% FCM = (0.432 + 0.165 × fat, %) × milk yield, kg/d. Milk samples were deproteinized with trichloroacetic acid solution (25%; 2:1 vol/vol; Shahani and Sommer, 1951) and stored at −20°C for MUN analyses.

Blood Metabolites, BW, and BCS

Blood samples were collected from the coccygeal vessels in 10-mL vacuum tubes without clot activator (BD Vacutainer, Becton Dickinson) and lithium heparin tubes 4 h after the morning feeding (1100 h) on d 21 in each experimental period. After clotting, blood samples from tubes without clot activator were centrifuged (2,000 × *g* for 15 min at room temperature), the serum was harvest-

ed and stored at −20°C for analysis of hepatic enzymes aspartate aminotransferase (**AST**), alanine transaminase (**ALT**), and gamma-glutamyl transpeptidase (**GGT**). Enzymes were analyzed by commercial colorimetric kits (Bioclin) and absorbances were measured on a semiautomatic biochemical analyzer (SBA-200, Celm). Serum haptoglobin concentration was analyzed in a commercial laboratory (VidaVet) using the method described by Cooke and Arthington (2013).

Whole blood samples from lithium heparin vacuum tubes were analyzed in portable biochemical blood analyzers VetScan VS2 (Zoetis Services LLC) for determination of albumin, amylase, BUN, calcium, creatinine, globulin, glucose, potassium, phosphorus, and total protein. The analysis of whole blood samples for hemoglobin, hematocrit, chloride, bicarbonate, pH, anion gap, base excess in the extracellular fluid compartment, partial pressure of carbon dioxide, and total carbon dioxide content were performed using the i-STAT EC8+ cartridge equipment (Abbot Point Care Inc.). The analysis of vitamin E and β-carotene were performed using the iCheck Vitamin E equipment (BioAnalyt) with the same whole blood samples.

Body weight was measured weekly before the morning feeding and after milking, using an electronic scale for large animals. Body condition score was assessed on the last day of each experimental period using a 5-point system (1 = emaciated to 5 = obese) according to Wildman et al. (1982).

Statistical Analysis

Data from the last 7 d of milk yield and DMI were averaged and used for statistical analysis. Milk composition data from each period were averaged and used for calculating yields of fat, protein, lactose, and FCM used in statistical analysis. Data were submitted to ANOVA using the PROC MIXED of SAS 9.4 (Statistical Analyses for Windows; SAS Institute Inc.) according to the following model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + TP_{ij} + TS_{ik} + \alpha_{kl} + \varepsilon_{ijkl},$$

with $\alpha_{kl} \approx N\left(0;\sigma_c^2\right)$ and $\varepsilon_{ijkl} \approx N\left(0;\sigma_e^2\right)$; where N = Gaussian distribution; σ_c^2 = estimated variance associated with cows; and σ_e^2 = estimated residual variance, Y_{ijkl} = observation on animal l, given treatment i, at period j, in square k; μ = overall mean, A_i = fixed effect of the ith treatment (i = 1 to 4); P_j = fixed effect of the jth period (j = 1 to 4); S_k = fixed effect of the kth Latin square (k = 1 to 3); TP_{ik} = interaction fixed effect between treatment and period; AS_{ik} = interaction fixed effect between treatment and Latin square; α_{kl} = random effect of animal within square (l = 1 to 12); and ε_{ijkl} = random error associated with each observation. Means were adjusted by LSMEANS and degrees of freedom were calculated using the Kenward and Roger (1997) method. LSMEANS were computed for each treatment level to estimate the mean response variable. Differences in response variables were evaluated by Tukey's test. The significance level was set at $P \leq 0.05$.

RESULTS

Contamination and Mycotoxin Intake

In this longitudinal assessment of the diets used for feeding cows, several mycotoxins were detected in the corn silage and in the diet before the artificial contamination (between d 1–14 of each period; Table 3). The daily intake of mycotoxins was slightly greater (1.0, 1.22, and 0.136% for AF, FUM, and ZEN, respectively) than planned due to natural mycotoxin contamination present in the feed (corn silage and concentrate) and the relatively high DMI by the cows. However, no differences were observed in the daily mycotoxin intake (between d 15–21 of each period) between treatments as proposed in the study (Table 4).

Concentrations of Mycotoxin in Milk, Urine, and Blood

Milk concentration of aflatoxin M_1 (**AFM1**) was lower ($P < 0.001$) when cows were fed additives compared with the MTX group. The MD15 and MD30 groups had lower excretion of AFM1 in the milk than HSCA ($P < 0.001$) and were similar to each other (Table 5). Deoxynivalenol, FUM, T2, or ZEN were not detected in the milk of cows fed MD15 and MD30. Concentrations of DON, FUM, T2, and ZEN were similar between MTX and HSCA groups.

Urine concentration of AFM1 was higher ($P < 0.001$) for MTX treatment than other treatments. Comparing HSCA with MD treatments, the concentration of AFM1 was greater for HSCA, and MD30 was more efficient at reducing AFM1 in urine than MD15. Urine concentrations of DON was greater ($P < 0.001$) for HSCA than

Table 3. Concentrations of mycotoxins in feeds and mycotoxin pool

	Mycotoxin,[1] mg/kg				
Item	AF	DON	FUM	T2	ZEN
Corn silage	2.1	<LOQ	61.6	<LOQ	2.0
Concentrate	1.0	<LOQ	16.4	<LOQ	<LOQ
Mycotoxins pool	404	5,025	8,046	195	2,008

[1]AF = aflatoxin; DON = deoxynivalenol; FUM = fumonisin; T2 = T2 toxin; ZEN = zearalenone; LOQ = limit of quantification.

Table 4. Daily intake of mycotoxins (aflatoxins, deoxynivalenol, fumonisins, T2 toxins, and zearalenone) by dairy cows

Treatment[1]	Daily intake, kg of DM/animal	Total daily intake of mycotoxin, µg/animal				
		AF[2]	DON[3]	FUM[4]	T2[5]	ZEN[6]
MTX	26.7	444.8	5,025	9,063	195	2,033.6
HSCA	26.6	444.6	5,025	9,059	195	2,034.5
MD15	27.1	445.4	5,025	9,078	195	2,034.0
MD30	27.1	445.4	5,025	8,960	195	2,034.0

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]AF = total aflatoxins (B_1, B_2, G_1, and G_2).

[3]DON = deoxynivalenol.

[4]FUM = fumonisins (FUM1, FUM2).

[5]T2 = T2 toxin.

[6]ZEN = zearalenone.

MTX, whereas DON was not detected in urine of cows in MD15 and MD30 groups. Fumonisins were not detected in urine of cows fed MD treatments, and similar concentrations of FUM were observed in MTX and HSCA groups. α-Zearalenol (**α-Zel**) or β-zearalenol (**β-Zel**) were not detected in urine of cows fed MD30. Urine concentration of α-Zel was lower ($P < 0.001$) in MD15 group in comparison with HSCA and MTX, whereas α-Zel concentration was the greatest in MTX group. Urine concentration of β-Zel was lower for MD15, whereas

Table 5. Concentrations of mycotoxins in blood, milk and urine of dairy cows fed different anti-mycotoxin feed additives

Item[3]	Treatment[1]				SEM	*P*-value[2]				
	MTX	HSCA	MD15	MD30		Trt	Per	LS	Trt × Per	Trt × LS
Milk, µg/L										
AFM1	1.31[a]	0.792[b]	0.075[c]	0.029[c]	0.096	<0.001	0.067	0.939	0.269	0.943
DON	0.301[a]	0.287[a]	<LOQ	<LOQ	0.010	<0.001	0.000	0.353	0.010	0.879
FUM	0.758[a]	0.718[a]	<LOQ	<LOQ	0.034	<0.001	0.040	0.583	0.216	0.947
T2	0.006[a]	0.005[a]	<LOQ	<LOQ	<0.0001	<0.001	0.103	0.258	0.570	0.107
ZEN	0.095[a]	0.092[a]	<LOQ	<LOQ	0.005	<0.001	0.165	0.436	0.507	0.049
Urine, µg/L										
AFM1	0.249[a]	0.222[b]	0.205[c]	0.184[d]	0.002	<0.001	0.912	0.680	0.701	0.558
DON	54.3[b]	55.0[a]	<LOQ	<LOQ	0.129	<0.001	0.061	0.310	0.157	0.767
FUM	40.9[a]	41.2[a]	<LOQ	<LOQ	0.117	<0.001	0.174	0.855	0.113	0.239
T2	<LOQ	<LOQ	<LOQ	<LOQ	—	1.00	1.000	1.000	1.000	1.000
α-Zel	0.954[a]	1.904[b]	0.023[c]	<LOQ	0.006	<0.001	0.624	0.989	0.951	1.000
β-Zel	1.10[a]	1.09[a]	0.083[b]	<LOQ	0.023	<0.001	0.672	0.998	0.940	1.000
Blood plasma, µg/L										
AFM1	0.281[a]	0.182[b]	0.025[c]	<LOQ	0.005	<0.001	0.986	0.130	1.000	0.069
DON	0.153[a]	0.151[a]	<LOQ	<LOQ	0.001	<0.001	0.270	0.038	0.666	0.286
FUM	0.299[a]	0.276[a]	0.016[b]	<LOQ	0.008	<0.001	0.387	0.459	0.535	0.433
T2	<LOQ	<LOQ	<LOQ	<LOQ	—	1.00	1.000	1.000	1.000	1.000
ZEN	0.269[a]	0.267[a]	0.006[b]	<LOQ	0.003	<0.001	0.169	0.413	0.596	0.436

[a–d]Within a row, means with different superscript letters were significantly different ($P < 0.05$). ND = nondetected or below the limit of detection.

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]*P*-values for effects of treatment (Trt), period (Per), Latin square (LS), interaction between treatment and period (Trt × Per), interaction between treatment and Latin square (Trt × LS).

[3]AFM1 = aflatoxin M_1; DON = deoxynivalenol; FUM = fumonisin; T2 = T2 toxin; ZEN = zearalenone; α-Zel = α-zearalenol; β-Zel = β-zearalenol; LOQ = limit of quantification. The averages have been calculated on the assumption that some animals have values below the LOQ, which has resulted in an overall average value below the LOQ.

urine β-Zel concentration was similar between MTX and HSCA groups.

Aflatoxin M_1, DON, FUM, and ZEN were not detected in plasma of cows fed MD30, and DON was also not detected in MD15 group. The T2 toxin was not detected in the plasma of any of the cows. Blood concentration of AFM1 was reduced ($P < 0.001$) with additives whereas MD15 was more efficient in reducing AFM1 in blood than the HSCA treatment. Plasma concentration of DON was similar between MTX and HSCA groups. Plasma concentration of FUM was lower ($P < 0.001$) in cows fed MD15, whereas similar plasma FUM concentration was observed between HSCA and MTX groups. Plasma concentration of ZEN was lower ($P < 0.001$) for MD15 in comparison with MTX and HSCA.

Performance, Total-Tract Digestibility, and Blood Metabolites

Dry matter intake, or nutrient intake and digestibility were not affected by treatments (Table 6). Feed sorting index, BW, and BCS were similar among treatments. Milk yield, FCM yield, or milk composition were not affected by treatments (Table 7).

Treatment comparisons did not reveal differences in serum concentrations of haptoglobin and hepatic enzymes (ALT, GGT, AST, and alkaline phosphatase [**ALP**]; Table 8). Blood creatinine concentration was greater ($P = 0.009$) in MD15 group in comparison with other treatments, which exhibited similar blood creatinine concentration to each other (Table 9). Blood concentrations of albumin, globulin, hemoglobin, total protein, urea-N, glucose, amylase, and bicarbonate were similar among treatments. Similar blood hematocrit, total carbon dioxide, partial pressure of carbon dioxide, pH, and base excess in the extracellular fluid compartment were observed among treatment groups.

Blood concentration of sodium was lower ($P \leq 0.012$) in HSCA compared with MD15 and MD30 groups (Table 10). Blood concentrations of chlorine, calcium, phosphorus, potassium, vitamin E, and β-carotene were similar among treatments. An interaction effect between treatment and Latin square was observed ($P = 0.022$) for blood vitamin E concentration where cows in the Latin square with the lowest initial milk yield and DMI tended to exhibit different ($P = 0.065$) concentrations for HSCA and MD15 (6.07 and 7.65 mg/L, respectively).

DISCUSSION

It was postulated that feeding MDP treatments would reduce the absorption of mycotoxin and consequently the

Table 6. Intake and total-tract apparent digestibility of nutrients, feed sorting index, and BW of dairy cows submitted to a multi-mycotoxin challenge and fed different anti-mycotoxin feed additives

	Treatment[1]					*P*-value[2]				
Item	MTX	HSCA	MD15	MD30	SEM	Trt	Per	LS	Trt × Per	Trt × LS
Intake, kg/d										
DM	26.9	26.9	27.1	27.1	1.31	0.972	0.434	0.118	0.902	0.755
CP	4.93	4.95	4.96	5.01	0.243	0.922	0.059	0.126	0.924	0.763
NDF	12.1	12.1	12.2	12.1	0.45	0.885	<.001	0.146	0.769	0.378
OM	25.8	25.9	26.0	26.1	1.26	0.969	0.348	0.119	0.898	0.757
Apparent digestibility, %										
DM	62.2	63.8	62.5	62.7	1.51	0.833	<.001	0.271	0.464	0.798
CP	60.2	62.5	60.6	61.0	1.89	0.772	<.001	0.449	0.294	0.558
NDF	50.6	53.2	51.6	50.9	2.45	0.813	0.002	0.196	0.936	0.996
OM	64.6	66.4	65.0	65.3	1.46	0.785	<.001	0.343	0.454	0.783
Sorting index[3]										
>19 mm	0.889	0.882	0.863	0.872	0.029	0.920	0.255	0.043	0.041	0.246
19–8 mm	0.999	1.00	1.00	0.994	0.005	0.564	0.508	0.848	0.272	0.630
8–4 mm	1.01	1.02	1.01	1.01	0.004	0.534	0.035	0.162	0.048	0.513
<4 mm	1.03	1.02	1.03	1.04	0.011	0.803	0.833	0.192	0.124	0.641
BCS	2.50	2.50	2.48	2.50	0.027	0.933	0.933	0.087	0.998	0.853
BW, kg	559	570	568	578	13.8	0.813	<.001	<.001	0.841	0.998

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15: basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]*P*-values for effects of treatment (Trt), period (Per), Latin square (LS), interaction between treatment and period (Trt × Per), interaction between treatment and Latin square (Trt × LS).

[3]No sorting: 1, value 1 indicates sorting for particles on the particular particle size range; sorting index was calculated according to Silveira et al. (2007).

Table 7. Milk yield and composition of dairy cows submitted to a multi-mycotoxin challenge and fed different anti-mycotoxin feed additives

	Treatment[1]					*P*-value[2]				
Item	MTX	HSCA	MD15	MD30	SEM	Trt	Per	LS	Trt × Per	Trt × LS
Yield, kg/d										
Milk	31.4	31.8	32	31.4	1.22	0.688	0.052	0.355	0.812	0.456
3.5% FCM	35.5	35.7	36.6	35.9	1.26	0.674	0.670	0.095	0.552	0.778
Fat	1.34	1.33	1.38	1.36	0.055	0.705	0.994	0.075	0.551	0.754
Protein	0.977	0.977	0.988	0.975	0.035	0.930	0.112	0.299	0.968	0.564
Lactose	1.44	1.45	1.48	1.44	0.082	0.648	<.001	0.593	0.746	0.504
Composition, %										
Fat	4.23	4.19	4.33	4.36	0.166	0.526	0.415	0.492	0.785	0.631
Protein	3.11	3.11	3.09	3.11	0.024	0.299	<.001	0.655	0.375	0.141
Lactose	4.56	4.52	4.60	4.58	0.140	0.519	<.001	0.947	0.971	0.836

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]*P*-values for effects of treatment (Trt), period (Per), Latin square (LS), interaction between treatment and period (Trt × Per), interaction between treatment and Latin square (Trt × LS).

concentration in the blood, thereby reducing the secretion of mycotoxins in the milk and urine of dairy cows challenged with a mycotoxin blend in the diet. Indeed, cows fed with both doses of MD had lower concentrations of mycotoxins in milk, urine, and blood in comparison with MTX and HSCA treatments. In addition, it was expected that mycotoxins would have detrimental effects in the gastrointestinal tract that could impair digestibility (Liew and Mohd-Redzwan, 2018) and consequently milk production. However, no effects on animal performance and total apparent digestibility were observed, possibly due to the contamination levels not being sufficiently high and the short-term period of exposure (7 d). It is important to highlight that this study has no genuine control group (i.e., a group with cows not exposed to mycotoxin challenge) and the results should be interpreted with caution.

Concentrations of mycotoxins initially proposed in this study are very close to what was observed in the mycotoxicological analysis (Gruber-Dorninger et al., 2019). Treatments containing MDP reduced mycotoxins in milk of dairy cows. Studies have shown reduced AF in milk when anti-mycotoxin additives were fed (Kutz et al., 2009; Pietri et al., 2009), but further studies are warranted to determine the effectiveness of these additives in reducing the secretion of other mycotoxin in milk. As expected, HSCA was effective in reducing concentration of AFM1 in blood, milk, and urine. It is well known that HSCA is effective in binding AF and preventing its absorption by the intestine and entering the bloodstream

Table 8. Concentration of haptoglobin and hepatic enzymes in the blood of dairy cows submitted to a multi-mycotoxin challenge and fed different anti-mycotoxin feed additives

	Treatment[1]					*P*-value[2]				
Item[3]	MTX	HSCA	MD15	MD30	SEM	Trt	Per	LS	Trt × Per	Trt × LS
Hp, mg/mL	1.14	1.24	1.01	1.10	0.219	0.899	0.068	0.608	0.548	0.910
AST, U/L	69.9	69.9	68.8	70.4	2.94	0.953	0.005	0.653	0.016	0.626
GGT, U/L	28.3	26.4	27.7	23.7	2.18	0.359	0.117	0.139	0.170	0.346
ALT, U/L	49.8	50.9	49.2	49.1	2.34	0.851	0.091	0.479	0.274	0.462
ALP, U/L	64.9	63.4	73.3	71.9	10.3	0.081	0.269	0.474	0.153	0.222

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30: basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]*P*-values for effects of treatment (Trt), period (Per), Latin square (LS), interaction between treatment and period (Trt × Per), interaction between treatment and Latin square (Trt × LS).

[3]Hp = haptoglobin; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

Table 9. Blood parameters of dairy cows submitted to a multi-mycotoxin challenge and fed different anti-mycotoxin feed additives

	Treatment[1]					*P*-value[2]				
Item	MTX	HSCA	MD15	MD30	SEM	Trt	Per	LS	Trt × Per	Trt × LS
Albumin, g/dL	2.73	2.68	2.65	2.71	0.060	0.272	0.004	0.262	0.952	0.864
Globulin, g/L	58.8	56.7	58.6	57.2	1.72	0.082	0.003	0.065	0.230	0.171
Hemoglobin, g/dL	7.66	7.60	7.88	7.83	0.199	0.311	0.188	0.664	0.674	0.707
Hematocrit, % PCV[3]	22.5	22.3	23.2	23.0	0.585	0.293	0.188	0.696	0.706	0.693
Total protein, g/dL	8.61	8.33	8.53	8.43	0.139	0.094	0.115	0.045	0.260	0.403
Creatinine, mg/dL	0.858[b]	0.833[b]	0.975[a]	0.833[b]	0.042	0.009	0.116	0.094	0.173	0.819
Urea-N, mg/dL	14.8	14.8	15.1	14.5	0.691	0.740	<.0001	0.524	0.976	0.442
Glucose, mg/dL	66.9	66.8	66.8	66.2	1.13	0.757	0.696	0.570	0.358	0.329
Amylase, U/L	41.9	42.8	42.4	45.3	2.90	0.075	0.128	0.511	0.112	0.261
TCO_2,[4] mmol/L	31.0	30.8	30.8	30.8	0.574	0.992	0.793	0.565	0.389	0.587
PCO_2,[5] mm/Hg	41.2	40.3	40.2	42.4	1.23	0.523	0.242	0.539	0.216	0.532
Bicarbonate, mmol/L	29.7	29.6	29.6	29.5	0.545	0.985	0.899	0.423	0.300	0.687
pH	7.46	7.47	7.48	7.45	0.013	0.561	0.362	0.262	0.991	0.685
BEecf,[6] mmol/L	5.92	6.00	6.00	5.67	0.674	0.959	0.999	0.426	0.530	0.672
Anion gap, mmol/L	13.1	13.8	13.0	13.4	0.417	0.575	0.501	0.102	0.848	0.647

[a,b]Within a row, means with different superscript letters were significantly different ($P < 0.05$).

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]*P*-values for effects of treatment (Trt), period (Per), Latin square (LS), interaction between treatment and period (Trt × Per), interaction between treatment and Latin square (Trt × LS).

[3]PCV = packed cell volume.

[4]Total carbon dioxide.

[5]Partial pressure of carbon dioxide.

[6]Base excess in the extracellular fluid compartment.

(Kubena et al., 1993; Neeff et al., 2013; Awuchi et al., 2021). Although HSCA is an effective adsorbent for AF, it does not minimize the toxic effects of other mycotoxins such as DON and ZEN (Döll et al., 2005; Van Le Thanh et al., 2015; Liu et al., 2022). In this study, feeding MD15 and MD30 has been shown to be more effective in reducing AFM1 in milk, urine, and blood in comparison to feeding HSCA. A previous study showed that an earlier MDP was able to reduce the AF in milk by 31% and 41% when fed at 20 g/cow per day and 50 g/cow per day, respectively (Pietri et al., 2009). Several reviews have also described the effectiveness of MDP in counteract-

Table 10. Concentration of minerals, vitamin E and β-carotene in the blood of dairy cows submitted to a multi-mycotoxin challenge and fed different anti-mycotoxin feed additives

	Treatment[1]					*P*-value[2]				
Item	MTX	HSCA	MD15	MD30	SEM	Trt	Per	LS	Trt × Per	Trt × LS
Mineral, mmol/L										
Chlorine	100	99.1	101	101	0.650	0.112	0.243	0.695	0.747	0.689
Calcium	2.49	2.46	2.51	2.49	0.036	0.567	0.059	0.224	0.711	0.251
Phosphorus	2.20	2.32	2.31	2.29	0.090	0.401	0.584	0.648	0.811	0.399
Sodium	141[ab]	140[b]	142[a]	142[a]	0.565	0.043	0.034	0.146	0.378	0.103
Potassium	4.77	4.46	4.58	4.62	0.082	0.067	0.410	0.107	0.748	0.264
Vitamin E, mg/L	6.18	5.94	6.25	6.25	0.493	0.585	0.002	0.501	0.639	0.022
β-Carotene, mg/L	1.67	1.67	1.56	1.68	0.201	0.889	0.004	0.773	0.983	0.648

[a,b]Within a row, means with different superscript letters were significantly different ($P < 0.05$).

[1]MTX = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN. HSCA = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 25 g/cow per day of hydrated sodium calcium aluminosilicate. MD15 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 15 g/cow per day of Mycofix Plus (dsm-firmenich). MD30 = basal diet + 404 µg of AFB + 5,025 µg of DON + 8,046 µg of FUM + 195 µg of T2 + 2,008 µg of ZEN + 30 g/cow per day of Mycofix Plus (dsm-firmenich).

[2]*P*-values for effects of treatment (Trt), period (Per), Latin square (LS), interaction between treatment and period (Trt × Per), interaction between treatment and Latin square (Trt × LS).

ing the negative effects of mycotoxins in poultry, swine, and dairy cows (Cheng et al., 2006; Gallo et al., 2020; Kehinde et al., 2020).

The US Food and Drug Administration has set an action level for AFM1 of 0.50 μg/kg in liquid milk, total AF of 20 μg/kg in feed ingredients offered to dairy and breeding cattle (FDA, 2019). The European Commission set up an action level for AFM1 of 0.05 μg/kg in liquid milk, AFB1 of 20 μg/kg in all feedstuffs, 10 μg/kg in complete feeds for cattle, sheep, and goats, and 5 μg/kg in complete feeds for dairy cattle (EC, 2003, 2006). In this study, the level of AFM1 found in milk was 1.31, 0.79, 0.08, and 0.03 μg/kg for the MTX, HSCA, MD15, and MD30 groups, respectively. It is worth noting that the cows in the MTX group were also contaminated with mycotoxins, which is the reason why they exhibited such a high level of mycotoxin in their milk. However, only MD30 has been able to reduce AFM1 in milk below the limit allowed by the European Commission.

Regarding the other mycotoxins, HSCA presented no difference compared with the MTX, meaning it did not reduce the concentration of FUM, T2, or ZEN in the blood, milk, and urine due to the varying affinities that different mycotoxins have for adsorbents. Hydrated sodium calcium aluminosilicate is an inorganic adsorbent that shows a remarkable ability to bind with nonpolar mycotoxins such as AF (Vekiru et al., 2007), being inefficient to bind other kinds of mycotoxins. However, treatment MD15 was able to reduce other mycotoxin (DON, FUM, T2, and ZEN) concentrations in milk to levels below the detection limit. The same occurred to DON, FUM, and T2 in the urine. α-Zearalenol and β-Zel are metabolites of ZEN that was measured in the urine, and the MD15 treatment has caused 97.5% and 92.4% reduction of α-Zel and β-Zel, respectively. The results of the mycotoxins in the blood resemble those found in milk and urine. Plasma levels of DON and T2 were reduced below the limit of quantification (**LOQ**) for MD15 group, whereas FUM and ZEN decreased by a remarkable 96.3% and 97.7% for MD15 and MD30, respectively. The MD30 treatment was the most effective in reducing the levels of mycotoxins; except for AFM1 in milk and urine, mycotoxins analyzed in this study were below the detection limit for milk, urine, and blood in cows fed MD30.

Mycotoxin deactivator has 3 mechanisms of action that supports its anti-mycotoxin effect. Inorganic constituents, specifically a blend of bentonites and diatomaceous earths present in MD, serve to adsorb polar mycotoxins such as AF (Vekiru et al., 2007). Non-adsorbable mycotoxins (e.g., trichothecenes, ZEN) are biotransformed by genus nov. species nov. of the family *Coriobacteriaceae* strain (BBSH7) and a yeast strain affiliated to the *Trichosporon genus* (*Trichosporon mycotoxinivorans* MTV; Schatzmayr et al., 2006). Ultimately, phycophytic compounds sourced from marine algae (*Ascophyllum nodosum*) and extracts from plants (*Silybum marianum*) function to protect vulnerable organs such as the liver and strengthen the immune system (Murugesan et al., 2015). The reduction of AF in the blood was observed for HSCA and MD15 (35% and 91%, respectively), although MD30 decreased AF concentration in plasma below LOD (0.033 μg/L).

Hepatic enzymes (GGT, AST, ALT, and ALP) are indicators of liver function (Santos and Fink-Gremmels, 2014; Xiong et al., 2015). There were no significant differences in most serum biochemical parameters among the treatments. Similar results have been observed in dairy cows fed with a BD contaminated with AF (0, 20, and 40 μg/kg of AF), and no changes were found in hepatic enzymes (Wang et al., 2019). Data on levels of hepatic enzymes in healthy bovine is still controversial in the literature, probably because AST, ALT, and GGT are not constant during the lactation and pregnancy phases (Tainturier et al., 1984; Stojević et al., 2005) and it depends on breed, sex, age, feeding, and so on (Zaitsev et al., 2020). Kaneko et al. (2008) suggest adequate blood concentrations of 78 to 132 U/L for AST, 6.1 to 17.4 U/L for GGT, and 11 to 40 U/L for ALT; however, the cattle breed data are not available. Stojević et al. (2005) studied the hepatic enzymes levels of 120 clinically healthy cows at different stages of lactation and found 44.9 ± 6.9, 20.08 ± 3.7, and 14.72 ± 3.7 U/L for AST, ALT, and GGT respectively for cows after the peak of lactation. In the current study, serum concentrations of AST, GGT, and ALT were 69.7, 26.9, and 49.8 U/L, respectively, slightly higher than reported in the literature (Stojević et al., 2005; Kaneko et al., 2008), as diets from all treatments were contaminated with mycotoxins. An increase in these enzymes after the mycotoxin challenge was expected, at least in MTX and HSCA groups, as they can cause damage photosensitization in cattle (Casteel et al., 1995). Serum amylase is considered a biomarker of acute pancreatitis (Salt and Schenker, 1976; Furey et al., 2020). Creatinine serves as an indicator of glomerular filtration rate, which is considered the most reliable measure of kidney function (KDIGO, 2024). In this trial, serum creatinine concentration was greater in MD15 compared with other treatment groups. However, serum creatinine remained within the range of normal concentration (Titgemeyer and Löest, 2001). The reasons for different serum creatinine concentrations between MD15 and other groups are unclear.

Mycotoxin adsorbents can bind to vitamins and minerals (Ward et al., 1991; Huwig et al., 2001; Liu et al., 2022), but no treatment differences were found in blood concentrations of β-carotene, Ca, or P. Despite reports of mycotoxin adsorbents binding to vitamins and minerals, the results of our study did not show differences in

plasma concentrations of vitamin E, β-carotene, Ca, or P. Mycotoxin deactivator has shown not to bind to vitamins in minerals in other studies also (Gallo et al., 2020). Blood sodium concentration was lower in the HSCA group compared with MD15 and MD30. Despite HSCA having the capacity to absorb vitamins and minerals as well (Moshtaghian et al., 1991; Huwig et al., 2001; Liu et al., 2022), we are not able to attribute this effect to HSCA binding properties, as blood levels of sodium in healthy animals on the same diet but without mycotoxins were not evaluated. However, cows with the highest mycotoxin challenge relative to DMI (i.e., cows with the lowest DMI) showed marginal differences in blood vitamin E concentration, whereas cows fed MD15 tended to present greater concentrations than those fed HSCA.

No differences were found in mycotoxin intake because the amounts of mycotoxins offered to the cows were carefully controlled, and the supply was administered through top dressing. Dry matter intake and milk yield were not affected by treatments. Similar results on these aspects are found in the literature (Kutz et al., 2009; Pietri et al., 2009). In a previous study evaluating the effects of a long period of exposure to mycotoxins (54 d) in a naturally contaminated diet, no differences were found in performance of dairy cows (n = 30) either among control group, diet with low contamination, and diet with high contamination and 100 g/cow per day of anti-mycotoxin feed additive (Catellani et al., 2023). These results can be explained by the fact that ruminants are less sensitive to mycotoxins, they are generally protected against toxins by the rumen microbiota (Fink-Gremmels, 2008a,b). However, Santos and Fink-Gremmels (2014) affirm that the exposure of dairy cows for a long period of the time (2 mo) can have negative effects on milk production. Therefore, the short-term exposure period of this trial can explain the lack of effects on performance and digestibility, but it reflects what can happen in dairy farms.

CONCLUSIONS

Feeding a mycotoxin deactivator over a short-term contamination period effectively reduces AFM1, DON, FUM, T2, and ZEN in blood, milk, and urine of dairy cows in both doses. The MD30 treatment presented the greatest reduction of mycotoxins in blood, urine, and milk, followed by MD15 and HSCA. The MD30 treatment reduced all other mycotoxins below the limit of detection in blood, urine, and milk, except for AFM1 in milk and urine. The lower dose of mycotoxin deactivator (MD15) presented a significant reduction in all mycotoxins, whereas HSCA presented small reduction in AF, and it was not able to reduce other mycotoxins secretions in blood, urine, and milk. This study did not detect differences in performance and digestibility of dairy cows. However, even during a short-term exposure to contaminated diets, dairy cows are capable of absorbing and secreting mycotoxins in milk, which are harmful to human health. This study demonstrated the effectiveness of the MD in reducing absorption by the cow and producing milk that is safer for consumers.

NOTES

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Nonstandard abbreviations used: α-Zel = α-zearalenol; β-Zel = β-zearalenol; AF = aflatoxins; AFB1 = aflatoxin B_1; AFM1 = aflatoxin M_1; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; BD = basal diet; DON = deoxynivalenol; FUM = fumonisins; GGT = gamma-glutamyl transpeptidase; HSCA = hydrated sodium calcium aluminosilicate; LOQ = limit of quantification; LS = Latin square; MD = mycotoxin deactivator; MDP = mycotoxin-deactivating products; MD15 = MD added to the BD at 15 g/cow per day; MD30 = MD added to the BD at 30 g/cow per day; MRM = multiple reaction monitoring; MTX = mycotoxin group; OTA = ochratoxin A; Per = period; T2 = T2 toxin; Trt = treatment; uNDF = undigested NDF; UPLC = ultra-performance liquid chromatography; ZEN = zearalenone.

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