A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of *Fusarium* mycotoxins in dairy cows

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ABSTRACT

Little is known about the effects of commonly found levels of *Fusarium* mycotoxins on the performance, metabolism, and immunity of dairy cattle. We investigated the effects of regular contamination levels, meaning contamination levels that can be commonly detected in dairy feeds, of deoxynivalenol (DON) and fumonisins (FB) in total mixed ration (TMR) on the performance, diet digestibility, milk quality, and plasma liver enzymes in dairy cows. This trial examined 12 lactating Holstein dairy cows using a 3-period × 3-treatment Latin square design. The experimental period was 21 d of mycotoxin exposure followed by 14 d of washout. During treatment periods, cows received one of 3 diets: (1) CTR (control) diet of TMR contaminated with 340.5 µg of DON/kg of dry matter (DM) and 127.9 µg FB/kg of DM; (2) MTX diet of TMR contaminated with *Fusarium* mycotoxins at levels higher than CTR but below US and European Union guidelines (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); or (3) MDP diet, which was MTX diet supplemented with a mycotoxin deactivator product (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM; Mycofix, 35 g/animal per day). During washout, all animals were fed the same CTR diet. Body weight, body condition score, DM intake, dietary nutrient digestibility, milk production, milk composition and rennet coagulation properties, somatic cell count, blood serum chemistry, hematology, serum immunoglobulin concentrations, and expression of multiple genes in circulating leucocytes were measured. Milk production was significantly greater in the CTR group (37.73 kg/d) than in the MTX (36.39 kg/d) and the MDP (36.55 kg/d) groups. Curd firmness and curd firming time were negatively affected by the MTX diet compared with the other 2 diets. Furthermore, DM and neutral detergent fiber digestibility were lower after the

MTX diet than after the CTR diet (67.3 vs. 71.0% and 42.8 vs. 52.3%). The MDP diet had the highest digestibility coefficients for DM (72.4%) and neutral detergent fiber (53.6%) compared with the other 2 diets. The activities of plasma liver transaminases were higher after the MTX diet than after the CTR and MDP diets. Compared with the CTR diet, the MTX diet led to slightly lower expression of genes related to immune and inflammatory functions, indicating that *Fusarium* mycotoxins had an immunosuppressive effect. Our results indicated that feed contaminated with regular levels of *Fusarium* mycotoxins adversely affected the performance, milk quality, diet digestibility, metabolic variables, and immunity of dairy cows, and that supplementation with mycotoxin deactivator product counteracted most of these negative effects.

Key words: milk, mycotoxin, silage, digestibility

INTRODUCTION

Mycotoxins are a diverse group of secondary metabolites produced by many filamentous fungi in the genera *Aspergillus*, *Fusarium*, and *Penicillium* that can cause toxic responses when ingested by humans and other vertebrates (Zinedine et al., 2007; Fink-Gremmels, 2008a; Zain, 2011). Mycotoxins are generally very stable and can be detected in animal feeds and home-grown forage (Kabak et al., 2006; Cheli et al., 2013; Gallo et al., 2015). Among mycotoxins, those produced by *Fusarium* spp. are usually detected in several feeds because mycotoxigenic *Fusarium* molds are widespread and able to contaminate field crops in the temperate and warm climate zones (EFSA, 2013a). Previous studies have described the toxicological effects of *Fusarium* toxins in farm animals (Fink-Gremmels and Malekinejad, 2007; Pestka, 2007; Voss et al., 2007; Bernabucci et al., 2011). Animals develop numerous symptoms following consumption of feed contaminated with the mycotoxin deoxynivalenol (DON), including gastrointestinal problems, soft stools, diarrhea, immunosuppression, and a general decrease of performance, prob-

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ably due to feed refusal (EFSA, 2004; Pestka, 2007). The biological mechanisms underlying these responses are not well understood, but dysbiosis in the rumen or gut milieu, increased permeability of the rumen or gut epithelia, and damage of gut epithelium are common signs (Antonissen et al., 2014). The fumonisins (**FB**) are mycotoxins that are cytotoxic, hepatotoxic, and nephrotoxic. Although their mechanism of action is uncertain (Caloni et al., 2000; Sabatini et al., 2010; Abeni et al., 2014), the animal gut absorbs FB (Riley and Pestka, 2005), and they can alter some metabolic pathways. Generally, an immunosuppressive condition in the intestine involves molecular systems including the downregulation of MyD88 and TLR, which are important players to act in the first phase of the pathogens' recognition, starting the inflammatory cascade necessary in responding to the pathogen (Sugiyama et al., 2016; Dąbrowski et al., 2017). There are no data in ruminants to investigate the effect of mycotoxins on circulating leukocytes.

As reviewed by Fink-Gremmels (2008b), several mycotoxins could be inactivated by the rumen flora (e.g., DON, which is converted almost completely into the less toxic de-epoxidized metabolite given physiological rumen conditions), whereas others pass the rumen-reticulum compartment unchanged (e.g., FB) or are converted into metabolites that retain biological activity. Hence, various mycotoxins have the ability to modify the rumen flora due to their antimicrobial activity and exert a modulating effect on the immune system of host animal, even at low doses. There is limited conclusive scientific evidence regarding the real effect of mycotoxins on digestive, physiological, and pathophysiological traits of dairy cows (Fink-Gremmels, 2008a; Gallo et al., 2015). For instance, in the opinion of the European Food Safety Authority concerning DON as an undesirable substance in the agro-food chain (EFSA, 2004), effects ascribable to this mycotoxin ingestion in dairy cows are described as loss of appetite, reduced feed intake and rumination activity, immunosuppression, and upregulation of pro-inflammatory cytokines, but it was also stated that only few feeding studies with dairy cows were available. Similarly, a recent scientific opinion of Knutsen et al. (2018) concerning FB, stated there is scarce information concerning the adverse effects of these mycotoxins in ruminants, particularly dairy cows. However, changes in organ macro- and microscopic appearance and serum enzymes, biochemistry consistent with an impairment of liver, and possibly kidney function were reported and associated with adverse effects of FB ingestion. The effects of mycotoxins on cheese-making characteristics have been poorly investigated (Migliorati et al., 2007), in particular when some kind

of mycotoxin-deactivating products are used in diets. Consequently, evaluation of the economic effect of mycotoxins, such as aflatoxins and *Fusarium*-produced mycotoxins, on ruminant livestock production deserves further investigation (Dunière et al., 2013; Santos et al., 2013; Pulina et al., 2014).

Studies of this topic may help field practitioners in advising dairy farmers who experience nonspecific health and performance problems on their farms. A previous study tested the effect of an MDP (Mycofix) on lactating dairy cows fed diets naturally contaminated by aflatoxins (**AF**; Pietri et al., 2009) or AF and several *Fusarium* toxins, such as DON, zearalenone (**ZE**), FB1, ochratoxin A (**OTA**), T-2 toxin, and HT-2 toxin (Kiyothong et al., 2012). The European Union has approved this product for the use in pig and poultry diets (Murugesan et al., 2015). This product is based on 3 principal properties (Pietri et al., 2009; Grenier et al., 2013; Murugesan et al., 2015): (1) its inorganic components (e.g., bentonite) adsorb polar, planar mycotoxins such as AF; (2) for mycotoxins unaffected by the inorganic components (e.g., trichothecenes, ZEA, FB), *Eu-bacterium* strain Biomin BBSH797 deactivates trichothecenes, a biological constituent deactivates ZEA, and a purified enzyme (fumonisin-esterase, FUMzyme 30,000 U/kg of Mycofix) biotransforms FB into nontoxic metabolites; and (3) its phycophytic substances (usually a blend of extracts from plants and algae) provide protective effects. In particular, FUMzyme is able to convert FB in their hydrolyzed forms (**HFB**) or partially hydrolyzed forms (**pHFB**) by acting an enzymatic degradation using fumonisin esterase FumD (EC 3.1.1.87; Heintz et al., 2010; Hartinger and Moll, 2011). This enzyme has been showed to eliminate FB parent toxin and to act a detoxification process that facilitates the formation of nontoxic metabolites (Grenier et al., 2017; Hahn et al., 2015).

The aims of this study on lactating dairy cows were to investigate the effects of a diet contaminated by regular concentrations of different *Fusarium* mycotoxins on feeding behavior, rumination activity, milk yield, its composition, milk coagulation properties, hematological, immunological, and biochemical traits and to determine the effect of a mycotoxin-deactivating product in preventing the negative effects of these mycotoxins.

MATERIALS AND METHODS

The study was authorized by Italian Health regulations that pertain to the accommodation and care of animals used for experimental and other scientific purposes (authorization n. 232/2017-PR issued on March 17, 2017).

Table 1. Chemical composition of ingredients and experimental diets (TMR; mean $\pm$ SD)

Ingredient	kg as fed	Chemical ¹					
		DM	CP	EE	Starch	Ash	aNDF _{om}
Corn silage	28.00	35.05 $\pm$ 0.51	9.30 $\pm$ 0.24	2.71 $\pm$ 0.21	32.05 $\pm$ 1.66	4.30 $\pm$ 0.22	43.85 $\pm$ 0.95
Sunflower dehulled meal 34%	1.00	89.25 $\pm$ 0.04	40.25 $\pm$ 1.32	0.72 $\pm$ 0.10	—	7.13 $\pm$ 0.03	—
Hydrogenated palm oil	0.40	—	—	99.9 $\pm$ 0.01	—	—	—
Corn meal	5.00	88.02 $\pm$ 0.28	10.73 $\pm$ 0.14	4.34 $\pm$ 0.15	69.47 $\pm$ 1.73	1.53 $\pm$ 0.11	—
Rye grass hay	0.80	94.61 $\pm$ 0.61	10.15 $\pm$ 0.32	1.88 $\pm$ 0.86	—	8.99 $\pm$ 1.46	62.16 $\pm$ 3.15
Dehydrated alfalfa hay	5.00	91.32 $\pm$ 0.76	19.15 $\pm$ 1.08	1.21 $\pm$ 0.29	—	9.58 $\pm$ 0.57	44.67 $\pm$ 1.50
Water	4.00	—	—	—	—	—	—
Soybean meal 44%	2.50	88.83 $\pm$ 0.04	48.41 $\pm$ 0.71	3.70 $\pm$ 0.14	—	7.17 $\pm$ 0.48	—
Premix ²	0.30	85.05 $\pm$ 0.51	9.30 $\pm$ 0.24	2.71 $\pm$ 0.21	32.05 $\pm$ 1.66	4.30 $\pm$ 0.22	23.85 $\pm$ 0.95
TMR composition ³							
CTR		50.68 $\pm$ 1.95	17.22 $\pm$ 2.02	4.14 $\pm$ 0.04	25.19 $\pm$ 2.37	6.14 $\pm$ 0.16	37.92 $\pm$ 0.78
MTX		50.45 $\pm$ 1.59	17.41 $\pm$ 1.31	4.03 $\pm$ 0.26	25.89 $\pm$ 1.76	6.20 $\pm$ 0.30	37.59 $\pm$ 0.61
MDP		50.33 $\pm$ 1.63	17.48 $\pm$ 2.21	4.14 $\pm$ 0.26	25.97 $\pm$ 3.68	5.79 $\pm$ 0.20	36.70 $\pm$ 0.90

¹EE = ether extract; aNDF_{om} = solution containing sodium sulfite and a heat-stable amylase and corrected for residual ash.

²Each kg contains 1,500,000 IU of vitamin A, 150,000 IU of vitamin D, 3,000 mg of vitamin E, 50 mg of vitamin B₃, 0.5 mg of vitamin B₁₂, 500 mg of vitamin P, 4,000 mg of choline, 350 mg of Mn, 800 mg of Zn, 40 mg of Cu, 20 mg of I, 1 mg of Co, 1 mg of Se, and 2 g of DL-methionine.

³CTR = TMR contaminated with regular level of *Fusarium* mycotoxins; MTX = TMR contaminated with a higher level of *Fusarium* mycotoxins compared with to CTR, but below US and European Union guidelines; MDP = MTX diet supplemented with mycotoxin deactivator product.

Experimental Cows and Diets

A total of 12 multiparous mid-lactation Holstein cows of different parity (2.92 ± 1.08 parities; 114 ± 16 DIM; milk yield of 42.2 ± 5.2 kg at study onset) were used in the experiment, conducted at the CERZOO research and experimental center (San Bonico, Piacenza, Italy). Cows were kept in a common pen, had free access to water, and were individually fed assigned diets formulated according to the nutrient requirements of dairy cattle (NRC, 2001) using the Calan Broadbent feeding system (American Calan Inc., Northwood, NH). Cows were randomly assigned to 1 specific feeder station before the start of the trial. During the study period, the climatic conditions and lighting scheme were natural. Temperature and relative humidity data were provided by the Regional Agency for Prevention, Environment, and Energy (ARPAE-Emilia Romagna).

Each TMR used in this study had the same composition, cows were fed once a day at 0800 h, and 5% expectedorts were collected individually and weighed daily. The components (Table 1) were mixed in an experimental mixer wagon (Storti model Labrador 70 horizontal mixer, capacity of 8 m³, Storti, Belfiore, Verona, Italy) in the following order: corn silage, hays, soybean meal (44%); dehulled sunflower meal (34%); and water. Then, corn meals (normal or supplemented by a mycotoxin contaminated culture medium, **CornMS**), were individually added to each diet, manually mixed, and fed to animals in specific feeding stations. The CornMS had fumonisin from *F. verticillioides* (Desjardins and Plattner, 2000) that was grown

on rice, homogenized, and freeze-dried, and DON from *F. graminearum* (Altpeter and Posselt, 1994) that was grown on maize, homogenized, and freeze-dried. The whole TMR with 1 or 2 corn meals (depending on the specific treatment) was provided individually to each animal based on measurement of DMI on the previous day. During the adaptation period and clearance period, all animals received the same TMR diet, which was similar in composition to the experimental control diet (described below). Representative TMR samples were collected weekly and analyzed for nutrient composition and mycotoxins (described below). Samples of individual ingredients were analyzed at the beginning of each experimental period, and their chemical compositions were determined (Table 1).

The whole study lasted 114 d and consisted of a group formation and adaptation period (21 d) and three 21-d experimental periods (in which each cow was assigned to a specific treatment) that were separated by two 14-d clearance periods (in which all animals received the same TMR, as described above). The animals were allocated before the start of the study to 1 of 4 Latin squares based on parity, BW, and milk yield. The experimental design was a 3 periods $\times$ 3 treatments Latin square design that consisted of 4 replicates. In each Latin square, 3 cows were grouped, and treatments consisted of: (1) the control diet (**CTR**), TMR contaminated by a regular level of *Fusarium* mycotoxins (i.e., 340.5 μ g of DON/kg of DM and 127.9 μ g of FB/kg of DM in the whole diet); (2) the mycotoxin (**MTX**) diet, TMR contaminated by a higher level of *Fusarium* mycotoxins compared with CTR (i.e., 733.0 μ g of

DON/kg of DM and 994.4 µg of FB/kg of DM); or (3) the mycotoxin-deactivating product (**MDP**) diet, the MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin-deactivating product (Mycofix, BIOMIN Holding GmbH, Tulln, Austria).

Analysis of Feeds, Diets, and Mycotoxins

Feeds and TMR were sampled once during clearance periods and each week of experimental periods. Then, samples were subjected to chemical analysis and measurement of mycotoxin contamination, as previously described (Gallo et al., 2016). Briefly, samples were dried at 60°C in a ventilated oven for 48 h, milled through a 1-mm screen using a laboratory mill (Thomas-Wiley, Arthur H. Thomas Co., Philadelphia, PA), and then stored for subsequent analysis. Uncorrected DM was determined by the gravimetric loss of free water after heating at 105°C for 3 h (AOAC International, 2000; method 945.15). Then, the DM concentration was corrected for volatile losses that occurred during oven drying using the equations adopted by NorFor (2011). Ash concentration was determined as gravimetric residue after incineration at 550°C for 2 h (AOAC International, 2000; method 942.05), an ether extract (**EE**) was obtained using AOAC method 920.29 (AOAC International, 2000), and CP ($N \times 6.25$) was determined using the Kjeldahl method (AOAC International, 2000; method 984.13). The soluble fraction of CP (expressed on a DM basis) was determined according to Licitra et al. (1996). Neutral detergent (**ND**), acid detergent (**AD**), and lignin sulfuric acid (**sa**) fiber fractions were sequentially determined using the AnkomII Fiber Analyzer (Ankom Technology Corporation, Fairport, NY), as described by Van Soest et al. (1991). The ND solution contained sodium sulfite and a heat stable amylase (activity: 17,400 Liquefon-U/mL, Ankom Technology). All fiber fractions were corrected for residual ash (aNDFom, ADFom, lignin sa). Starch content was determined by polarimetry (Polax 2 L, Atago, Tokyo, Japan).

Measurements of mycotoxins followed the methods of Gallo et al. (2010) for AF, the methods of Pietri and Bertuzzi (2012) for FB, the methods of Bertuzzi et al. (2014) for ZEA, DON, T-2, and HT-2 toxin, and the methods of Rossi et al. (2006) for OTA. Briefly, AFB1 was extracted using acetone:water (7:3 vol/vol) and purified using an immuno-affinity column (R-Biopharm Rhône LTD, Glasgow, Scotland). Mycotoxins were then measured using an HPLC instrument with a fluorescence detector (**FLD**). The limit of detection (**LOD**) and the limit of quantification (**LOQ**) were 0.05 and

0.15 µg/kg, respectively. After extraction with a phosphate buffer and purification using an immuno-affinity column (R-Biopharm Rhône LTD), FB were quantified by HPLC coupled with a mass spectrometer. The LOD and the LOQ were 10 and 30 µg/kg, respectively. The ZEA, DON, T-2, and HT-2 toxin were extracted with acetonitrile:water (86:14 vol/vol), and purified using an immuno-affinity column (ZEA) or a Trilogy-Puritox Trichothecenes column (DON, T-2 and HT-2 toxins) from R-Biopharm (Rhône LTD). Quantification was performed using HPLC-FLD (ZEA), GC-MS (DON), or LC-MS/MS (T-2 toxin and HT-2 toxin). The LOD and LOQ values were 2 and 5 µg/kg for ZEA; 10 and 30 µg/kg for DON; and 0.5 and 1.5 µg/kg for T-2 toxin and HT-2 toxin, respectively. OTA was extracted from samples using methanol-3% aqueous sodium hydrogen carbonate (50:50 vol/vol), purified using an immuno-affinity column (R-Biopharm Rhône LTD), and quantified using HPLC-FLD. The LOD and LOQ for OTA were 0.2 and 0.6 µg/kg, respectively. Mycotoxins were analyzed in corn meal and TMR samples that were completely dried before extraction.

Rumination Time, BW, and BCS

The daily rumination time of each cow was recorded using an acoustic sensor collar (RuminAct, SCR Hea-time, Netanya, Israel), and average times are reported. The cows were weighed on 2 consecutive days every week at the same time of day using an electronic scale; the averages of these 2 measurements are reported. Metabolic BW was calculated using the formula: $BW^{0.75}$ (NRC, 2001). The BCS was determined once every 2 wk using a 4-point scoring system (ADAS, 1986).

Health Status of Cows

The health status of each cow was monitored daily throughout the study. Mastitis was diagnosed by visual evaluation of abnormal milk from each quarter, and SCC analysis was performed for suspicious cases. Diarrhea was diagnosed by visual evaluation of the consistency and color of feces using the fecal score method (Ireland-Perry and Stallings, 1993), with diarrheic feces defined as those with a fecal score of 2 or less. A veterinarian considered all animals to be healthy, the husbandry to be generally good, and the feces to have normal consistency. No animals were included if they had any severe diseases. Some health problems were recorded (Supplemental Table S1, <https://doi.org/10.3168/jds.2020-18197>), but they did not have a marked effect on milk production or animal behavior. Consequently, no animals were excluded during the trial.

Milk Yield and Composition

The milk yield of each cow was recorded at each milking by an automatic milking system (Westfalia, Norwell Dairy Systems, Drayton, ON, Canada). Representative milk samples were taken at 0, 14, and 21 d during each experimental period at the morning milking using an automatic sampling unit. Butterfat, protein, lactose, casein content, titratable acidity, and coagulation properties [rennet clotting time (**r**), curd firming time (**K20**), and curd firmness at 30 min (**a30**)] were measured using infrared measurements (MilkoScan FT 120, Foss Electric, Hillerød, Denmark) according to Chessa et al. (2014). The fat and protein content and the fat-to-protein ratio were also calculated. Urea nitrogen was determined in skim milk using a spectrophotometric assay and a urea nitrogen kit (cat# 0018255440, Instrumentation Laboratory, Milano, Italy) in association with an auto-analyzer (ILAB-650, Instrumentation Laboratory, Lexington, MA). The SCC was determined using an optical fluorimetric method with an automated cell counter (Fossomatic 180, Foss Electric).

Blood Sampling and Blood Biochemistry

Blood samples were collected for chemical analysis on d 0, 14, and 21 of each experimental period. The samples were taken in the morning (before feeding) by venipuncture of the jugular vein using 10-mL lithium heparin tubes (Vacuette, containing 18 IU of Li-heparin/mL, Kremsmünster, Austria) and immediately cooled in an ice water bath. A small amount of blood was used to calculate packed cell volume (Centrifuge 4203; ALC International Srl, Cologno Monzese, Italy), the remaining blood was centrifuged ($3,500 \times g$ for 16 min at 4°C), and the resulting plasma was separated into aliquots and stored at -20°C until analysis.

Plasma metabolites were analyzed at 37°C using an automated clinical analyzer (ILAB 650, Instrumentation Laboratory, Lexington, MA) as described by Calamari et al. (2016). Commercial kits from Instrumentation Laboratory SpA (Werfen, Italy) were used to measure glucose, total cholesterol, urea, Ca, P, Mg, total protein, albumin, total bilirubin, and creatinine. Kits from Wako (Chemicals GmbH, Neuss, Germany) were used to measure nonesterified fatty acids (**NEFA**), BHBA, and Zn. Electrolytes (Na, K, and Cl) were measured using a potentiometer method (ion-selective electrode connected to ILAB 650). Kinetic analysis was used to determine the activities of alkaline phosphatase (ALP; EC 3.1.3.1), aspartate aminotransferase (AST; EC 2.6.1.1), and γ -glutamyltransferase (GGT; EC 2.3.2.2), with kits from Instrumentation Laboratory SpA. Ceru-

loplasmin and haptoglobin were measured as described by Calamari et al. (2016), paraoxonase activity as described by Bionaz et al. (2007), myeloperoxidase activity as described by Bradley et al. (1982), and ferric reducing antioxidant power as described by Benzie and Strain (1999).

Gene Expression in Circulating Leukocytes

For evaluation of gene expression in circulating leukocytes, blood samples of each cow were collected before the morning feeding on the last day of each experimental period (d 21) in 10-mL PAXgene blood RNA tubes (Qiagen, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). The tubes were incubated at room temperature for 6 h, maintained overnight at -20°C, and then stored at -80°C until extraction of total RNA.

RNA extraction was performed using a kit according to the manufacturer's protocol (Blood RNA Kit Handbook, PreAnalytix GmbH, Qiagen, Hilden, Germany), including the DNase digestion step for 15 min (RNase-Free DNase Set Qiagen cat. no. 79254). After extraction, RNA was quantified using the Qubit RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA) and RNA quality was assessed by the Experion Automated Electrophoresis System (Bio-Rad, Hercules, CA). The average of RNA quality index for the 36 samples was 9.3 (range: 8.7–9.6).

Synthesis of cDNA was performed using a reverse transcription kit (RevertAid RT Reverse Transcription Kit; Thermo Fisher Scientific). Briefly, each reaction mixture (1 μ L of 100 ng/ μ L of total RNA solution, 1 μ L of random hexamer primer, and 9.5 μ L of DNase/RNase-free water) was incubated for 5 min at 65°C. Then, 8.5 μ L of master mix [5 μ L of 5 $\times$ First-Strand Buffer, 1 μ L of 0.1 M DTT, 0.25 μ L of RevertAid RT (200 U/ μ L), and 0.25 μ L of RiboLock RNase Inhibitor (20 U/ μ L)] was added. The reaction was performed in an Eppendorf Mastercycler gradient (Westbury, NY), using the following temperature program: 25°C for 5 min, 42°C for 60 min, and 70°C for 5 min. The resulting cDNA was diluted 1:4 (vol/vol) with DNase/RNase-free water, and then stored before quantitative PCR (qPCR).

The qPCR was performed in an Optical 384-Well Reaction Plate (CFX384 Touch; Bio-Rad, Hercules, CA, USA) using the following protocol: 2 min at 50°C, 10 min at 95°C, and 40 cycles with 15 s at 95°C followed by 1 min at 60°C. A dissociation curve was plotted (gradient from 95°C to 60°C to 95°C) to check amplification quality. Within each well, 4 μ L of diluted cDNA was added to 6 μ L of a solution consisting of 5 μ L 1 $\times$ SYBR Green master mix (Cat# 4367659, Applied

Biosystems, Woolston Warrington, UK), 0.4 μ L each of 10 μ M forward and reverse primers, and 0.2 μ L of DNase/RNase-free water. Three replicates were used for each sample. The qPCR efficiency and quantification of cycles were obtained for each reaction using LinRegPCR (Version 2017.1; Ruijter et al., 2009), a program for the analysis of quantitative real-time PCR data based on measurements from SYBR green and similar fluorescent dyes. This program measures and subtracts the baseline fluorescence, and then sets a window-of-linearity for calculation of PCR efficiency. Based on mean PCR efficiency per amplicon, the quantitation cycle (**Cq**) value per sample, the fluorescence threshold (used to determine the Cq), and the starting concentration per sample (in arbitrary fluorescence units) were calculated. The final data were normalized using the geometric mean of 3 internal control genes (*ACTB*, *SDHA*, and *YWHAZ*). The stability of the normalization factor was tested across all samples using GeNorm software (Vandesompele et al., 2002), and the final pairwise variation was 0.08.

The target genes are related to recognition and immune mediation functions (*MYD88* and *TLR2*), the inflammatory cascade (*CASP8*, *IL1B*, *IL1R*, *IL8*, *IL18*, *IRAK1*, *TNF*, and *NRLP3*), and the leukotrienes pathway (*ALOX5* and *ALOX15*). The primers were designed to span an exon-exon junction. The primers and sequences of genes used for transcript profiling were previously reported (Lopreiato et al., 2020) and listed in Supplemental Tables S2 and S3 (<https://doi.org/10.3168/jds.2020-18197>).

Ruminal Fluid Collection and Composition

Ruminal fluid samples (200 mL) were collected from each cow at 5 to 6 h after the morning feeding on the last day of each experimental period (d 21). These samples were collected using a stomach tube that was connected to a manual vacuum pump. Ruminal pH was recorded, and samples were then filtered through 3 layers of cheesecloth, immediately frozen in dry ice, and then stored at -20°C in 3 aliquots for measurements of pH, ammonia, VFA (acetate, propionate, butyrate, isobutyrate, valeric, isovaleric, hexanoic, heptanoic), and lactic acid (L and D stereoisomers), as previously described (Trevisi et al., 2014).

Feces Collection and Nutrient Digestibility

Fecal samples were collected from the rectum of each cow on d 0 and d 21 of each experimental period. The feces were mixed thoroughly, and subsamples were taken. The first subsample was dried at 60°C in a forced-draft oven for 48 h, ground with a knife mill (1-mm

screen), and then DM, OM, ash, EE, NDF, ADF, and ADL were measured. The acid-insoluble ash in this first subsample was used as an internal marker to calculate digestion coefficients of the feeds on fecal samples collected in d 21. The pH was measured in these second subsamples immediately after collection.

FB and Their Fully or Partially Hydrolyzed Forms in Rumen and Fecal Samples

The FB, HFB, or pHFB were quantified in rumen and fecal samples collected on d 21 of each experimental period in agreement to the method previously reported by Hahn et al. (2015). In particular, FB, HFB, and pHFB were extracted from homogenized fresh samples (thorough stirring with a spoon) with 10 mL, 10 mL, and 5 mL of acetonitrile/water/formic acid (74/25/1, vol/vol/vol) by shaking for 30 min, 20 min, and 10 min, respectively. Shaking was performed on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany) at 200 rpm at room temperature. Supernatants obtained by centrifugation ($2,655 \times g$, 10 min, room temperature) were combined, vortexed, diluted 1 + 3 with extraction solvent, and centrifuged at $14,000 \times g$ for 10 min at room temperature before LC-MS/MS analysis as described by Hahn et al. (2015).

Statistical Analysis

Variables with non-normal distributions (such as SCC) were log-transformed before statistical analysis. There were 4 replicates of 3 periods $\times$ 3 treatments in a Latin square design. For the analysis of the gene expression experiment, we calculated the fold change between the levels of gene expression of cows in the CTR and MDP against the MTX group (reference group), and the fold changes were submitted to ANOVA.

Data that were measured once in each cow during each experimental period were tested for normality and analyzed using the MIXED procedure in SAS (release 9.3, 2002–2010, SAS Inst. Inc., Cary, NC) according to the model:

$$Y_{ijklm} = \mu + T_i + L_j + p_k + c_{l(k)} + e_{ijklm},$$

where Y_{ijklm} is the dependent variable, μ is the population mean, T_i is the fixed effect of treatments, L_j is the fixed effect of the Latin square, p_k is the random effect of a period nested within the Latin square, $c_{l(k)}$ is the random effect of a cow nested within the Latin square, and e_{ijklm} is the residual error.

Data that were measured more than once in each cow during each experimental period were tested for normality and analyzed as repeated measurements using

the MIXED procedure in SAS (release 9.3, 2002–2010) according to the model:

$$Y_{ijklmn} = \mu + T_i + D_j + L_k + TD_{ij} + TL_{ik} + p_m + c_{n(m)} + e_{ijklmn},$$

where Y_{ijklmn} is the dependent variable, μ is the population mean, T_i is the fixed effect of treatment, D_j is the fixed effect of daily or weekly repeated measurements, L_k is the fixed effect of the Latin square, TD_{ij} is the fixed effect of treatment daily or weekly interactions, p_m is the random effect of a period nested within the Latin square, $c_{n(m)}$ is the random effect of a cow nested within the Latin square, and e_{ijklmn} is the residual error.

One of 5 covariate model structures was used based on the finite-sample corrected Akaike information criterion and the Schwarz Bayesian criterion for the best fitting model. The 5 tested structures were compound symmetry, heterogeneous compound symmetry, unstructured, autoregressive, and ante-dependence (Littell et al., 1998; Wang and Goonewardene, 2004). Comparisons of least squares means for fixed factors were obtained with the DIFF option in the LSMEANS statement by using t -tests for the statistical significance of the difference between the means (Littell et al., 2006). A P -value < 0.05 was considered significant.

RESULTS

Table 1 shows the chemical composition of the TMR. All chemicals analyzed in feeds used during the trial were typical for the specific feed categories. The CornMS was contaminated by several mycotoxins (aflatoxin B1: 0.6 ± 0.1 $\mu\text{g/kg}$ of DM, DON: $2,647 \pm 718$ $\mu\text{g/kg}$ of DM, ZEA: 60 ± 17 $\mu\text{g/kg}$ of DM, OTA 0.1 ± 0.2 $\mu\text{g/kg}$ of DM, FB1: $6,648 \pm 3,770$ $\mu\text{g/kg}$ of DM, FB2: $3,699 \pm 1,823$ $\mu\text{g/kg}$ of DM, HT-2: 6.7 ± 6.0 $\mu\text{g/kg}$ of DM, and T-2: 5.6 ± 4.2 $\mu\text{g/kg}$ of DM). Table 2 shows the levels of mycotoxins in the different experimental diets. The DON concentrations in MTX and MDP diets were respectively 2.1 and 2.6 times higher than in the CTR diet. Similarly, the FB concentration was about 8 to 10 times greater in MTX and MDP diets than in the CTR diet. Differences in mycotoxin concentrations between MTX and MDP diets were observed in particular for DON and FB concentrations, being respectively 22% and 25% greater in the MDP diet than in the MTX diet.

We measured the BW, DMI, feed efficiency, BCS, rumination time, and nutrient digestibility of dairy cows during the trial (Table 3). The 3 groups had no significant differences in BW (i.e., on average 607 kg),

DMI (i.e., on average 25.32 kg of DM/d), and BCS (i.e., on average 2.28). The DMI decreased ($P < 0.05$) by approximately 10 and 13%, respectively, in experimental periods 2 and 3, when compared with the greatest value measured in period 1. The daily rumination time was 11 min/d longer in the MTX group and 12 min/d shorter in the MDP group than CTR group. Cows spent more time in ruminating in period 1 than period 3 ($P < 0.05$). Relative to the CTR group, the MTX group had lower feed efficiency (-2.7% , $P < 0.05$), DM digestibility (-3.7% , $P < 0.05$), and NDF digestibility (-9.5% ; $P < 0.05$). The MDP group had the highest digestibility coefficients ($P < 0.05$) for both DM and NDF, with increases of 2.0% and 2.5% when compared with CTR group, respectively. For both digestibility observations, the values increased ($P < 0.05$) when passing from period 1 to period 3, by 14% for DM digestibility, or by 37% for NDF digestibility.

Measurements of the milk yield, milk composition, and cheese-making traits are shown in Table 4. The milk yield was lower ($P < 0.05$) in the MTX and MDP groups than in the CTR group (-1.34 and -1.18 kg/d, respectively). Furthermore, both milk yield and its composition changed among experimental periods. In particular, the milk yield decreased ($P < 0.05$) by 11% in period 2 with respect period 1 and by 6% in period 3 respecting to period 2. The groups had no differences in milk casein and titratable acidity and no differences in r, whereas these traits showed the highest values in period 1 ($P < 0.05$). The a30 was about 22% lower in the MTX group than in the CTR and MDP groups ($P < 0.05$). The K20 had a tendency ($P = 0.05$) to be about 32% lower in the CTR and MDP groups than in the

Table 2. Mycotoxin concentrations ($\mu\text{g/kg}$ of DM, mean $\pm$ SD) of the experimental diets

Item	Experimental diet ¹		
	CTR (n = 18)	MTX (n = 18)	MDP (n = 18)
Aflatoxin B ₁	<0.05	0.37 ± 0.21	0.51 ± 0.35
Deoxynivalenol	340.5 ± 161.0	733.0 ± 213.6	897.3 ± 230.4
Zearalenone	7.8 ± 4.2	33.0 ± 8.2	30.9 ± 8.8
Ochratoxin A	1.9 ± 1.8	2.1 ± 1.7	1.5 ± 1.4
Fumonisin B ₁	98.4 ± 31.4	699.7 ± 220.4	857.8 ± 278.8
Fumonisin B ₂	29.5 ± 21.4	295.2 ± 115.7	389.3 ± 114.3
Fumonisin B ₁ + B ₂	127.9 ± 43.9	994.4 ± 323.2	$1,247.1 \pm 370.2$
HT-2 toxin	0.8 ± 1.5	3.4 ± 3.0	6.1 ± 5.5
T-2 toxin	3.3 ± 4.6	36.9 ± 22.3	47.0 ± 23.0

¹CTR = TMR contaminated with regular level of *Fusarium* mycotoxins; MTX = TMR contaminated with a higher level of *Fusarium* mycotoxins compared with CTR, but below US and European Union guidelines; MDP = MTX diet supplemented with mycotoxin deactivator product.

Table 3. Body weight, DMI, BCS, rumination time, DM digestibility, and NDF digestibility of lactating dairy cows that received 3 different experimental diets during 3 experimental periods of Latin square design

Characteristic	Period			Treatment ¹				P-value			
	1	2	3	CTR	MTX	MDP	SEM	Latin square	Period	Treatment	Treatment × day
BW, kg	609	602	609	609 ^a	605 ^b	606 ^b	19.354	0.919	0.356	0.066	0.753
DMI, kg/d	27.51 ^a	24.57 ^b	23.86 ^b	25.41	25.02	25.52	0.217	0.180	<0.05	0.200	0.147
Feed efficiency, dmm ²	1.48 ^a	1.48 ^a	1.42 ^b	1.49 ^a	1.45 ^b	1.44 ^b	0.001	0.241	<0.05	<0.05	<0.05
BCS, n	2.30	2.29	2.26	2.29	2.28	2.28	0.0008	0.741	0.301	0.893	—
Rumination, min/d	562 ^a	547 ^{ab}	507 ^b	539 ^b	550 ^a	527 ^c	176.42	0.461	<0.05	<0.05	0.235
DM digestibility, %	66.1 ^c	68.8 ^b	75.9 ^a	71.0 ^b	67.3 ^c	72.4 ^a	0.037	0.387	<0.05	<0.05	—
NDF digestibility, %	43.3 ^b	45.9 ^{ab}	59.5 ^a	52.3 ^b	42.8 ^c	53.6 ^a	0.165	0.383	<0.05	<0.05	—

^{a-c}Within a period or a treatment, means with different superscript letters were significantly different ($P < 0.05$).

¹CTR = TMR contaminated by regular level of *Fusarium* mycotoxins [i.e., 340.5 µg of deoxynivalenol (DON)/kg of DM and 127.9 µg of fumonisins (FB)/kg of DM in the whole diet]; MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin deactivator product (Mycotfix, BIOMIN Holding GmbH, Tulln, Austria).

²dmm = dimensionless.

MTX group. Differences ($P < 0.05$) in milk coagulation properties were observed among experimental periods, with period 1 characterized by the lowest r and K20 and the highest a30 values.

Measurements of blood variables (Table 5) indicated the packed cell volume tended to be lower in the MDP group than in the CTR and MTX groups ($P = 0.068$), whereas the ceruloplasmin level tended to be greater in the CTR group than in the MTX and MDP groups ($P = 0.090$). Some blood variables such as glucose, cholesterol and minerals (i.e., Mg, K or Zn) decreased ($P < 0.05$) when advancing in the experimental periods. On average, the ceruloplasmin was approximately 13% greater ($P < 0.05$) in period 2 than other experimental periods. The aspartate amino transferase and γ-glutamyl transferase levels were greater in the MTX group than in the CTR and MDP groups ($P < 0.05$ and $P = 0.059$ respectively). The bilirubin level was lower in the MTX group than in the CTR and MDP groups ($P < 0.05$). No differences between groups for other biomarkers were highlighted. Differences among periods were measured for creatinine and myeloperoxidase that increased ($P < 0.05$) by passing from period 1 to period 3, whereas paraoxonase and ferric reducing antioxidant power decreased ($P < 0.05$).

Data of gene expression in blood leukocytes using qPCR are shown in Figure 1. Considering the level of mycotoxin in the diet (comparison between MTX and CTR), cows that received a regular amount of mycotoxin (CTR group) have shown a higher expression of *MYD88*, *IL1R*, and *TLR2* ($P < 0.05$ for all), but a lower expression of *CASP8* ($P < 0.05$) compared with MTX group. When the deactivator was administered together with the mycotoxin (comparison between MTX and MDP), MDP cows have shown only a higher expression of *IL1R* ($P < 0.05$) compared with MTX group. There were no differences among CTR and MDP groups and there were no significant differences in the expression of the other investigated genes (data not shown).

Measurements of rumen traits (Table 6) indicated the isovaleric acid (C5i) level was different among experimental periods ($P < 0.05$), whereas hexanoic acid (C6) tended ($P = 0.068$) to be different among treatments, with an increase in concentration of about 28% in MTX than CTR groups. All other evaluated rumen traits had no differences in the CTR, MDP, and MTX groups.

Measurements of fecal variables (Table 7) indicated the crude protein level was lower in the CTR group than in the MTX group (14.09 vs. 15.30%; $P < 0.05$). The VFA contents indicated that the butyrate level was lower in the MTX and MDP groups than in the CTR group (9.26 and 8.88 vs. 10.34% mol; $P < 0.05$). Both fecal isobutyric acid (C4i) and valeric acid (C5) concentrations resulted higher ($P < 0.05$) in period 3

Table 4. Milk yields, milk composition, and milk rennet coagulation properties of lactating dairy cows that received 3 experimental diets during 3 experimental periods of Latin square design

Characteristic ¹	Period			Treatment ²				P-value			
	1	2	3	CTR	MTX	MDP	SEM	Latin square	Period	Treatment	Treatment × day
Milk production, kg/d	40.69 ^a	36.16 ^b	33.82 ^c	37.73 ^a	36.39 ^b	36.55 ^b	0.699	0.085	<0.05	<0.05	0.213
Fat, % wt/wt	4.11	4.20	4.16	4.04	4.19	4.25	0.0293	0.288	0.873	0.147	0.363
Fat, % kg/d	1.71 ^a	1.52 ^b	1.41 ^b	1.54	1.55	1.55	0.005	0.079	<0.05	0.965	0.726
Protein, % wt/wt	3.60 ^a	3.46 ^b	3.51 ^b	3.50	3.52	3.54	0.002	0.884	<0.05	0.374	0.388
Protein, % kg/d	1.49 ^a	1.25 ^b	1.19 ^b	1.33	1.31	1.30	0.002	0.172	<0.05	0.426	0.527
Lactose, % wt/wt	5.04	5.02	5.08	5.05	5.04	5.05	0.002	0.220	0.230	0.944	0.601
Lactose, % kg/d	2.09 ^a	1.82 ^b	1.73 ^b	1.92	1.88	1.84	0.004	0.250	<0.05	0.189	0.555
Urea, mg/100 mL	28.95	30.30	29.71	29.13	29.28	30.55	2.788	0.903	0.504	0.375	0.093
SCC, log ₁₀ (cells/mL × 1,000)	1.82	1.83	1.79	1.75	1.68	2.00	0.021	0.339	0.166	0.873	0.459
Casein, % wt/wt	2.71 ^a	2.62 ^a	2.67 ^a	2.65	2.67	2.69	0.001	0.854	<0.05	0.158	0.673
Casein, % kg/d	1.12 ^a	0.95 ^b	0.91 ^b	1.01	0.99	0.98	0.001	0.176	<0.05	0.498	0.608
SH, %H/50 mL	3.11 ^a	2.85 ^b	2.95 ^b	2.95	2.96	3.01	0.007	0.956	<0.05	0.563	<0.05
Clotting time (r), min	16.73 ^b	21.19 ^a	18.21 ^{ab}	18.53	19.47	18.12	3.071	0.587	<0.05	0.484	0.223
Curd firmness (a30), mm	36.45 ^a	22.68 ^b	30.44 ^{ab}	32.08 ^a	25.05 ^b	32.44 ^a	18.448	0.566	<0.05	<0.05	0.199
Curd firming time (K20), min	6.04 ^b	14.54 ^a	10.20 ^{ab}	8.07 ^b	13.00 ^a	9.71 ^{ab}	9.659	0.584	<0.05	0.051	0.688

^a Within a period or a treatment, means with different superscript letters were significantly different ($P < 0.05$).

¹ Fat (kg/d), protein (kg/d), lactose (kg/d), and casein (kg/d) calculated on d 0, -14, and -21 of each experimental period when milk was sampled. SH = Soxhlet-Henkel degree.

² CTR = TMR contaminated by regular level of *Fusarium* mycotoxins [i.e., 340.5 µg of deoxynivalenol (DON)/kg of DM and 127.9 µg of fumonisins (FB)/kg of DM in the whole diet]; MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g of mycotoxin deactivator product (Mycotex, BIOMIN Holding GmbH, Tulln, Austria).

than other experimental periods. There were no other differences related to dietary treatments or experimental periods in all other evaluated fecal variables.

Ruminal and fecal quantifications of FB, HFB, and pHFB are reported in Table 8. In rumen fluid, sums of FB, HFB, and pHFB did not differ among periods, whereas FB increased ($P < 0.05$) and both HFB and pHFB decreased ($P < 0.05$) in the MTX group when compared with the MDP group. The lowest concentrations ($P < 0.05$) of FB, HFB and pHFB were measured in the CTR group. In fecal samples of the MDP group, the sum of FB decreased ($P < 0.05$) by 107% whereas the sums of HFB and pHFB increased ($P < 0.05$) by 59 and 180%, respectively, compared with the MTX group. A higher HFB fecal content ($P < 0.05$) was measured in period 2 than period 1.

DISCUSSION

Contamination of Animal Diets

The levels of mycotoxin contamination in the diets used in this study are typical mycotoxin levels found in the feed of dairy cows in Europe (EFSA, 2013b). Further, meta-analysis of Grenier and Applegate (2013) verified the effects of mycotoxin exposure on digestive and absorptive functions, intestinal defense, and microbiome composition in animals evaluating these effects according to experimental adopted dose. In particular, the mycotoxin doses were placed in 3 different categories (i.e., realistic, occasional and unrealistic doses) based on worldwide mycotoxin surveys of Binder et al. (2007) and Rodrigues and Naehrer (2012). As stated by Grenier and Applegate (2013), realistic DON and FB dietary contamination levels are defined respectively for levels lower than 5,000 and 10,000 µg/kg of diet. The DON contaminations were 340.5, 733.0, and 897.3 µg/kg for CTR, MTX and MDP diets, respectively. The FB (i.e., sum of FB1 and FB2) were 127.9, 994.4, and 1,247.1 µg/kg, respectively. Unexpectedly, the concentrations of DON and FB were about 22% or 25% greater in MDP than MTX diets, and this could be related to a poor homogeneity among different batches of the mycotoxin contaminated culture media corn meals.

Feed Intake and Rumen Activities

Cows that received the different diets had similar DMI. Different authors reported similar findings for animals exposed to similar (McKay et al., 2019) or greater (Korosteleva et al., 2007; Winkler et al., 2014) levels of DON in TMR than those measured in current trial. However, a study by Keese et al. (2008) that examined a contamination level much higher than in the

Table 5. Blood variables of lactating dairy cows that received the 3 experimental diets during 3 experimental periods of Latin square design

Item ¹	Period			Treatment ²				P-value			
	1	2	3	CTR	MTX	MDP	SEM	Latin square	Period	Treatment	Day
Packed cell volume, vol/vol	0.298	0.294	0.288	0.297 ^a	0.295 ^a	0.289 ^b	0.00001	0.801	0.201	0.068	0.881
Glucose, mmol/L	4.101 ^a	3.797 ^b	3.837 ^b	3.931	3.940	3.863	0.016	0.334	<0.05	0.599	0.987
Cholesterol, mmol/L	5.396 ^a	5.058 ^{ab}	4.916 ^b	5.168	5.156	5.046	0.442	0.976	<0.05	0.607	0.558
Urea, mmol/L	4.974	5.686	5.175	5.199	5.366	5.270	0.072	0.639	0.078	0.626	<0.05
Ca, mmol/L	2.571	2.630	2.585	2.603	2.582	2.603	0.0023	0.171	0.224	0.748	0.850
P, mmol/L	1.837	1.757	1.680	1.728	1.778	1.778	0.013	0.792	0.358	0.760	0.408
Mg, mmol/L	1.136 ^a	1.054 ^b	1.086 ^{ab}	1.087	1.105	1.085	0.0013	0.137	<0.05	0.633	0.950
Na, mmol/L	140.74	138.7	139.13	139.86	139.38	139.33	1.870	0.683	0.212	0.791	0.144
K, mmol/L	4.186 ^a	3.967 ^b	3.9299 ^b	4.104	3.977	4.002	0.0104	<0.05	<0.05	0.138	0.932
Cl, mmol/L	100.34	99.51	100.73	100.56	99.978	100.03	1.153	0.770	0.279	0.656	0.948
Zn, µmol/L	14.070 ^a	13.417 ^{ab}	12.177 ^b	13.222	12.989	13.453	0.693	0.108	<0.05	0.693	0.627
Ceruloplasmin, µmol/L	1.956 ^b	1.772 ^b	2.142 ^a	2.037 ^a	1.920 ^b	1.913 ^b	0.0097	0.724	<0.05	0.090	0.686
Total protein, g/L	80.842	82.562	83.453	81.663	82.368	82.826	1.215	0.401	0.130	0.255	0.288
Albumin, g/L	39.161	39.497	39.344	39.369	39.269	39.364	0.183	0.244	0.680	0.921	0.516
Globulin, g/L	41.681	43.065	44.109	42.293	43.164	43.398	1.074	0.163	0.091	0.214	0.437
Aspartate amino transferase, U/L	110.65	115.82	107.25	106.62 ^b	117.09 ^a	110.01 ^b	28.184	0.853	0.125	<0.05	0.436
γ-Glutamyl transferase, U/L	29.501	29.969	30.335	29.604 ^b	30.726 ^a	29.475 ^b	0.788	0.077	0.399	0.059	0.652
Bilirubin, µmol/L	1.393	1.572	1.7402	1.615 ^a	1.368 ^b	1.723 ^a	0.026	0.527	0.424	<0.05	0.900
ALP, U/L	53.895 ^a	42.959 ^b	42.126 ^b	46.143	44.744	48.094	7.229	0.366	<0.05	0.169	0.479
Haptoglobin, g/L	0.107	0.096	0.203	0.134	0.14	0.132	0.0037	0.579	0.326	0.977	0.948
NEFA, mmol/L	0.135	0.153	0.144	0.156	0.128	0.150	0.0008	0.885	0.866	0.261	0.618
BHB, mmol/L	0.413	0.374	0.499	0.348	0.4202	0.517	0.0168	0.362	0.377	0.143	0.724
Creatinine, µmol/L	82.622 ^b	84.829 ^b	87.752 ^a	84.961	85.317	84.924	1.573	0.8302	<0.05	0.873	<0.05
Paraoxonase, U/mL	87.534 ^a	81.829 ^b	81.178 ^b	85.040	82.992	82.508	4.934	0.599	<0.05	0.170	0.472
ROMt, mgH ₂ O ₂ /100 mL	14.804	14.034	15.674	15.054	14.851	14.608	0.495	0.754	0.102	0.615	0.998
Myeloperoxidase, U/L	359.6 ^b	371.51 ^b	417.79 ^a	379.46	385.87	383.57	276.17	0.1299	<0.05	0.827	0.109
FRAP, µmol/L	151.01 ^a	135.76 ^b	127.38 ^b	137.18	140.07	136.9	34.383	0.565	<0.05	0.656	0.413

^{a,b}Within a period or a treatment, means with different superscript letters were significantly different ($P < 0.05$).¹NEFA = nonesterified fatty acids; FRAP = ferric reducing antioxidant power; ALP = alkaline phosphatase; ROMt = reactive oxygen metabolites.²CTR = TMR contaminated by regular level of *Fusarium* mycotoxins [i.e., 340.5 µg of deoxynivalenol (DON)/kg of DM and 127.9 µg of fumonisins (FB)/kg of DM in the whole diet]; MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin deactivator product (Mycotex, BIOMIN Holding GmbH, Tulln, Austria).

Figure 1. Average fold change (relative to MDP) in the expression of genes in the circulating leucocytes of lactating dairy cows that received 3 different experimental diets. CTR = TMR contaminated by regular level of *Fusarium* mycotoxins [i.e., 340.5 μg of deoxynivalenol (DON)/kg of DM and 127.9 μg of fumonisins (FB)/kg of DM in the whole diet]; MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 μg of DON/kg of DM and 994.4 μg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 μg of DON/kg of DM and 1,247.1 μg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin deactivator product (Mycofix, BIOMIN Holding GmbH, Tulln, Austria).

current study (approximately 4,400 to 5,300 $\mu\text{g}/\text{kg}$ of DMI) reported that mycotoxin contamination led to an increased DMI. Our MDP treatment had no apparent effect on DMI. The reductions in DMI among experimental periods appeared slightly higher with respect to those predictable for the specific milk production levels by applying equations 1 and 2 of NRC (2001), for which DMI decreases of approximately 6 or 9% could be estimated. The observed DMI decreases were equal to 10 and 13% in experimental periods 2 and 3, respectively, when compared to period 1.

The decrease of rumination time through the periods can be related to the decrease of DMI. In particular, rumination activity could be related to rumen content, a greater amount of fiber in rumen-reticulum compartment being able to stimulate bolus formation and to increase effective rumination, as reviewed by Nikkhah (2013). Consistently, the increases in either DM or NDF degradability measured among experimental periods corresponded to a reduction in time spent by animal for ruminating. Differences in rumination time were observed among treatments, with MTX group showing an increase in rumination time of 11 or 23 min/d compared with CTR and MDP groups, respectively. To the best of our knowledge, no information is available explaining relationships between mycotoxin ingestion and rumination activity, and this aspect deserves future investigation.

Digestibility Traits and Ruminal and Fecal Variables

Our findings indicated that the MTX diet impaired NDF and DM digestibility. Hildebrand et al. (2012) found similar effects in their study of dairy cows that received about 4,400 μg of DON/kg of feed, in that these cows had significantly decreased degradation of crude fiber and NDF. A previous study (Gurung et al., 1999) reported that FB had no effect on in vitro DM degradability. In the present study, mycotoxin-deactivating products counteracted the negative effects of mycotoxins on NDF and DM digestibility. However, the feed efficiency was similar in MTX and MDP diets and approximately 4 to 5% lower than CTR diet. Kiyothong et al. (2012) reported a similar effect in an in vivo study. In particular, mycotoxin-deactivating products significantly improved NDF digestibility coefficients when a mycotoxin-contaminated TMR was fed to dairy cows (containing 38 $\mu\text{g}/\text{kg}$ AFB1, 701 $\mu\text{g}/\text{kg}$ FB, 720 $\mu\text{g}/\text{kg}$ DON, 541 $\mu\text{g}/\text{kg}$ ZEA, and 501 $\mu\text{g}/\text{kg}$ OTA). The reduction in digestibility in MTX group did not affect feed intake, milk yield, or feed efficiency, in agreement to previous findings (Nefzaoui and Vanbelle, 1986). Beauchemin et al. (2000) also reported that the supplementation of a nonstarch polysaccharides

Table 6. Rumen variables of lactating dairy cows that received 3 experimental diets during 3 experimental periods of Latin square design

Item	Period			Treatment ¹			P-value		
	1	2	3	CTR	MTX	MDP	SEM	Latin square	Treatment
pH	6.167	6.309	6.165	6.295	6.095	6.251	0.044	0.230	0.370
Acetic acid (C2), mmol/L	71.310	67.407	66.721	66.426	71.326	67.686	20.920	0.259	0.291
Propionic acid (C3), mmol/L	28.933	24.680	25.319	24.647	28.189	26.096	9.848	0.319	0.382
Butyric acid (C4), mmol/L	14.609	14.409	15.687	14.095	15.527	15.083	2.056	0.784	0.341
Isobutyric acid (C4i), mmol/L	0.737	0.823	0.703	0.726	0.772	0.764	0.017	0.053	0.660
Valeric acid (C5), mmol/L	1.900	1.819	1.632	1.656	1.927	1.768	0.038	0.828	0.152
Isovaleric acid (C5i), mmol/L	1.423 ^a	1.927 ^b	1.373 ^a	1.592	1.632	1.499	0.042	0.429	0.667
Hexanoic acid (C6), mmol/L	0.791 ^b	0.586 ^a	0.584 ^a	0.563 ^a	0.746 ^b	0.651 ^{ab}	0.010	0.851	0.068
Heptanoic acid (C7), mmol/L	0.067	0.045	0.055	0.045	0.062	0.060	0.0002	0.405	0.264
Acetic acid (C2), % mol	59.694	60.500	59.545	60.580	59.542	59.616	1.812	0.857	0.512
Propionic acid (C3), % mol	24.001	22.001	22.555	22.395	23.145	23.017	3.632	0.934	0.847
Butyric acid (C4), % mol	12.202	12.824	13.985	12.831	12.973	13.207	0.713	0.595	0.828
Isobutyric acid (C4i), % mol	0.619	0.740	0.633	0.664	0.657	0.670	0.004	0.571	0.951
Valeric acid (C5), % mol	1.577	1.625	1.465	1.505	1.588	1.574	0.012	0.536	0.495
Isovaleric acid (C5i), % mol	1.189 ^a	1.754 ^b	1.236 ^a	1.473	1.388	1.317	0.039	0.507	0.564
Hexanoic acid (C6), % mol	0.662	0.517	0.531	0.510	0.624	0.575	0.007	0.498	0.218
Heptanoic acid (C7), % mol	0.056	0.040	0.050	0.041	0.052	0.054	0.0002	0.422	0.400
C2/C3 ratio, dimensionless	2.549	2.823	2.705	2.761	2.676	2.640	0.070	0.882	0.812
(C2+C4)/C3 ratio, dimensionless	3.071	3.421	3.349	3.345	3.265	3.230	0.113	0.851	0.890
NH ₃ , mmol/L	7.121	8.611	8.705	7.319	8.413	8.705	1.936	0.654	0.377
L-Lactate, mmol/L	0.718	0.889	0.652	0.6897	0.764	0.806	0.016	0.841	0.398
D-Lactate, mmol/L	0.684	0.922	0.618	0.704	0.759	0.761	0.017	0.949	0.753
Lactate total, mmol/L	1.402	1.811	1.2701	1.393	1.525	1.565	0.064	0.916	0.574

^{a,b}Within a period or a treatment, means with different superscript letters were significantly different ($P < 0.05$).

¹CTR = TMR contaminated by regular level of *Fusarium* mycotoxins [i.e., 340.5 µg of deoxynivalenol (DON)/kg of DM and 127.9 µg of fumonisins (FB)/kg of DM in the whole diet]; MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin deactivator product (Mycofix, BIOMIN Holding GmbH, Tulln, Austria).

Table 7. Fecal variables of lactating dairy cows that received 3 experimental diets during 3 experimental periods of Latin square design

Item	Period			Treatment ¹			P-value		
	1	2	3	CTR	MTX	MDP	SEM	Latin square	Treatment
pH	5.929	5.882	5.862	5.896	5.852	5.925	0.010	0.288	0.552
DM, %	13.365	13.418	13.566	13.318	13.618	13.413	0.187	0.057	0.630
OM, % DM	90.121	89.928	89.347	90.000	89.855	89.541	0.130	0.688	0.234
CP, % DM	14.236	14.786	14.990	14.08 ^a	15.299 ^b	14.625 ^{ab}	0.376	0.273	<0.05
NDF, % DM	60.630	63.334	61.355	62.036	62.257	61.026	2.924	0.286	0.584
ADF, % DM	34.905	37.617	36.817	37.079	37.070	35.267	1.807	0.098	0.158
ADL, % DM	9.754	12.300	11.226	11.591	11.282	10.407	1.274	0.942	0.314
Ash, % DM	9.879	10.072	10.653	10.000	10.145	10.460	0.130	0.688	0.234
VFA, mmol/100 g of DM	56.019	55.951	51.752	55.014	54.579	54.129	9.413	0.728	0.912
Acetic acid (C2), % mol	73.143	74.182	72.535	72.957	73.477	73.427	0.520	0.426	0.613
Propionic acid (C3), % mol	15.364	15.116	15.329	14.75	15.283	15.776	0.662	0.181	0.904
Butyric acid (C4), % mol	9.668	8.947	9.863	10.336 ^a	9.263 ^b	8.879 ^b	0.443	0.389	<0.05
Isobutyric acid (C4i), % mol	0.498 ^b	0.532 ^b	0.6499 ^a	0.5497	0.554	0.577	0.003	0.988	0.787
Valeric acid (C5), % mol	0.954 ^{ab}	0.849 ^b	1.139 ^a	0.9895	1.038	0.914	0.011	0.900	0.343
Isovaleric acid (C5i), % mol	0.374	0.374	0.484	0.418	0.386	0.428	0.004	0.978	0.619
C2/C3, dimensionless	4.799	4.967	4.769	4.968	4.848	4.719	0.056	0.143	0.434
(C2+C4)/C3, dimensionless	5.438	5.576	5.418	5.675	5.461	5.297	0.091	0.165	0.257

^{a,b}Within a period or a treatment, means with different superscript letters were significantly different ($P < 0.05$).

¹CTR = TMR contaminated by regular level of *Fusarium* mycotoxins (i.e., 340.5 µg of deoxynivalenol (DON)/kg of DM and 127.9 µg of fumonisins (FB)/kg of DM in the whole diet); MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin deactivator product (Mycotofix, BIOMIN Holding GmbH, Tulln, Austria).

enzyme to lactating cow diets modified the DM digestibility, with inconsistent effects on feed intake. Further, no effects of DM digestibility increase were observed on rumen traits, milk yield, and milk component yield, probably due to positive energy balance status of lactating dairy cows used in the Beauchemin et al. (2000) experiment. These aspects deserve future investigation to be fully elucidated. On the contrary, the increases in either DM or NDF digestibility observed in the different experimental periods appeared consistent with changes in animal feeding behaviors during the trial. In particular, when DMI decreases, a lower rate of passage of nutrients through the gastrointestinal tract and a consequent increase in mean retention time of feeds in rumen-reticulum compartment could be expected (Seo et al., 2006; Sauvante and Nozière, 2016).

Milk Yield and Its Quality

No previous studies have examined the effects of specific combinations of *Fusarium* mycotoxins FB and DON on the performance of dairy cattle, in particular their effect on milk rennet coagulation properties. Winkler et al. (2014) analyzed the effect of a diet contaminated with up to about 5,240 µg of DON per kg feed and found no effect on average milk yield. Another study in which dairy cows received a diet with about 3,200 µg of DON/kg of feed also reported no negative effect on milk yield (Korosteleva et al., 2007). Recently, McKay et al. (2019) carried out a trial on early-lactation dairy cows. These authors concluded that the level of mycotoxin contamination reported in their study (i.e., TMR contaminated at 1,966 µg of DON/kg of DM and 366 µg of ZEA/kg of DM and offered for 28-d period), did not cause changes in DMI, milk production, or composition between treated and control groups. As stated by these authors, the daily milk yield was reduced in treated group by 0.74 kg/d ($P = 0.38$) with respect to control, and this numeric difference can represent an important reduction in farm gate income. In accordance with these findings, the diets with a greater DON and FB contamination levels influenced milk yield, with average reductions being measured both in the MTX (i.e., -1.34 kg/d) and MDP (i.e., -1.18 kg/d) groups relative to the CTR group. In previous studies, with moderate-to-low mycotoxin contamination (a combination of AFB1, DON, ZEA, and a combination of DON, ZEA, and FB), it was reported an improvement in milk yield upon addition of mycotoxin-deactivating products (Jovaišiene et al., 2016; Zouagui et al., 2017).

Migliorati et al. (2007) evaluated the effects due to the use of a mycotoxin adsorbent (80% clinoptilolite + 20% sepiolite) in lactating dairy cow diets during 2 experimental periods (i.e., summer and spring periods)

for 84 d. Despite the relatively high adsorbent dosages (i.e., 160 g/cow per day), no changes in milk yield as well as fat, protein, or lactose contents, SCC, pH, titratable acidity, clotting time, and ionic and total Ca were reported. On the contrary, Johnson et al. (1988) reported a significant reduction in milk fat and protein yields using zeolite at 2% DM inclusion rate. Instead, little is known about the effect of *Fusarium* mycotoxins on milk rennet coagulation properties. A previous field study (Křížová et al., 2016) found that the milk from dairy cows with increased mycotoxin exposure (categorized in different co-contamination level clusters of DON, FBs, ZEA, Aflatoxin B1, and T-2 toxin) had lower cheese curd firmness and lower whey volume ($P < 0.05$ for both). In addition, rennet coagulation time was increased in cows with higher mycotoxin exposure, although this effect was not statistically significant (Křížová et al., 2016). The changes in milk coagulation properties associated with lactation stage are consistent with previous observations reviewed by Bittante et al. (2012), reporting these milk properties tend to be favorable at the beginning of the lactation and less favorable in mid lactation, with a tendency to recover at the end of lactation.

In the current study, the MTX diet had a significant negative effect on K20 and a30, but the CTR and MDP diets led to similar K20 and a30 values. These results suggest that mycotoxin-deactivating products alleviated the negative effects of mycotoxins. The milk coagulation properties are economically important traits, especially in regions where milk is processed to cheeses (Pretto et al., 2013; Cassandro et al., 2016). Moreover, the biological pathways underlying the adverse effects of mycotoxins on milk rennet coagulation properties are largely unknown. Thus, further investigations of these topics are urgently needed.

Physiological Responses, Blood Variables, and Gene Expression

The changes in blood variables in the different dietary groups were generally modest, although some of the differences were statistically significant. However, we found elevated liver transaminases (AST and GGT) in the MTX group, as previously reported for monogastric and ruminant animals fed with mycotoxin (Faixová et al., 2010; Gallo et al., 2015). This effect is likely attributable to the more intense detoxifying activity of the liver in these animals. The chelating agent in mycotoxin-deactivating products attenuated this effect, thus we could expect a lower level of mycotoxins in the blood stream in line with results measured in ruminal and fecal samples, and these results should be confirmed by quantification of mycotoxin and their metabolites into blood too.

Our results indicated that the positive acute phase proteins did not increase due to contamination with *Fusarium* mycotoxins, thus indicating that they did not elicit an acute inflammatory response. Indeed, the decline of plasma ceruloplasmin in the MTX group, although very modest, suggests a weakening of the inflammatory response. This is compatible with an attenuation of the systemic immune response, also in case of the release of proinflammatory cytokines, and is a known response in cases of liver failure due to trauma (Orzheshkovskiy and Trishchynska, 2019) or challenges of innate immune system. Interestingly, even if the differences were small, we found that the MTX diet altered the expression of several genes in circulating leucocytes. In particular, was observed a lower expression of genes that are important mediators of immune and inflammatory responses (*MYD88*, *IL1R*, and *TLR2*). The reduction of the expression of *MyD88* and

Table 8. Ruminal and fecal concentrations of fumonisins (FB), hydrolyzed FB (HFB), or partially hydrolyzed FB (pHFB) forms (mol/mL of extract) of lactating dairy cows that received 3 experimental diets during 3 experimental periods of Latin square design

Item	Period			Treatment ¹				P-value		
	1	2	3	CTR	MTX	MDP	SEM	Latin square	Period	Treatment
Ruminal samples										
Sum of FB	0.39	0.42	0.35	0.08 ^a	0.72 ^c	0.37 ^a	0.003	0.585	0.575	<0.05
Sum of HFB	0.14	0.11	0.11	0.10 ^a	0.12 ^a	0.15 ^b	0.001	0.954	0.102	<0.05
Sum of pHFB	0.13	0.20	0.16	0.03 ^a	0.10 ^b	0.37 ^c	0.002	0.786	0.277	<0.05
Fecal samples										
Sum of FB	8.95	7.82	8.28	2.14 ^a	15.44 ^c	7.46 ^b	0.790	0.262	0.531	<0.05
Sum of HFB	1.36 ^a	1.75 ^b	1.60 ^{ab}	1.31 ^a	1.31 ^a	2.09 ^b	0.023	0.829	<0.05	<0.05
Sum of pHFB	1.79	2.59	2.29	1.00 ^a	1.49 ^b	4.17 ^c	0.173	0.758	0.091	<0.05

^{a-c}Within a period or a treatment, means with different superscript letters were significantly different ($P < 0.05$).

¹CTR = TMR contaminated by regular level of *Fusarium* mycotoxins [i.e., 340.5 µg of deoxynivalenol (DON)/kg of DM and 127.9 µg of FB/kg of DM in the whole diet]; MTX = TMR contaminated by a higher level of *Fusarium* mycotoxins with respect to CTR (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); MDP = MTX diet (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM) supplemented with 35 g/animal per day of mycotoxin deactivator product (Mycofix, BIOMIN Holding GmbH, Tulln, Austria).

TLR2 in the intestine following mycotoxins challenge was observed also in previous studies (Sugiyama et al., 2016; Dąbrowski et al., 2017). These genes play a fundamental role in pathogen recognition and activation of innate immunity (Akira and Takeda, 2004). Their lower expression could be because the *Fusarium* mycotoxins had an immunosuppressive effect; this interpretation is supported by the higher expression of the *CASP8* gene in the MDP group. In fact, activation of the caspases plays a central role in the execution-phase of cell apoptosis, thus suggesting that mycotoxins may promote the apoptosis of immune cells. To point out the result in the MDP group, where the sequestering agent seems to stop the effects of mycotoxins on circulating leucocytes. Our study has limitations for clarifying the mechanism by which mycotoxins have a negative effect on circulating leukocyte functions and other more specific studies must be performed (better considering the connection between mycotoxins in the diet, functions in blood stream circulating leukocytes and liver metabolism). Previous in vitro studies proposed that *Fusarium* mycotoxins (DON in particular) had this effect on leukocytes (Pestka et al., 2004; Pestka, 2007). In this previous research, the immunosuppressive effect was affected by the dosage and duration of the stimulus. Although comparison of this previous in vitro study with our animal studies may be problematic, we observed that a dose of *Fusarium* mycotoxin that was administered for at least 21 d had an immunosuppressive effect on circulating leucocytes, and possibly activated apoptosis-related processes.

The differences for some blood variables between periods can be related to metabolic (i.e., progression of the lactation stage) and nutrition (i.e., reduction of feed intake) changes. It is difficult to infer separately these 2 factors because they are linked by the period (Table 3). However, these differences observed between the periods resulted in poor significance and did not relate to physiological problems during the study. For example, the reduction of plasma zinc through the study could be related to the reduction of mineral input by feed intake. The plasmatic cholesterol is a marker of lipoproteins necessary for the lipid transport in the blood (Trevisi et al., 2013), and its reduction from average lactation stage is associated with the lower amount of fat absorbed according with the decrease of feed intake (Piccioli-Cappelli et al., 2014). Paraoxonase, an enzyme linked to lipoproteins, showed a similar trend.

FB, HFB, and pHFB Concentration in Feces

The FB in general have a very low oral bioavailability in healthy animals and are poorly degraded in rumen-reticulum compartment (Voss et al., 2007; Fink-

Gremmels, 2008b; Gallo and Masoero, 2009). Previous studies assessed the efficacy of fumonisin esterase FumD (EC 3.1.1.87, commercial name FUMzyme; BIOMIN Holding GmbH, Getzersdorf, Austria) to cleave the tricarballic acid side chains of FB, leading to the formation of nontoxic HFB in the gastrointestinal tract of rats (Hahn et al., 2015), broiler chickens (Grenier et al., 2017), turkeys (Masching et al., 2016), and swine (Schertz et al., 2018). Rice and Ross (1994) designed a study to set up analytical methods to measure FB1, HFB1, and pHFB1 in feeds and animal excreta. The authors concluded that the FB1 was largely excreted via the fecal route and to a lesser extent via urine, with fecal samples containing a measurable amount of also hydrolyzed forms of FB1 in ruminants.

Fodor et al. (2007) reported that microbiota from swine ceca were able to transform FB1 to its fully and partially hydrolyzed forms. By contrast, when rumen fluid was incubated with FB1 for 72 h, no hydrolyzed metabolites were detected (Caloni et al., 2000), suggesting that FB1 is poorly metabolized in the rumen environment either in presence or absence of alfalfa as substrate for microbial growth. Prelusky et al. (1995) reported that, upon oral administration of FB1 to dairy cows (5 mg/kg of BW), neither FB1 nor HFB1 was detected in plasma (detection limits: 4 and 8 ng/mL, respectively). In particular, neither parent FB1 molecule nor its metabolite was detectable 120 min after dosing. The authors concluded that a low absorption or a very efficient pre-systemic metabolism might explain the observed results. Present findings indicated that a small fraction of FB could be partially or fully hydrolyzed in the gastrointestinal tract of lactating dairy cows. However, the use of fumonisin esterase drastically reduced the FB and increased their hydrolyzed metabolites in both rumen fluid and feces, thus this enzyme appears suitable for detoxifying FB in lactating dairy cows and safeguarding their gastrointestinal functions.

CONCLUSIONS

Dairy cows that received a diet contaminated with a regular level of *Fusarium* mycotoxins experienced adverse effects. In particular, the presence of some *Fusarium* mycotoxins, such as DON and FB, even when well below the guidelines of the United States and European Union, negatively affected diet digestibility, milk yield, milk rennet coagulation properties, and the activities of some liver enzymes. The mycotoxin-deactivating products counteracted the negative effects of the mycotoxin on diet digestibility, quality of milk (mainly clotting features), and immune-metabolic responses in lactating dairy animals, even if lactating dairy cows receiving the MDP-supplemented mycotoxin contaminated diet had

a lower milk yield and feed efficiency than the CTR diet. Our results provide a better understanding of the risk of mycotoxin regarding effects on the performances, quality of milk, and immune-metabolic responses in lactating dairy animals ingesting diets characterized by DON and FB contamination levels that can be commonly detected in normal farm conditions.

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