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Effect of Adding an Organic Binder on Health of Cows Fed with Mycotoxins Contaminated Diet

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ABSTRACT: Mycotoxins contamination occurring in dairy cow's diet is responsible for devastating effects on livestock health. Among different strategies, using organic adsorbents is a promising approach to reduce the toxicity of mycotoxins. This study investigated the effects of an organic adsorbent containing *Lactobacillus brevis* TD4, *Lactobacillus paracasei* TD3, and *Saccharomyces cerevisiae* cell wall on milk production, somatic cell count, blood parameters (white blood cell [WBC], lymphocyte [LYM], neutrophil, basophil, monocyte, eosinophil, red blood cell, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin) and liver function (alanine transaminase [ALT], gamma-glutamyl transferase [GGT], aspartate transaminase [AST], alkaline phosphatase [ALP], urea, total protein, albumin) in dairy cows fed a naturally contaminated diet with Zearalenone (ZEA) and Deoxynivalenol (DON). The amounts of mycotoxins ZEA and DON in feed ingredients were measured using the HPLC method. Ten Holstein dairy cows received organic adsorbents daily in their diets for four weeks. Milk and blood samples were taken from cows before the start of feeding adsorbent (CTRL), during the feeding period (FP), and one week after removal of adsorbent from the diet (RP). Totally, the amount of measured ZEA and DON toxins in the diet were 389 and 1254.6 ppb, respectively. Feeding of organic adsorbent significantly increased milk production, total serum protein, and albumin compared to CTRL ($P < 0.01$). Also, numerically lower somatic cell count in their milk and a significant decrease ($P < 0.01$) in serum urea were resulted. Among examined blood parameters, the number of WBC and LYM significantly decreased ($P < 0.01$) after feeding with the organic binder in comparison to the control period. Furthermore, except for a significant increase in the level of AST ($P < 0.05$), the other liver function examined parameters were not affected. The consumption of feed containing low-cost organic adsorbent including *Lactobacillus* bacteria and yeast cell wall can improve the physical condition and health of dairy cows and reduce economic losses in livestock production.

Keywords: Mycotoxins; Dairy cattle; Organic adsorbents; Blood parameters; Somatic cell counts

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INTRODUCTION

Mycotoxins are secondary metabolites produced by molds and occur in many dairy cow foodstuffs, including roughages and concentrate (Zouagui et al., 2017, Changwa et al., 2018). Zearalenone (ZEA) and Deoxynivalenol (DON) are *Fusarium* mycotoxins. Due to the high occurrence, these mycotoxins are the most challenging toxins for livestock (Binder 2007, Sobrova et al., 2010). Published data about the effects of feedborne *Fusarium* mycotoxins on the biochemical and haematological blood parameters are still rare (Jovaisiene et al., 2016). A naturally contaminated diet (with DON and ZEA) in supplementation with a mycotoxin binder improved liver function, increased blood total protein, and decreased blood urea in dairy cows (Zouagui et al., 2017).

The unavoidable problem of mycotoxin presence in dairy cow feedstuff is a tremendous economic and health challenge in dairy farms. Thus, various strategies have been developed to control mycotoxins in animal feed. Electronic and hand sorting to remove mycotoxin contaminated grains, washing with water or sodium carbonate, using mold inhibitors, or preservation by acid are some solutions for mycotoxin control. However, many of these methods are impractical. The most commonly used method to counteract the risk effects of mycotoxins is the decrease in their bioavailability with various mycotoxin binder agents in the dairy cow rations (Binder 2007, Franco et al., 2011).

Different substances have been evaluated for this purpose (Bočarov-Stančić et al., 2011). These substances can be divided into two categories, inorganic and organic binders. Organic adsorbents include microorganisms (such as yeasts and lactic acid bacteria [LAB]) and yeast cell walls. The detoxification potential of LAB might be related to the degradation through its metabolism or bacterial cell wall compounds (Dalić et al., 2010). Detoxification of DON by eight LAB isolated from wheat products and kefir seed was studied and the results revealed all of the bacteria have the detoxification potential of DON (Franco et al., 2011). Another study demonstrated the capacity of four strains of *Lactobacillus* spp. to adsorb ZEA (Vega et al., 2017). A significant decrease in the ZEA and aflatoxin concentration was observed in fermented cornmeal with LAB (Mokoena et al., 2005).

Our previous in vitro studies showed the adsorbent containing *Lactobacillus* strains and the yeast cell

wall of *Saccharomyces cerevisiae* was able to significantly adsorb and reduce DON mycotoxin (Adami Ghamsari et al., 2021). Due to little information about the efficiency of LAB binders to deactivation of ZEA and DON, the present work was designed to study the effects of an organic mycotoxin binder containing two *Lactobacillus* spp. and yeast cell wall on blood factors, liver enzymes, milk production, and composition in dairy cows fed a naturally contaminated diet with both ZEA and DON.

MATERIALS AND METHODS

Cows, management, and mycotoxin binder

The animal experiments were carried out in accordance with EU Directive 2010/63/EU and the Animal Research: Reporting of in vivo Experiments (ARRIVE) guidelines (Anonymous 2010, Percie du Sert et al., 2020). Also, the research ethics committee at Islamic Azad University, Science and Research Branch in Tehran, Iran reviewed all animal studies and declared that ethics approval was not required.

Ten lactating Holstein cows with average milk production 11.5 ± 3.5 kg (Mean \pm SD), parity 1.2 ± 0.42 (Mean \pm SD), weight 586 ± 35 kg (Mean \pm SD), and DIM 426.3 ± 166.9 were used in a nine-week experimental period. In the four weeks, ten dairy cows moved to a group pen for adaptation, fed with a basal diet without adsorbent, and considered as a control period (CTRL).

The mycotoxin binder comprised *Lactobacillus brevis* TD4 (IBRC-M10790), *Lactobacillus paracasei* TD3 (IBRC-M10784), and *S. Cerevisiae* cell wall at a ratio of 35%, 35%, and 30%, respectively. Our previous in vitro study indicated that this combination was able to adsorb and reduce DON mycotoxin significantly (Adami Ghamsari et al., 2021).

Twenty-five grams of an organic toxin binder per cow top-dressed to the cows' diet since the 5th week and continued until the 8th week (considered as a feeding period; FP). Toxin binder removed from the diet on the 9th week (considered as removing period; RP, Elis, 1994). The feed and water were supplied ad libitum during the experiment period. The cows were milked twice per day at 10:00 and 18:00 h.

A basal diet was formulated to meet the National Research Council nutrient requirements of dairy cows and fed at 08:00 and 17:00 h throughout the experiment (Anonymous 2001). The basal diet consisted

of (DM basis): 10.06% of ground barley, 8.26% of ground corn, 7.18% of soybean meal, 4.64% of cottonseed meal, 3.99% bran and 0.37% mineral-vitamin supplement, 6.11% corn silage, 40.93% alfalfa, and 18.47% straw. The nutrient and energy concentration (DM basis) were 14.8% crude protein, 43.56% neutral detergent fiber, 29.6% acid detergent fiber, and net lactation energy of 1.47 mg/kg diet.

Analysis of feed-borne mycotoxins

The feedstuff content of ZEA and DON were analysed by HPLC. Samples of concentrate, corn silage, alfalfa, and straw were taken before, during, and end of feeding organic toxin binder. Samples dried at 60°C and ground to pass through 1 mm screen. Twenty-five grams of each sample was mixed with 100 ml acetonitrile and distilled water by the ratio of 90:10 v/v and stirred at 5000 rpm for 1 h. The samples were then centrifuged (5000 rpm, 15°C, 5 min,) and the supernatant of each sample was filtered at first through Whatman No. 1 filter paper (Whatman, Inc., Clifton, New Jersey, USA) and then 0.45 µm syringe filter. After clean-up with immuno-affinity columns (PriboFast China), the toxins present in the filtered liquid were measured by HPLC (Zaied et al., 2012).

The HPLC device used included a C-R5A (HPLC CHROMATOPAC/KNAUER) system equipped with a Gilson 151 UV-Vis detector and Gilson 712 software. The chromatographic separation was achieved using the Ultrabase C18 column (250×4.6 mm, 5 µm). The mobile gradient phase for ZEA toxin consisted of acetonitrile (HPLC grade) and doubled-distilled water by the ratio of 55:45 v/v, and for DON toxin consisted of acetonitrile (HPLC grade), doubled-distilled water, and methanol (HPLC grade) by the ratio 10:80:10 v/v/v, which were prepared for injection in the device following the filtration with 0.45 µm Teflon filter. In isocratic mode with a flow rate of 1 ml/min, the chromatogram column was set at 30°C, and the injection volume was 20 µl. The wavelength of 236 nm and 220 nm was utilized to evaluate the presence of ZEA and DON toxins in the samples, respectively (Kotal and Radová 2002, Vega et al., 2017). The concentration of the ZEA and DON toxins in the feed ingredients was measured based on the linear equation calculated from the standard curve of the mentioned toxins.

Blood and milk sampling and measurements

Blood samples were collected from the tail vein before the morning feeding on d 28 (before adding adsorbent to the diet), 56 (after four weeks feeding

adsorbent), and 63 (one week after removal adsorbent) of experimental days. Blood samples from the tube without any anticoagulant were allowed to clot. After centrifugation (3000 × g for 20 min at 4°C), the serum was stored at -20°C until analysed for total protein, albumin (ALB), urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT). Blood parameters were determined using enzymatic kits (Pars Azmoon Co., Tehran, Iran) and a BT1500 automatic analyser (Biotecnica Instruments S. p. A, Italy).

Blood samples for complete blood count analyses were collected in EDTA-coated blood tubes. The total number of white blood cells (WBC), red blood cells (RBC), neutrophils (NEU), lymphocyte (LYM), basophils (BASO), eosinophils (EOS), monocytes (MONO), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined in the laboratory through both haematology analyser (Exigo-vet, Boule Diagnostics Co., Sweden) and microscopic counting under light microscope.

Milk yield of the last three consecutive days of each period (CTRL, FP, and RP) was recorded and sampled for compositional analysis. The milk samples were analysed for fat, protein, lactose, mineral, and density by LactoStare device (FUNKE GERBER, Germany). The somatic cell count (SCC) was determined manually.

Statistical analysis

Statistical analyses were carried out using SAS software (SAS Institute, 1999). Values are displayed as mean ± SD and the mean values of time periods were compared using t-test. Data normality was assessed by Proc Univariate and Shapiro-Wilk tests. Normalization was performed for data that did not have a normal distribution using data conversion. To this end, the Box-Cox method was used to determine λ. In cases where the data were not normalized by conversion, they were compared by Wilcoxon method. Differences in the means were considered to be significant when P<0.05 and P<0.01.

RESULTS

Analysis of feed-born mycotoxins

All feed ingredients used in the experimental diet were naturally contaminated with ZEA and DON (Ta-

ble 1). Contents of ZEA and DON toxins in the whole diet were 389 and 1254.6 ppb, respectively.

Table 1. Mycotoxin content of dietary ingredients

Feedstuffs	Mycotoxins*	
	ZEA (ppb)	DON (ppb)
Concentrate	178	591.8
Corn Silage	45.2	119.7
Alfalfa	126	406.4
Straw	39.8	136.7
Total ration†	389	1254.6

*DON= deoxynivalenol, ZEA= zearalenone.

† Content of mycotoxin in the total ration was calculated based on the ingredient ratio in the diet.

Evaluation of blood parameters

Complete blood cell count results revealed that feeding organic binder affects blood cell parameters (Table 2). The WBC and LYM significantly decreased after feeding by the organic binder compared to CTRL (P<0.01). The removal of the organic

binder from the cow's diet did not affect WBC and RBC, whereas LYM was significantly higher compared to those in the FP (P<0.01). Supplementation of organic toxin binder increased the NEU, BASO, and EOS concentration compared to the control period (P<0.01). Removing the binder decreased BASO and EOS (P<0.01); however, it did not affect HCT and NEU compared with the FP. The organic binder did not affect MONO and MCV.

The effect of adsorbent supplementation on liver enzymes is presented in Table 3. The results of the present experiment indicate feeding of adsorbent did not affect the concentration of ALT, GGT, and ALP enzymes compared to the control period. However, in the blood of cows, the concentration of AST by adding adsorbent to their diets was higher than the control period (P<0.01). After cessation of adsorbent consumption, AST concentration decreased significantly compared to the adsorbent consumption period (P<0.01).

Table 2. The effect of organic adsorbent on the blood cells in dairy cows fed diets naturally contaminated with ZEA and DON (Mean ± Standard deviation)

Item	Experimental periods *			P-value of paired comparison		
	CTRL	FP	RP	CTRL vs. FP	CTRL vs. RP	FP vs. RP
WBC, 10 ³ /μl	22.26 ± 9.95	9.64 ± 1.7	10.29 ± 2.40	0.02	0.03	0.83
LYM, %	83.7 ± 8.13	60.7 ± 8.08	68.2 ± 8.55	0.00	0.00	0.00
NEU, %	13.8 ± 7.78	30.6 ± 8.15	28.7 ± 8.91	0.00	0.00	0.37
BASO, %	0.1 ± 0.33	1.88 ± 0.56	0.88 ± 0.31	0.00	0.00	0.00
MONO, %	0.6 ± 1.32	1.3 ± 0.86	0.4 ± 0.70	0.22	1.00	0.05
EOS, %	1.8 ± 1.22	5.2 ± 1.47	2.1 ± 1.66	0.00	0.73	0.00
RBC, 10 ⁶ /μl	5.8 ± 0.65	6.4 ± 0.69	5.20 ± 0.68	0.60	0.08	0.06
Hb, g/dl	9.8 ± 0.65	9.35 ± 0.39	9.01 ± 0.7	0.23	0.08	0.23
HCT, %	26.1 ± 3.56	25.1 ± 0.74	27 ± 3.00	0.39	0.47	0.09
MCV, fl	51.35 ± 6.64	53.45 ± 1.62	56.08 ± 2.83	0.50	0.20	0.10
MCH, pg	16.7 ± 1.54	16.51 ± 0.99	16.58 ± 1.25	0.26	0.93	0.41
MCHC, g/dl	33.43 ± 0.79	34.26 ± 0.96	33.12 ± 0.42	0.16	0.20	0.03

*CTRL= adaptation period (no mycotoxin binder, 4 wk), FP= Feeding period by organic mycotoxin binder (4 wk), RP= mycotoxin binder removal period (1 wk). WBC= White Blood Cell, LYM= Lymphocyte, Neu= Neutrophil, BASO= Basophil, MONO= Monocyte, EOS= Eosinophil, RBC= Red Blood Cell, Hb= Haemoglobin, HCT= Haematocrit, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration.

Table 3. The effect of organic adsorbent on the activity of liver enzymes in dairy cows fed diets naturally contaminated with ZEA and DON (Mean ± Standard deviation)

Item †	Experimental periods *			P-value of paired comparison		
	CTRL	FP	RP	CTRL vs. FP	CTRL vs. RP	FP vs. RP
ALT, U/l	55.2 ± 12.0	61.8 ± 8.98	60.6 ± 13.78	0.14	0.32	0.55
GGT, U/l	14.97 ± 2.02	14.58 ± 3.31	16.89 ± 2.15	0.81	0.18	0.24
AST, U/l	70.6 ± 8.38	77.3 ± 10.97	64.1 ± 13.63	0.01	0.30	0.01
ALP, U/l	130.9 ± 54.00	107.6 ± 30.35	117.4 ± 33.84	0.12	0.40	0.67

*CTRL= adaptation period (no mycotoxin binder, 4 wk), FP= Feeding period by organic mycotoxin binder (4 wk), RP= mycotoxin binder removal period (1 wk); †ALT= Alanine transaminase, GGT= Gamma-glutamyl transferase, AST= Aspartate transaminase, ALP= Alkaline phosphatase.

Table 4. The effect of organic adsorbent on the activity of blood parameters in dairy cows fed diets naturally contaminated with ZEA and DON (Mean \pm Standard deviation)

Item	Experimental periods *			P-value of paired comparison		
	CTRL	FP	RP	CTRL vs. FP	CTRL vs. RP	FP vs. RP
Urea, mg/dl	17.67 \pm 2.24	15.97 \pm 1.23	14.31 \pm 1.17	0.02	0.00	0.06
Total protein, g/dl	6.01 \pm 0.39	7.24 \pm 0.39	6.83 \pm 0.62	0.00	0.00	0.10
Albumin, g/dl	3.38 \pm 0.29	3.75 \pm 0.21	3.27 \pm 0.08	0.00	0.84	0.06

*CTRL= Adaptation period (no mycotoxin binder, 4 wk), FP= Feeding period by organic mycotoxin binder (4 wk), RP= mycotoxin binder removal period (1 wk).

Table 5. The effect of organic adsorbent on milk production and composition in dairy cows fed diets naturally contaminated with ZEA and DON (Mean \pm Standard deviation)

Item	Experimental periods *			P-value of paired comparison		
	CTRL	FP	RP	CTRL vs. FP	CTRL vs. RP	FP vs. RP
Milk yield (kg/d)	11.5 \pm 3.6	16.21 \pm 2.54	14.70 \pm 1.55	0.03	0.20	0.40
Milk compositions						
Fat (%)	2.8 \pm 0.49	2.9 \pm 0.15	3.0 \pm 0.10	0.47	0.52	0.11
Protein (%)	4.1 \pm 0.37	4.2 \pm 0.28	4.3 \pm 0.28	0.96	0.59	0.86
Lactose (%)	5.5 \pm 0.21	5.4 \pm 0.26	5.4 \pm 0.16	0.56	0.39	0.42
Minerals (%)	0.62 \pm 0.02	0.61 \pm 0.03	0.66 \pm 0.03	0.68	0.22	0.08
Density (%)	1.2 \pm 0.04	1.2 \pm 0.01	1.2 \pm 0.01	0.6	1	1
SCC, cells/ml $\times 10^4$	17.38 \pm 4.92	12.34 \pm 3.97	10.19 \pm 3.47	0.09	0.01	0.40

*CTRL= Adaptation period (no mycotoxin binder, 4 wk), FP= Feeding period by organic mycotoxin binder (4 wk), RP= mycotoxin binder removal period (1 wk). SCC= somatic cell count.

Serum urea content, total protein, and ALB were significantly affected by feeding mycotoxin binder (Table 4). Total protein and ALB concentrations were higher, and urea content was lower when cows received an organic binder in their rations ($P < 0.01$). After removing the binder from the diet, the amount of total protein and urea remained significantly higher and lower than the control period, respectively ($P < 0.01$).

Evaluation of milk production and its compounds

Mycotoxin binder supplementation was improved milk production compared to the control period ($P < 0.03$, Table 5). Fat, protein, lactose, mineral percentage, and milk density were not affected by adding a binder to the ration. Binder supplementation numerically reduced milk SCC compared to the CTRL, which continued during the RP and showed a significant decrease ($P < 0.03$).

DISCUSSION

Supplementation of dairy cattle ration with some sequestering agents is an economic strategy to overcome the detrimental effects of mycotoxins contaminated feedstuffs (Bočarov-Stančić et al., 2011, Kong et al., 2014). In recent years, LAB has shown the most

tremendous potential for both fungal growth inhibition and detoxification of mycotoxins (Franco et al., 2011). The mycotoxin adsorption properties of the cell wall and living cells of *S. cerevisiae* and some *Lactobacillus* strains subjected them to mycotoxin detoxification-related researches to be employed as an effective tool in the development of animal husbandry (El-Nezami et al., 2002, Wan et al., 2016).

In the current study, we evaluated the potential of two Iranian native probiotic bacteria, *L. paracasei* TD3 and *L. brevis* TD4, and yeast cell wall of *S. cerevisiae*, to decline adverse effects of feed-borne ZEA and DON on blood parameters, liver function, and milk yield of dairy cows. Ration ingredients mycotoxins analysis showed that all items have ZEA and DON contamination. The concentration of ZEA and DON in the diet was 389 and 1254.6 ppb, respectively, which was lower than previously reported data (Korosteleva et al., 2007, Kiyothong et al., 2012).

In most research on mycotoxin binders, pure toxins have been added to the diet; however, feeding a naturally contaminated diet allows us to study synergistic effects of minor toxins and mycotoxins metabolites. Meanwhile, some toxins may be conjugated with another compound in the diet and escape from

detection procedures; however, they can be released into the gastrointestinal tract and cause a mycotoxicosis even if the concentration of the major mycotoxin is lower than the threshold level (Korosteleva et al., 2007). A complex of DON with glycoside was detected previously in naturally and artificially contaminated wheat (Berthiller et al., 2005).

Because of the synergistic effect in naturally contaminated diets, a lower dose of mycotoxins can show a deleterious effect. ZEA with concentrations of about 400 ppb causes reproductive problems in dairy cattle (Towers et al., 1995). Furthermore, vaginitis, vaginal secretions, poor reproductive performance, and mammary gland enlargement of virgin heifers, decreased milk production, diarrhea, increased reproductive tract infections, and total reproductive failure occurred following consumption of a diet contaminated simultaneously with about 660 ppb of ZEA and 440 ppb of DON or alone with 25 ppm of ZEA. In addition, 4 to 7 ppm of ZEA in feedstuff is responsible for enlarged vulva but no abortion was observed in most cases (Weaver et al., 1986, Coppock et al., 1990, Adams et al., 1993). Off-fed, ketosis, pronounced milk decrease, and sometimes diarrhea were observed with the consumption of 1.5 to 2.5 ppm or even less of DON-contaminated diet (Adams et al., 1993, Diaz et al., 2001). Also, the reduced utilizable protein flow to the duodenum and altering in the rumen fermentation were shown in the cows consuming 2.6 to 6.5 ppm of DON (Charmley et al., 1993, Dänicke et al., 2005, Seeling et al., 2006).

The increased number of WBC and RBC was revealed following fed with DON and ZEA contaminated feed (Marczuk et al., 2012, Düringer et al., 2020). Adding the combination of mineral and biological adsorbent was shown to not reduce WBC and RBC levels, significantly (Marczuk et al., 2012, Fushimi et al., 2014, Jovaisiene et al., 2016). In the present study, the level of WBC and Lymphocytes reduced to near the normal range level compared to the control group while RBC and other blood parameters were not affected.

An increased level of serum ALT, AST, ALP, LDH, and GGT were reported in dairy cows fed with the *Fusarium* mycotoxin-contaminated diet (Seeling et al., 2006, Tripathi et al., 2008, Du et al., 2017, Zouagui et al., 2017). In the present study, examination of the level of liver enzymes such as ALP, ALT, AST, and GGT showed only serum GGT and ALP level decreased after the use of the organic adsorbent

in cows, although it was not significant. No alteration of liver enzymes concentrations can be explained by a lower concentration of mycotoxins compared to previous studies (Korosteleva et al., 2007, 2009, Jovaisiene et al., 2016, Zouagui et al., 2017). A significant increase of AST in cows fed toxin binder that was in agreement with previous data had no biological importance and was within the range of standard value (Jovaisiene et al., 2016).

Furthermore, blood urea significantly decreased in cows fed toxin binder compared to the control period and this trend continued after removing binder from the ration. Similar to our results, supplementation with mycotoxin binders decreased blood urea levels in dairy cows fed by naturally contaminated diets (Jovaisiene et al., 2016, Zouagui et al., 2017). In general, the serum concentration of urea is affected by the adsorption of ammonia from the rumen and protein metabolism in the liver. In mycotoxicosis, rumen microbiota and protein anabolism in the liver can be inhibited (Fuentes et al., 1998, Korosteleva et al., 2007). The supplemented diet with a mycotoxin deactivator resulted in higher populations of amylolytic, cellulolytic, and proteolytic bacteria as well as fungal zoospores (Kiyothong et al., 2012). Moreover, rumen ammonia concentration was increased by the feeding of DON and ZEA-contaminated wheat (Dänicke et al., 2005). When protein synthesis is inhibited in the liver, free amino acids are increased and used to produce energy, thus increasing blood urea concentration. Increased concentration of uric acid in the blood of laying hens fed *Fusarium* contaminated feed has been previously reported (Chowdhury and Smith 2004). Unlike to present study, serum urea and creatinine concentrations increased after using adsorbent in cows (Jovaisiene et al., 2016).

In the current study, the higher level of total protein and ALB in FP compared to the control period suggests better protein anabolism in the liver. The albumin and protein levels are other indicators of liver function (Tripathi et al., 2008). Korosteleva et al. (2007) showed total serum protein and albumin concentrations were not significantly increased following the consumption of adsorbent. In contrast to our data, a higher protein level of serum was reported by feeding mycotoxin to the dairy cow (Korosteleva et al., 2007). It seems that *Fusarium* toxin-contaminated diets can alter microbial protein synthesis in the rumen (Korosteleva et al., 2007), and supplementing the diet with a mycotoxin binder in our study can mo-

dify the negative effects of mycotoxins on the rumen microbiome.

Improving milk production also confirms better fermentation during the adsorbent consumption period. Similar to the results of the present study, milk production (kg/cow) in dairy cows significantly increased with the use of adsorbent (Whitlow and Hagler, 2005). A diet with about 660 ppb of ZEA and DON can result in a low feed intake and depressed milk production (Sultana and Hanif, 2009). In contrast, milk production and composition were not affected by diets naturally contaminated with ZEA and DON (Korosteleva et al., 2007, 2009). Supplementation of contaminated diet with mycotoxin binder had a variety of effects on milk production. The findings of the present study, in line with other studies, showed that the addition of a mycotoxin binder to mycotoxin-contaminated rations improved milk production of cows (Zouagui et al., 2017). Variation in the results might relate to the type of binder, binder's dose, the level of contamination, the experimental duration, and the physiological stage of the animal. In a field study, it was found that foods with about 750 ppb ZEA and 500 ppb DON cause weakness, decreased milk production, diarrhea, increased genital infections, and infertility (Whitlow and Hagler 2005).

The milk SCC is a widely used marker for both milk quality and udder health (Stocco et al., 2020). In the present study, the milk SCC was lower in cows fed with organic binder compared to the control group,

which is in agreement with some reports (Kiyothong et al., 2012, Jovaisiene et al., 2016, Zouagui et al., 2017) and can be explained through decreased immunosuppressive and hepatotoxicity effect of mycotoxins by the binder (Zouagui et al., 2017). Contrary to our findings, no effect of a supplemented diet with a mycotoxin binder on milk SCC was reported (Korosteleva et al., 2007).

CONCLUSION

In conclusion, this study evaluated the effect of an organic mycotoxin binder to minimize the negative impact of feedstuff naturally contaminated with ZEA and DON mycotoxins on dairy cows. The better results obtained from haematological parameters, liver function, and milk production after being fed with this product suggested its ability to decrease the deleterious effect of mycotoxins.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Adami Ghamsari F, Tajabadi Ebrahimi M, Bagheri Varzaneh M, Iranbakhsh A, Akhavan Sepahi A (2021) In vitro Reduction of Mycotoxin Deoxynivalenol by Organic Adsorbent. *J Food Process Preserv*:e15212.
- Adams R S, Kephart K B, Ishler V A, Hutchinson L J, Roth G W (1993) Mold and mycotoxin problems in livestock feeding. Dept of Dairy and Animal Science, Extension Publ. DAS:93-21.
- Anonymous. (2001). National Research Council. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition. Washington, DC: The National Academies Press.
- Anonymous (2010) Council Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union*53:33-79.
- Berthiller F, Dall'Asta C, Schuhmacher R, Lemmens M, Adam G, Krska R (2005) Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography–tandem mass spectrometry. *J Agric Food Chem*53:3421-3425.
- Binder E M (2007) Managing the risk of mycotoxins in modern feed production. *Anim Feed Sci Technol*133:149-166.
- Bočarov-Stančić A, Adamović M, Salma N, Bodroža-Solarov M, Vučković J, Pantić V (2011) In vitro efficacy of mycotoxins adsorption by natural mineral adsorbents. *Biotechnology in Animal Husbandry*27:1241-1251.
- Changwa R, Abia W, Msagati T, Nyoni H, Ndleve K, Njobeh P (2018) Multi-Mycotoxin Occurrence in Dairy Cattle Feeds from the Gauteng Province of South Africa: A Pilot Study Using UHPLC-QTOF-MS/MS. *Toxins*10:294.
- Charmley E, Trenholm H L, Thompson B K, Vudathala D, Nicholson J W G, Prelusky D B, Charmley L L (1993) Influence of Level of Deoxynivalenol in the Diet of Dairy Cows on Feed Intake, Milk Production, and Its Composition I. *J Dairy Sci*76:3580-3587.
- Chowdhury S, Smith T (2004) Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on performance and metabolism of laying hens. *Poultry science*83:1849-1856.
- Coppock R W, Mostrom M S, Sparling C G, Jacobsen B, Ross S C (1990) Apparent zearalenone intoxication in a dairy herd from feeding spoiled acid-treated corn. *Veterinary and human toxicology*32:246-248.
- Dalić D, Deschamps A, Richard-Forget F (2010) Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. *Food control*21:370-380.

- Dänicke S, Matthäus K, Lebzien P, Valenta H, Stemme K, Ueberschär K H, Razzazi-Fazeli E, Böhm J, Flachowsky G (2005) Effects of Fusarium toxin-contaminated wheat grain on nutrient turnover, microbial protein synthesis and metabolism of deoxynivalenol and zearalenone in the rumen of dairy cows. *J Anim Physiol Anim Nutr (Berl)*89:303-315.
- Diaz D, Hagler Jr W, Hopkins B, Patton R, Brownie C, Whitlow L (2001) The effect of inclusion of a clay type sequestering agent on milk production of dairy cattle consuming mycotoxins contaminated feeds. *J Dairy Sci*84:1554.
- Du X, Chen L, Huang D, Peng Z, Zhao C, Zhang Y, Zhu Y, Wang Z, Li X, Liu G (2017) Elevated apoptosis in the liver of dairy cows with ketosis. *Cell Physiol Biochem*43:568-578.
- Duringer J, Roberts H, Doupovec B, Faas J, Estill C, Jiang D, Schatzmayr D (2020) Effects of deoxynivalenol and fumonisins fed in combination on beef cattle: health and performance indices. *World Mycotoxin Journal*13:533-543.
- El-Nezami H, Polychronaki N, Salminen S, Mykkänen H (2002) Binding rather than metabolism may explain the interaction of two food-Grade Lactobacillus strains with zearalenone and its derivative (†) alpha-earalenol. *Appl Environ Microbiol*68:3545-3549.
- Franco T S, Garcia S, Hirooka E Y, Ono Y S, Dos Santos J S (2011) Lactic acid bacteria in the inhibition of Fusarium graminearum and deoxynivalenol detoxification. *J Appl Microbiol*111:739-748.
- Fuertes J, Gonzalo C, Carriedo J, San Primitivo F (1998) Parameters of test day milk yield and milk components for dairy ewes. *J Dairy Sci*81:1300-1307.
- Fushimi Y, Takagi M, Hasunuma H, Uno S, Kokushi E, Watanabe U, Liu J, Marey M, Miyamoto A, Otoi T (2014) Application of mycotoxin adsorbent to cattle feed contaminated with zearalenone: Urinary zearalenone excretion and association with anti-Müllerian hormone. *World Mycotoxin Journal*7:367-378.
- Jovaisiene J, Bakutis B, Baliukoniene V, Gerulis G (2016) Fusarium and Aspergillus mycotoxins effects on dairy cow health, performance and the efficacy of Anti-Mycotoxin Additive. *Pol J Vet Sci*19:79-87.
- Kiyothong K, Rowlinson P, Wanapat M, Khampa S (2012) Effect of mycotoxin deactivator product supplementation on dairy cows. *Animal production science*52:832-841.
- Kong C, Shin S Y, Kim B G (2014) Evaluation of mycotoxin sequestering agents for aflatoxin and deoxynivalenol: an in vitro approach. *SpringerPlus*3:346.
- Korosteleva S N, Smith T K, Boermans H J (2007) Effects of feedborne Fusarium mycotoxins on the performance, metabolism, and immunity of dairy cows. *J Dairy Sci*90:3867-3873.
- Korosteleva S N, Smith T K, Boermans H J (2009) Effects of feed naturally contaminated with Fusarium mycotoxins on metabolism and immunity of dairy cows. *J Dairy Sci*92:1585-1593.
- Kotal F, Radová Z (2002) A Simple Method for Determination of Deoxynivalenol in Cereals and Flours. *Czech Journal of Food Sciences*20:63-68.
- Marczuk J, Obremski K, Lutnicki K, Gajeczka M, Gajeccki M (2012) Zearalenone and deoxynivalenol mycotoxicosis in dairy cattle herds. *Polish Journal of Veterinary Sciences*15:365-372.
- Mokoena M P, Chelule P K, Gqaleni N (2005) Reduction of fumonisin B1 and zearalenone by lactic acid bacteria in fermented maize meal. *J Food Prot*68:2095-2099.
- Percie du Sert N, Ahluwalia A, Alam S, Avey M T, Baker M, Browne W J, Clark A, Cuthill I C, Dirnagl U, Emerson M, Garner P, Holgate S T, Howells D W, Hurst V, Karp N A, Lazic S E, Lidster K, MacCallum C J, Macleod M, Pearl E J, Petersen O H, Rawle F, Reynolds P, Rooney K, Sena E S, Silberberg S D, Steckler T, Würbel H (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol*18:e3000411.
- Seeling K, Lebzien P, Dänicke S, Spilke J, Südekum K H, Flachowsky G (2006) Effects of level of feed intake and Fusarium toxin-contaminated wheat on rumen fermentation as well as on blood and milk parameters in cows. *J Anim Physiol Anim Nutr (Berl)*90:103-115.
- Sobrova P, Adam V, Vasatkova A, Beklova M, Zeman L, Kizek R (2010) Deoxynivalenol and its toxicity. *Interdisciplinary toxicology*3:94-99.
- Stocco G, Summer A, Cipolat-Gotet C, Zanini L, Vairani D, Dadousis C, Zecconi A (2020) Differential Somatic Cell Count as a Novel Indicator of Milk Quality in Dairy Cows. *Animals*10:753.
- Sultana N, Hanif N (2009) Mycotoxin contamination in cattle feed and feed ingredients. *Pakistan Veterinary Journal*29:211-213.
- Towers N, Sprosen J, Webber W. (1995). Zearalenone metabolites in cycling and non-cycling cows. In *Toxinology and Food Safety* (pp. 46-47). New Zealand: Toxinology and Food Safety Research Group, Ruakura Research Centre, Hamilton.
- Tripathi M K, Mondal D, Karim S A (2008) Growth, haematology, blood constituents and immunological status of lambs fed graded levels of animal feed grade damaged wheat as substitute of maize. *Journal of animal physiology and animal nutrition*92:75-85.
- Vega M F, Dieguez S N, Riccio B, Aranguren S, Giordano A, Denzoin L, Soraci A L, Tapia M O, Ross R, Apás A (2017) Zearalenone adsorption capacity of lactic acid bacteria isolated from pigs. *Braz J Microbiol*48:715-723.
- Wan M L Y, Turner P C, Allen K J, El-Nezami H (2016) Lactobacillus rhamnosus GG modulates intestinal mucosal barrier and inflammation in mice following combined dietary exposure to deoxynivalenol and zearalenone. *Journal of Functional Foods*22:34-43.
- Weaver G A, Kurtz H J, Behrens J C, Robison T S, Seguin B E, Bates F Y, Mirocha C J (1986) Effect of zearalenone on dairy cows. *American journal of veterinary research*47:1826-1828.
- Whitlow L W, Hagler W. (2005). Mycotoxins in dairy cattle: Occurrence, toxicity, prevention and treatment. Paper presented at the Proc. South-west Nutr. Conf.
- Zaied C, Zouaoui N, Bacha H, Abid S (2012) Natural occurrence of zearalenone in Tunisian wheat grains. *Food control*25:773-777.
- Zouagui Z, Asrar M, Lakhdissi H, Abdennebi E (2017) Prevention of mycotoxin effects in dairy cows by adding an anti-mycotoxin product in feed. *Journal of Materials and Environmental Sciences*8:3766-3770.