ORIGINAL ARTICLE

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Influence of bentonite adsorbents associated with hepatic antioxidants on the health and production of dairy cows fed diets containing naturally produced mycotoxins

Influência do adsorvente bentonita associado a antioxidantes hepáticos na saúde e produção de vacas leiteiras alimentadas com dietas contendo micotoxinas naturalmente produzidas

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ABSTRACT: As mycotoxins are consistent contaminants in the dairy cow diet, the use of adsorbents is recommended, although there are no ideal adsorbents. Although there are studies on this subject, few have focused on chronic natural intoxications. Here, we evaluated the effect of bentonite adsorbents associated with liver antioxidants on the health and milk production of dairy cows fed a diet containing naturally-produced fumonisin, zearalenone, and deoxynivalenol. Eighteen dairy cows (bodyweight 550 ± 50 kg, 5 ± 2 years old, and 30 ± 1,25 kg/day milk production) in the middle of lactation were divided into groups: treatment (TG, n = 9, 22 g/day of supplement added to diet) and control (CG, n = 9, without supplement). A physical examination was performed, weekly over 56 days and blood was collected for liver and immune assessments. Milk was harvested to evaluate milk production and content (fat protein, somatic cell count, and lactose). The additive promoted beneficial effects on the liver from the 24th day due to a decrease in the enzymatic activities of gamma-glutamyltransferase and lactate dehydrogenase and increased serum protein and albumin levels. There were improvements in health, evidenced as fewer clinical manifestations of the disease, greater leukocyte oxidative metabolism capacity, and a lower neutrophil lymphocytes ratio. The treatment also promoted a 19% increase in milk volume. It was concluded that the additive promoted health benefits and milk production in dairy cows.

KEYWORDS: immunity; somatic cell count; reactive oxygen species; albumin, zearalenone.

RESUMO: Como as micotoxinas são contaminantes constantes na dieta de vacas leiteiras, o uso de adsorventes é recomendado, embora não existam adsorventes ideiais. Ainda que existam estudos sobre o assunto, poucos são direcionados às intoxicações naturais crônicas. Nosso experimento avaliou o efeito do adsorvente bentonita associado a antioxidantes hepáticos sobre a saúde e produção de leite de vacas leiteiras alimentadas com dieta contendo fumonisina, zearalenona e desoxinivalenol produzidos naturalmente. Dezoito vacas leiteiras (peso corporal 550 ±50 kg, 5±2 anos de idade, e 30 ± 1,25 kg/dia de produção de leite) no meio da lactação, foram divididas em grupos: Tratamento (GT, n=9, 22g/dia do suplemento adicionado à dieta) e controle (GC, n =9, sem suplemento). Em intervalos semanais, durante 56 dias, foi realizado exame físico, coleta de sangue para avaliação hepática e imunológica. O leite foi colhido para avaliar a produção e análises de leite para (proteína, gordura, contagem de células somáticas e lactose). O aditivo promoveu efeitos benéficos no fígado à partir do 24º dia devido à diminuição das atividades enzimáticas da gama-glutamiltransferase e lactato desidrogenase e aumento dos níveis séricos de proteína e albumina. Houve melhora na saúde, evidenciada por menor número de manifestações clínicas de doença, maior capacidade de metabolismo oxidativo dos leucócitos e menor razão neutrófilos/linfócitos. O tratamento também promoveu aumento de 19% no volume de leite. Concluiu-se que o aditivo promoveu benefícios à saúde e à produção de leite em vacas leiteiras.

PALAVRAS-CHAVE: imunidade; contagem de células somáticas; espécie reativa de oxigênio; albumina, zearalenona.

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INTRODUCTION

The use of energy foods has become routine in dairy cattle. Although it is advantageous for productivity, it creates challenges related to mycotoxicosis. This is especially true in the southern region of Brazil, where climatic conditions of high relative humidity and sudden temperature changes are favorable for fungal growth and toxin production at the time of planting, harvesting, and storage of crops (KELLER et al., 2013; CUSTODIO et al., 2017).

Approximately 90%–100% of Brazilian animal feed is believed to have some mycotoxin contamination, particularly fumonisin (FB), zearalenone (Zea), deoxynivalenol (Don), and aflatoxin (AF) (CUSTODIO et al., 2017; DADALT; PRIMIERI, 2020). Although intoxication with high doses of these mycotoxins causes liver failure and cancerous lesions (FINK-GREMMELS, 2008a; CUSTODIO et al., 2017), lower doses interfere with animal immunity and productivity. They can alter the genetic material of highly proliferative cells, such as those in the immune system, increasing susceptibility to diseases and interfering with liver function by deregulating the metabolism of carbohydrates, lipids, and proteins (CORRIER, 1991; CUSTODIO et al., 2017).

Therefore, mycotoxin adsorbents are important to prevent intoxication. Although inorganic adsorbents, such as bentonites, are effective for AF absorption, inorganic adsorbents, such as yeast, are more effective against Zea and Don (SABATER-VILAR et al., 2007). There are also antioxidant compounds, such as vitamins, amino acids, and enzymes, that can help both in the degradation and metabolization of mycotoxins, and in liver regeneration (NASEER et al., 2016; GUO et al., 2019).

Considering that most studies on mycotoxin adsorbents in cattle have been conducted with experimental intoxication with only one mycotoxin (NASEER et al., 2016; CUSTODIO et al., 2017, GUO et al., 2019), it is necessary to verify whether compound adsorbents will attenuate the deleterious effects of diets naturally contaminated by mycotoxins, thereby allowing the animal to express its maximum productive potential.

Currently, there is an antioxidant adsorbent formulation available containing milk thistle extract (silymarin), yeast cell walls, bentonite, charcoal, vitamin E, and choline chloride. It acts as an adsorbent for mycotoxins, causes liver regeneration, and improves the use of nutrients. Thus, the objective of this experiment was to verify whether this compound additive improves the health and production of diet-fed cows containing naturally produced mycotoxins.

MATERIAL AND METHODS

The experimental procedures were approved by the Committee for Ethical Conduct in the Use of Experimental Animals (N° 023/2019 CEUA/UNICENTRO; 25/06/2019).

The experiment was conducted on a commercial farm and consisted of 60 lactating animals in a compost barn system located in the district of Jordão, approximately 50 km from Guarapuava, PR, in the south-central region of the state of Paraná, at the geographic coordinates 25°23′26 S, 51°27′15 W, Greenwich. The region has an altitude of approximately 1100 m, has a moderately humid subtropical climate, an annual rainfall of 1,944 mm, minimum and maximum temperatures of 12.7 °C and 23.5 °C, respectively, and a mean relative humidity of 77.9%.

Using adopted management, the animals were fed twice a day with a diet consisting of 30.8 kg/animal/day of total ration, based on dry matter, consisting of 13.46% of corn silage, 40.86% commercial concentrate (Milk Max Avant 20T GP, Cooperative AGRÁRIA, Entre Rios, PR, Brazil), 8.65% soybean meal, 23.24% pre-dried oats, and 0.98% mineral core (Bovigold®, DSM). The total diet formulation was based on the NRC (2001) requirements for dairy cattle (Table 1). To detect mycotoxins, 500 g samples of the diet were collected at two different times, at the beginning and in the middle of the experiment, and were sent to the Lamic SAMITEC Laboratory (Santa Maria, RS, Brazil). In foods, the mycotoxins AF, FB, Don, and Zea were measured by liquid chromatography-sequential mass spectrometry (LC-MS/MS). The results showed an average of 990.3 ppb FB (Fb1, 683.7 ppb; Fb2, 306.6 ppb); Zea 503.0 ppb, and Don 724.61 ppb, which were naturally produced.

Table 1. Chemical composition of foods used in the total animal ration, based on dry matter.

Parameter	Corn Silage 13,46%	Concentrate 40,86%	Soy Bran 8,65%	Pre-Drying of Oats 23,14%	Total Feed
DM %	42	89,98	89	53,7	68,5
MM % DM	3,49	9,16	6,84	6,5	7,05
CP % DM	7,98	22,23	49,72	9,2	16,7
EE % DM	3,57	4,73	4,51	5,0	4,7
NDF % DM	35,01	20,82	9,28	64,2	37,15
AFD % DM	21,03	8,39	7,76	38,9	19,86
TDN % DM	76,39	83,99	4,45	60,61	67,79

DM= dry matter; MM= mineral matter; CP= crude protein; EE= ether extract; NDF= neutral-detergent fibre; AFD= acid-detergent fibre; TDN = total digestible nutrients.

From the total number of animals, 18 clinically healthy Holstein cows were randomly selected, with a mean age of 5 ± 2 years, 550 ± 50 kg of bodyweight (BW), between the 3rd and 5th month of lactation, 30 ± 1, 24 kg/day of milk production obtained in two mechanical milkings, and an body condition scorebetween 3.5 and 4.0. The health of the animals was verified by physical examination and blood count before inclusion in the experiment.

The experimental design was completely randomized and performed with a blind study model in relation to treatment. The control group (CG) (n = 9) received a diet without adsorbent, and the treatment group (TG) (n = 9) received a commercial adsorbent complex based on bentonite (min 650 g/kg), beta-glucans (min 54 g/kg), choline (min 2600 mg/kg), mannan oligosaccharide (min 59.4 g/kg), mineral matter (max 720 g/kg), and vitamin E (min 27.5 IU/kg) (*Safetox plus* R®, Safeeds S.A., Cascavel, Brazil, registration in MAPA Nº. 21034.016243/2019-61, SEI Nº. 9706806). The total dose was 22 g/animal/day, as recommended by the manufacturer, for 56 days. The product was added directly in the trough with the total feed and a small portion of concentrate (approximately 50 g) in the morning, ensuring that the cows ingested the product when the feeder was closed. Later, the feeder was opened, and the rest of the total mixed ration was administered.

The animals were subjected to a physical examination and blood and milk collection at weekly intervals for 2 months on days D0, D8, D16, D24, D32, D40, D48, and D56. Physical examinations consisted of inspection, cardiac and pulmonary auscultation, and measurement of rectal temperature.

Blood samples were collected by superficial epigastric venipuncture with needles (25 × 0.8 mm) coupled to plastic vacuum tubes containing heparin (4 mL) to assess the oxidative metabolism of leukocytes. Tubes containing EDTA were used for blood to assess the hemogram (4 mL), and dry tubes were used for blood to assess liver biochemical measurements of aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), serum protein, and albumin (8 mL).

The oxidative metabolism of leukocytes was measured using the colorimetric technique of nitroblue tetrazolium (NBT), as described by Flores et al. (2019). The heparinized blood was kept cold using ice and processed within 3 h of collection. Then, 100 µL of whole blood was incubated with equal parts with 1% NBT solution (Sigma®, SP, Brazil) stimulated with 5 µL of 12-myristate-13-phorbol acetate (PMA at 300 ng/mL, Sigma®, SP, Brazil) at 37 °C in a water bath. After 30 min, the reaction of phagocytosis and oxidation of the internalized NBT was interrupted by the addition of 2000 µL of ice-cold ethylenediaminetetraacetic acid (EDTA) (3 mM), and the red blood cells were disrupted by osmotic lysis. Extracellular NBT was then removed by washing with phosphate-buffered saline (PBS). The samples were centrifuged (1200 rpm, 8 min at 4 °C), the supernatant was discarded, and the leukocyte pellets were fixed with methanol (Synth®, SP, Brazil). Leukocytes were then dissolved in KOH (Synth®, SP, Brazil, 3M, 120 µL) and DMSO (Dimesol® - MarcoLab, RJ, Brazil, 99%, 140 µL), and the suspension was read at 630 nm with a spectrophotometer (Thermo Plate®, MG, Brazil) in duplicate, with intra-variability confidence lower than 0.05%.

The blood count was analyzed using an automatic hematology counter (SDH-3 VET, Labtest®, MG, Brazil), and the differential leukocyte count was performed using blood smears, based on morphological and metric characteristics under an optical microscope.

For liver analyses, serum was extracted from whole blood after centrifugation (3000 rpm for 15 min at 25^{0}C, in which total serum protein (g/dL), albumin (g/dL), and enzyme activity, AST (U/L), GGT (U/L), and LDH (U/L), were measured by biochemical tests using the methodology of continuously descending kinetics and colorimetric reactions in a Bioplus® semi-automatic device with commercial kits (total proteins, albumin, AST/GOT Liquiform, GAMA-GT Liquiform, and LDH Liquiform, Labtest® Diagnóstica S.A., Lagoa Santa, MG, Brazil).

Milk collection was conducted directly in the milking line (closed system) in tubes containing bronopol, a preservative for milk samples (Broad Spectrum Microtabs® II, Advanced Instruments®, Norwood, Massachusetts, USA). For each animal, an individual milk sample was collected from four quarters at each time point. Milk samples were subjected to analyses: somatic cell count (SCC) (cells × 10^3/mL), lactose (%), fat (%), and protein (%), at an outsourced laboratory, using FTIR and flow cytometry (APCBRH methods, PARLEITE, Curitiba, Brazil). The volume of milk produced (kg/day) was measured using an individual digital automatic milk flow meter after milking each animal.

The data collected for each variable were evaluated using the InStat Graphpad statistical software. Data were submitted to normality (Kolmogorov Smirnov) and homogeneity of variance (Bartlett test) tests. Only the CCS data did not pass the normality tests; therefore, they were transformed using $\log_{10}$. To evaluate the effect of time, means were analyzed using the one-way analysis of variance for repeated samples and Tukey's test. For comparison between treatments, the results were analyzed using the Student's *t*-test for unpaired samples.

RESULTS AND DISCUSSION

Our results showed that the combined use of bentonite and hepatic antioxidants was beneficial to animal health and milk production in cows fed a diet containing naturally produced mycotoxins.

Although the levels of mycotoxins contained in the diet were below those recommended by Brazilian legislation (BRASIL, 2011), the levels of Zea were higher, and the levels

of FB and Don were close to the upper limits recommended by the Laboratory of Mycotoxicological Analysis (2020) and by the Research Center in Forage Culture at UFPR (SOUZA et al., 2011). Notably, mycotoxins act synergistically and potentiate the toxic effects of the diet (CUSTODIO et al., 2017).

The present study demonstrated the benefits of the treatment on the liver health of cattle from D24 (Figure 1). Although AST activity was within the normal clinical limits, GGT and LDH activities were above the reference values in both groups (KANEKO et al., 2008). Although AST was not influenced by treatment or time, GGT and LDH decreased ($p < 0.05$) with TG for interaction and treatment on D24 and D32.

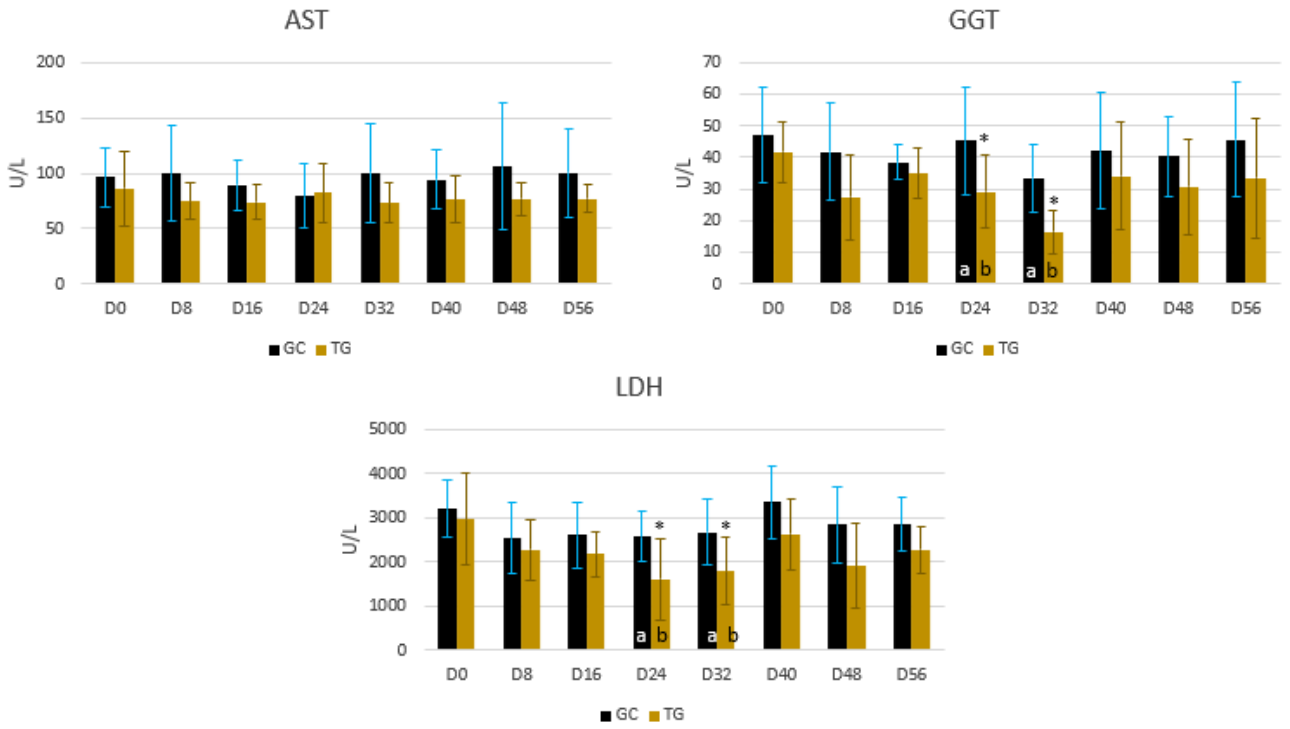
AST is an enzyme that determines the integrity of hepatocytes, but it is only elevated in the acute phase of liver injury (KANEKO et al., 2008). The normal enzymatic activity of AST in both groups at all times indicated that the intoxication was probably chronic, because the levels of mycotoxins contained in the diet were lower than those used in previous experiments of experimental intoxication (NASEER et al., 2016; GUO et al., 2019). However, they were not innocuous because the other liver enzymes, serum protein, and albumin were altered.

According to Stockham and Scott (2008), an increase in GGT enzyme activity occurs in hepatobiliary disorders with cholestasis, with or without biliary hyperplasia. LDH is an enzyme that catalyzes the reversible oxidation of lactate to pyruvate in the presence of the coenzyme, NAD+, which acts as a hydrogen donor or receptor (STOCKHAM & SCOTT, 2008). This enzyme is present in several body tissues such as cardiac, skeletal, and liver muscles, and is highly nonspecific, but together with other liver enzymes, it indicates liver damage (KANEKO et al., 2008).

The increase in GGT and LDH activity reflected liver changes, mainly related to cholestasis because of mycotoxicosis, with the possibility of changes in liver metabolism, resulting in lower protein anabolism. It interferes with animal production and immunity, as previously reported by Jovaisiene et al. (2016), Naseer et al. (2016), and Custodio et al. (2017).

As these enzymes decreased in TG even for a short time, it is believed that the adsorbent additive of mycotoxins and hepatic antioxidants could regenerate liver health. Such findings have already been reported by Jovaisiene et al. (2016), Naseer et al. (2016), and Guo et al. (2019), who used different adsorbent compounds in cattle experimentally intoxicated with mycotoxins.

Figure 2 shows the levels of serum albumin, total serum protein, and serum globulin. Although the reference values for these variables in cattle show variations in the literature (LOPES et al., 2007; KANEKO et al., 2008), it is noted that on D0, the protein and albumin serum values of most animals were below the reference values for the species in both groups, but over time, these two variables reached the reference parameters or were positioned slightly above in the TG (KANEKO et al., 2008).



Values expressed as means and standard deviation. Different lowercase letters indicate statistical difference for treatment interaction. * indicates statistical difference for time interaction $P \leq 0.05$, Tukey test. Reference Values= AST- aspartate amino transferase 78-132 U/L, GGT-gamma-glutamyl transferase 6.1-17.4 U/L, LDH-lactate dehydrogenase 692-1445 U/L (KANEKO et al., 2008).

Figure 1. Serum liver enzymes (AST, LDH, and GGT) from the control group (GC) and treatment group (TG), with the latter given a bentonite adsorbent and liver antioxidants for mycotoxins.

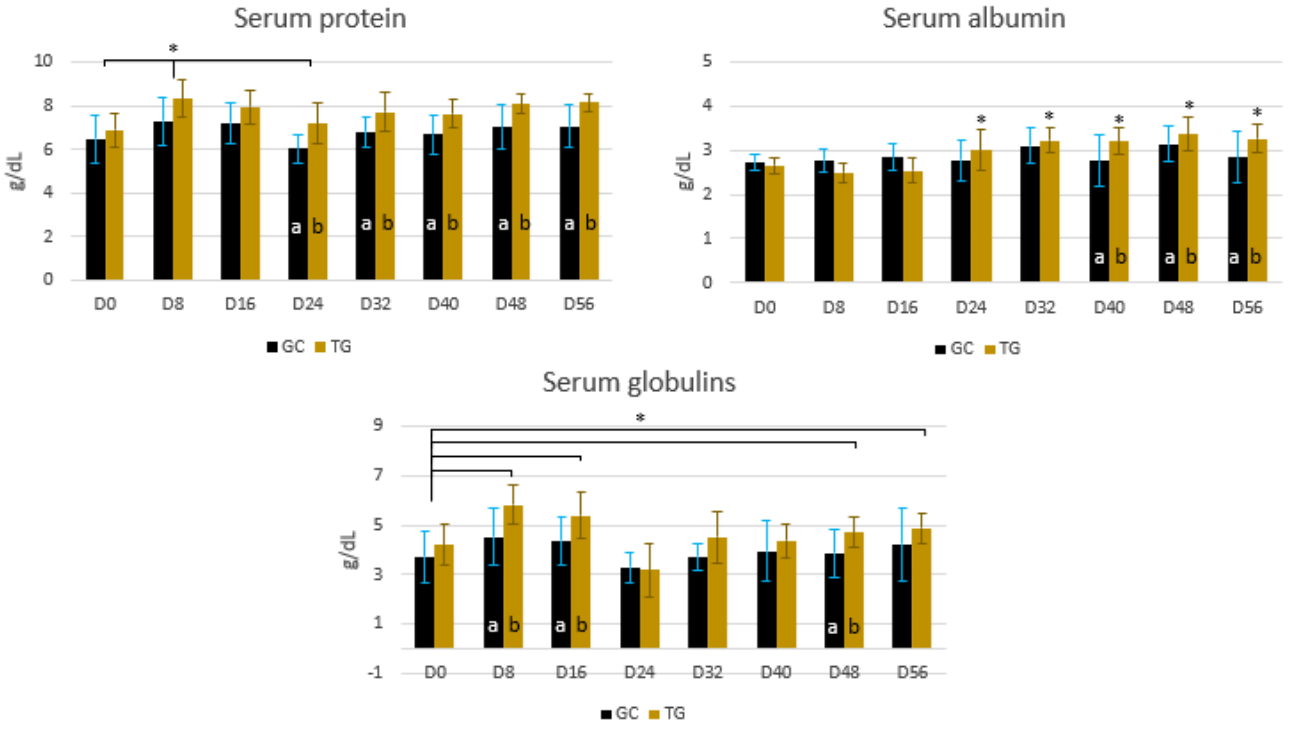
Furthermore, there was a punctual increase in serum protein levels in both groups on D8 compared with D0 and D24 ($p = 0.05$). At other times, protein content remained stable in both groups, with no effect of time. Regarding the treatment effect, there was an increase in the serum protein concentration in the TG compared to that in the CG from D24 ($p < 0.05$). Similarly, serum albumin levels increased from D24 for TG in the time effect ($p < 0.05$) and from D40 for the treatment effect ($p < 0.05$), which may indicate higher effectiveness of protein synthesis in TG than in GC (GONZÁLEZ & SILVA, 2006). There was a significant increase in serum globulin in treated animals on D8, D16, D48, and D56 in relation to the initial moments (time effect, $p = 0.0017$) and on D8, D16, and D48 compared to CG (treatment effect, $p < 0.05$), which could indicate higher immune effectiveness (KANEKO et al., 2008).

The nutritional status of the animal, as well as failure in ingestion, absorption, or protein loss because of gastrointestinal, urinary, dermal, or blood loss changes, could also be the cause of the decreased protein and albumin serum (LOPES et al., 2007); however, there were no changes in diet. None of the animals showed visible disease conditions, such as hemorrhages, throughout the experiment. Few animals had softened feces, and there was not anthelminthic treatments. It is believed that the reason for the decreased protein and albumin serum values was a lower protein anabolism because of liver changes or an overload caused by the metabolization of mycotoxins circulating during the initial moments for both groups and throughout the experiment for the animals in the CG (KANEKO et al., 2008). Unfortunately, the mycotoxin serum was not measured because it is an expensive technique and requires specific equipment, being performed in very few studies (WINKLER et al., 2014).

Another possible explanation is that the hepatic oxidizing agents contained in the adsorbent compounds, such as vitamins, amino acids, and enzymes, can assist in the degradation and/or metabolization of mycotoxins and act as hepatic antioxidants, resulting in higher protein anabolism, which suggests an improvement in liver function (NASEER et al., 2016; GUO et al., 2019).

Regarding immunity, there was a significant increase in the leukocyte oxidative metabolism in treated cows at D24 in relation to the initial time point (time effect, $p < 0.0001$), whereas the CG showed no change in this parameter. Regarding the treatment effect, an increase in leukocyte oxidative metabolism from D24 to D48 ($p < 0.05$) in the TG compared to the CG (Figure 3).

Oxidative metabolism is a respiratory leukocyte burst that blood phagocytes perform, such as neutrophils and monocytes. It occurs mainly during phagocytosis by oxidizing compounds called reactive oxygen species (ROS). Their main function is to eliminate internalized pathogens (TIZARD, 2017). The NBT colorimetric assay mimics an infection, where PMA-stimulated

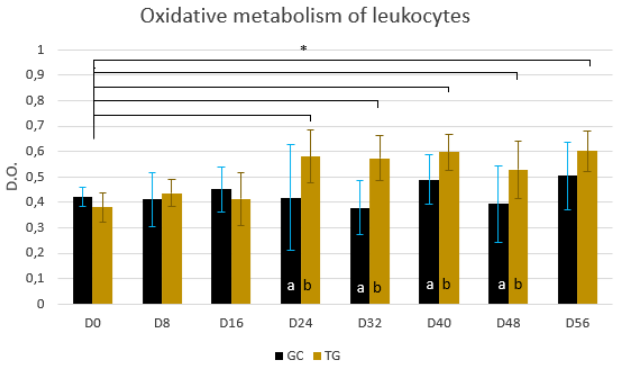


Values expressed as mean and standard deviation. Different lowercase letters indicate statistical difference for treatment interaction * indicates statistical difference in time effect; significance level $p<0.05$. Reference Values = Serum protein - 6.74 - 7.76 g/dL, Serum albumin 3.03 - 3.55 g/dL. Serum globulin – 3.0 - 3.5g/dL (KANEKO et al., 2008).

Figure 2. Serum protein, serum albumin and serum globulin from the control group (GC) and treatment group (TG), with the latter given a bentonite adsorbent and liver antioxidants for mycotoxins.

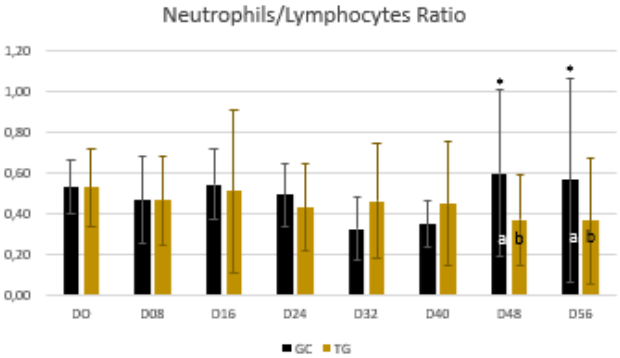
blood phagocytes phagocytose the yellow-colored reagent and oxidize it to form a bluish compound called formazan. At the end of the reaction, the leukocytes are dissolved, and the compound is released into the solution, stained blue proportional to the magnitude of the oxidative metabolism (FLORES et al., 2019). Thus, the increased leukocyte oxidative metabolism in TG indicated that the treatment increased the ability of animals to fight pathogens, thereby becoming less susceptible to infectious diseases (SCHALM et al., 2010).

Regarding blood counts, the animals showed no change in these variables throughout the experiment. There was an influence of time and treatment on the neutrophil/lymphocyte ratio, where the CG had a higher proportion of neutrophils/lymphocytes than the TG, both at D48 and D56 (p = 0.05, Figure 4). At the same time point, there was a higher frequency of animals with mucopurulent nasal secretion in the CG (22.2%) than in the TG (0%), although none of these cows had fever, pulmonary auscultation alteration, or reduced milk production. Associated with white blood cell data and clinical signs of nasal secretion,



Values expressed as mean and standard deviation. O.D.- optical density. Different lowercase letters indicate statistical difference in treatment effect, * indicates statistical difference in time effect; significance level p<0.05, Tukey test.

Figure 3. Oxidative metabolism of blood leukocytes from the control group (GC) and treatment group (TG), with the latter given a bentonite adsorbent and liver antioxidants for mycotoxins.



Values expressed as mean and standard deviation. Different lowercase letters indicate statistical difference in treatment effect, * indicates statistical difference in time effect, p < 0.05.

Figure 4. Blood neutrophils/lymphocytes ratio from the control group (GC) and treatment group (TG), with the latter given a bentonite adsorbent and liver antioxidants for mycotoxins.

it is believed that animals in the CG showed alterations in the anterior respiratory tract. In contrast, in the TG, the higher efficiency of phagocytes and globulin production prevented diseases.

Figure 5 shows the production and analysis of CCS, protein, fat, and lactose in milk. Only TG exhibited increased milk production from day D24 compared to the initial moments (p = 0.0002). This increase was higher than that in CG on days D24, D32, D40, and D48 (p = 0.02, 0.01, 0.01, and 0.03, respectively).

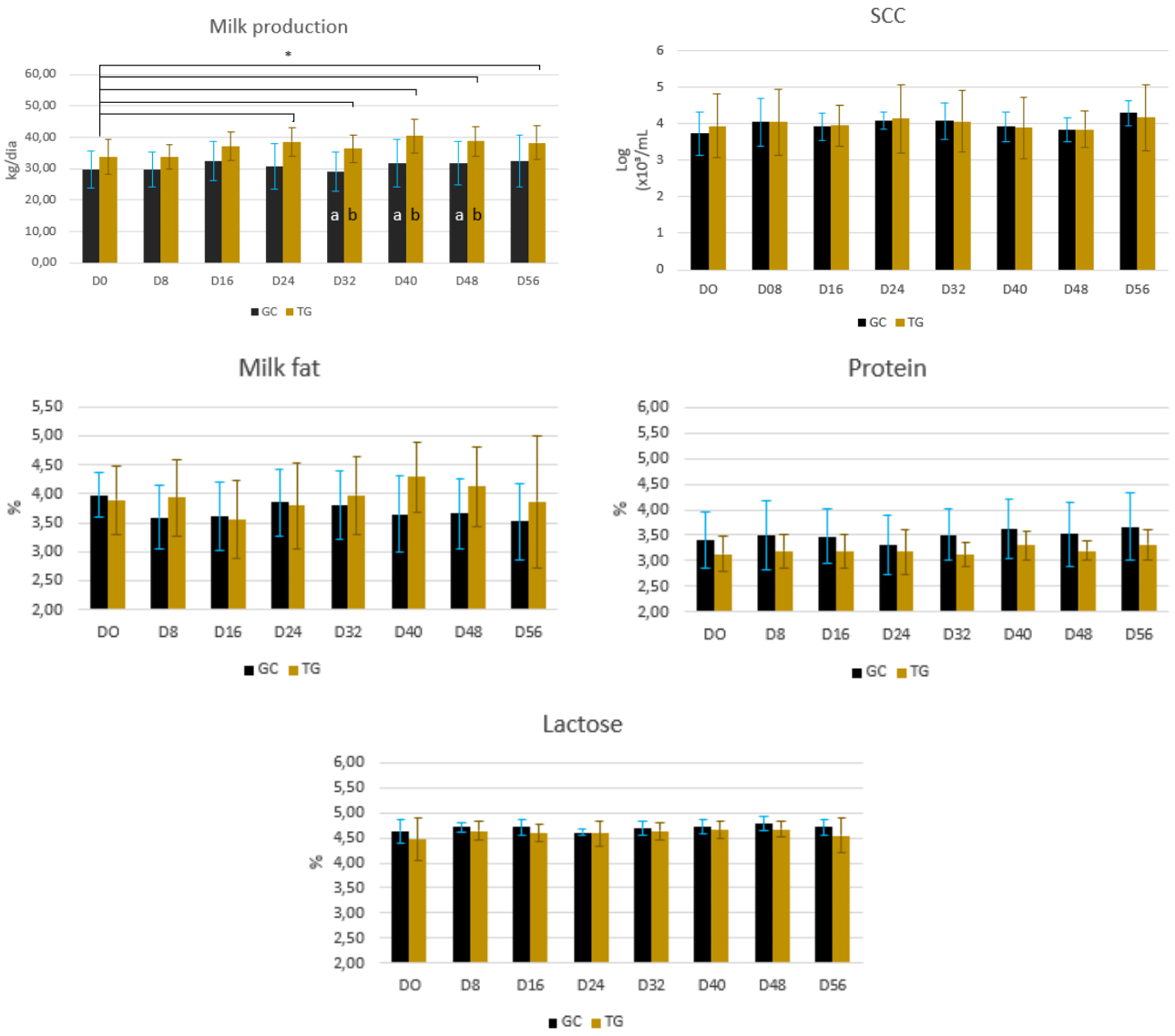
As the increase in milk production was perpetuated over time, it is believed that this occurred because of the use of the adsorbent additive with hepatic antioxidants, responsible for minimizing hepatic cholestasis. This effect might increase the anabolism of carbohydrates, lipids, and proteins, and these are the main precursors of milk (CUSTODIO et al., 2017; FONSECA & SANTOS, 2000).

In our experiment, food consumption was not measured, and although the animals received the same amount of the diet throughout the experiment, management was conducted with an open feeder after consumption of the additive, which allowed the animal to regulate its consumption. Fink-Gremmels (2008b) reported that chronic contamination by mycotoxins mainly implies hidden disturbances, such as reduced voluntary food intake by animals.

It is known that both choline and silymarin, present in the adsorbent, are capable of decreasing liver enzymes in calves intoxicated with AF (NASEER et al., 2016), indicating that these compounds improve liver health, which could reflect an increase in both liver health and milk production. Yeasts can also promote an increase in milk production because of the greater digestibility of the diet, increased microbial protein flow to the small intestine (BITENCOURT et al., 2011), and increased dry matter consumption (OLIVEIRA et al., 2010).

Regarding mastitis, it was noted that no animal presented clinical mastitis throughout the experiment. Regarding SCC, the treatment did not influence this variable, and only the CG showed a punctual increase in CCS on D24 compared to D0 and D40. The other variables were not influenced by time or treatment. The adsorbent compound did not interfere with SCC, probably because this parameter was already low for most animals in both groups (below 300,000 cell/mL of milk), indicating that the management conducted on the farm avoided the inoculation of infectious agents in the mammary gland, with no need to increase defense cells in this region (SORDILLO, 2016).

In contrast, milk solids remained practically stable even with an increase in the volume of milk produced. Milk proteins are synthesized in alveolar cells from blood amino acids, except for albumin and immunoglobulins, which are synthesized outside the mammary gland and transported to the secretory cells by the bloodstream. Thus, the increase in



Values expressed as means and standard deviation. Different lowercase letters indicate treatment effect statistical difference, * indicates statistical difference in time effect; significance level p<0.05 Tukey test.

Figure 5. Milk production, somatic cell count (SCC) and analysis and chemistry of milk from the control group (GC) and treatment group (TG), with the latter given a bentonite adsorbent and liver antioxidants for mycotoxins.

serum albumin would not reflect the increase in milk protein, because it represents a very small fraction of milk composition, approximately 9.2% (300 mg/L). The fact that the mammary glands of most animals are healthy also contributed to the stable protein levels throughout the experiment, given that casein and immunoglobulins, which correspond to 78% of the total milk protein, only increase in case of infection (FONSECA; SANTOS, 2000).

Finally, it was possible to verify that the adsorbent complex for mycotoxins and hepatic antioxidants benefitted the animals from the 24th day of use. Additionally, the increase in milk production by 19.9% (6.16 L/day) economically compensates for the use of the product, the cost of which is estimated to be R$ 0.22 per animal per day for the producer.

CONCLUSION

The adsorbent complex and hepatic antioxidants improved the health and production of dairy cows fed diets containing naturally produced FB, Zea, and Don, because they improved indicators of liver injury and function after 24 days of use. Directly or indirectly, this effect improved animal immunity, reduced the rate of respiratory infections, and also promoted an increase in milk production, although it did not interfere with the composition of the milk or the health of the mammary glands.

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