


Mycotoxin-contaminated diets and an adsorbent affect the performance of Nellore bulls finished in feedlots

L. Custodio¹, L. F. Prados^{2†} , D. N. Figueira¹, A. Yiannikouris³, E. M. Gloria⁴, V. B. Holder³, J. E. Pettigrew⁵, E. Santin⁶, F. D. Resende^{1,2} and G. R. Siqueira^{1,2}

¹Department of Animal Science, Universidade Estadual Paulista (UNESP), 14884-900 Jaboticabal, SP, Brazil; ²Agência Paulista de Tecnologia dos Agronegócios (APTA), 14770-000 Colina, SP, Brazil; ³Alltech, Nicholasville, KY 40356, USA; ⁴Biological Science Department, College of Agriculture Luiz de Queiroz (ESALQ), University of São Paulo (USP), 13418-900 Piracicaba, SP, Brazil; ⁵Pettigrew Research Services, Inc., Champaign, IL 61820, USA; ⁶Department of Medicine Veterinary, Universidade Federal do Paraná (UFPR), 80060-240 Curitiba, PR, Brazil

(Received 14 October 2019; Accepted 16 March 2020; First published online 15 April 2020)

Mycotoxins are present in almost all feedstuffs used in animal nutrition but are often ignored in beef cattle systems, even though they can affect animal performance. The objective of this study was to evaluate the effects of mycotoxins and a mycotoxin adsorbent (ADS) on performance of Nellore cattle finished in a feedlot. One hundred Nellore cattle (430 ± 13 kg) were used in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. The factors consisted of two diets with either natural contamination (NC) or exogenous contamination (EC) and the presence (1 g/kg of DM; ADS) or absence of a mycotoxin adsorbent. The NC and EC diets had the following contaminations, respectively: 0.00 and 10.0 µg/kg aflatoxins, 5114 and 5754 µg/kg fumonisins, 0.00 and 42.1 µg/kg trichothecenes B, 0.00 and 22.1 µg/kg trichothecenes A and 42.9 and 42.9 µg/kg fusaric acid. At the beginning of the experiment, all animals were weighed, and four randomly selected animals were slaughtered to evaluate the initial carcass weight. After 97 days of treatment, all animals were weighed and slaughtered. There was no interaction among factors for the DM intake (DMI; P = 0.92); however, there was a tendency for the EC diets to decrease the DMI by 650 g/day compared to animals fed NC diets (P = 0.09). There was a trend for interaction among factors (P = 0.08) for the average daily gain (ADG), where the greatest ADG was observed for cattle fed the NC diet (1.77 kg), and the lowest was observed for those fed the EC diet (1.51 kg). The NC + ADS and EC + ADS treatments presented intermediate values for ADG. The animals fed the NC diet had a greater final BW (596 kg) than animals fed the EC treatment (582 kg; P = 0.04). There was a tendency for interaction among factors for carcass gain (P = 0.08). Similarly to ADG, the highest carcass gain was observed for animals fed the NC diet (1.20 kg), and the lowest was observed for those fed the EC diet (1.05 kg). The NC + ADS and EC + ADS treatments presented intermediate values. The natural contamination groups had greater carcass gain than that of the EC groups, and the use of the ADS recovered part of the weight gain in animals fed the EC diet. In conclusion, mycotoxins at the levels evaluated affected the performance of beef cattle, and adsorbents may mitigate their impact.

Keywords: aflatoxins, carcass, fumonisins, fungi, mycotoxin production

Implications

Mycotoxins are present in almost all feedstuffs used in animal nutrition, and the effects can be manifested in reproductive, immunological and performance disorders. The data suggest that feedlot Nellore bulls fed finishing diets containing exogenous contamination of mycotoxins exhibited poorer performance compared to those fed diets that were naturally contaminated. When a mycotoxin adsorbent was added to the diet with exogenous contamination, the performance of the animal was partially recovered. Therefore, our results may be useful for improving performance in beef cattle.

† E-mail: laurafrancoprados@hotmail.com

Introduction

The effects of mycotoxins in animals depend on the amount consumed, exposure time, type of toxin and interaction among different toxins (Upadhaya *et al.*, 2010). These effects may manifest as reproductive, immunological and performance disorders (Zain, 2011). Since 1968, it is known that mycotoxins affect the performance and liver and kidney weights of beef cattle (Garrett *et al.*, 1968). Additionally, a recent study by Custodio *et al.* (2019) outlined that at least one mycotoxin is present in samples of feedlot diets collected in Brazil. However, studies evaluating the effects of mycotoxins on ruminants were developed mainly with dairy cattle

(Fink-Gremmels, 2008; Chaiyotwittayakun, 2010; Santos and Fink-Gremmels, 2014). Fink-Gremmels (2008) concluded that the major effects of the mycotoxins present in feed included reduced DM intake and, consequently, reduced milk production and reproduction in dairy cattle. Moreover, Osweiler *et al.* (1993) observed that the ingestion of fumonisins could elevate the serum enzyme activity of liver enzymes, suggesting impairment of liver function.

In a review, Jouany (2007) explained that mycotoxin adsorbents can be an effective strategy to reduce or even control the harmful effects of these toxins. Adsorbents can decrease mycotoxin absorption by animals, creating stable chemical interactions with toxins and further limiting their bioavailability in the gastrointestinal tract (Yiannikouris and Jouany, 2002). Merrill *et al.* (2007) studied the milk production of beef cattle (Angus × Hereford) fed mycotoxin-contaminated diets and observed a linear increase in milk production (9.8 to 14.2 kg/day) as the intake of a yeast cell wall adsorbent increased (0 to 60 g/day). However, studies with beef cattle and the effects of mycotoxin adsorbents on performance are scarce.

The hypothesis of this experiment was that the consumption of mycotoxins would decrease the performance of Nellore bulls finished in the feedlot and that the yeast cell wall adsorbent may attenuate this damage. The objective of this experiment was to evaluate the effects of mycotoxin levels and adsorbent usage on the intake and performance of Nellore cattle finished in feedlots.

Material and methods

Experimental animals and treatments

This experiment was conducted at Agência Paulista de Tecnologia dos Agronegócios (APTA) in Colina, SP, Brazil. The experimental period lasted 97 days, divided into an adaptation period of 28 days and a finishing period of 69 days. The animals received treatment diets during the entire experimental period (97 days). In total, 100 contemporary Nellore bulls (430 ± 13 kg BW; 24 ± 4 months old) were used in a randomized complete block design (blocked by initial BW; animals were stratified and assigned to eight blocks) in a 2 × 2 factorial arrangement of treatments. Animals were then assigned randomly within the block to pens, and each pen was randomly assigned to one of the four treatments. Four animals, representative of different BW blocks, were slaughtered at the beginning as the baseline group to represent the initial carcass weight of the remaining animals. The remaining animals were allocated into 32 (three animals per pen) 4 m × 15 m (totaling area 60 m², feed bunk of 4 m) open-air feedlot pens that were equipped with individual water fountains with 100 l capacity, with a high flow valve and covered trough.

The treatments were evaluated in a 2 × 2 factorial arrangement to investigate the main effects of diets and adsorbents and their interactions. The main effect of diet was evaluated by implementing two different diets with

Table 1 The most frequent mycotoxins found in diets and their concentration in the natural and exogenous contaminated diets of Nellore cattle finished in feedlot

Mycotoxin ¹ (µg/kg)	NC ²	Exogenous contamination	EC ³
Aflatoxin	–	10.0	10.0
Fumonisin	5114	640	5754
Trichothecenes B	–	42.1	42.1
Trichothecenes A	–	22.1	22.1
Fusaric acid	42.9	–	42.9

DON = deoxynivalenol; NC = diet with natural contamination; EC = diet with exogenous contamination; REQ = risk equivalent quantities.

¹Aflatoxin: B1 + B2 + G1 + G2; fumonisin: B1 + B2; trichothecenes B: DON, 15-acetyl DON, 3-acetyl DON, fusarenon X, nivalenol, and DON 3-glycoside; trichothecenes A: T-2, H-T2, diacetoxyscirpenol and neosolaniol.

²NC: REQ = 15.0 µg/kg.

³EC: REQ = 45.0 µg/kg.

different mycotoxin contaminations (Table 1). The main effect of the adsorbent was established by the presence or absence of the adsorbent in the diet (1 g/kg of DM). The organic mycotoxin adsorbent (ADS; Mycosorb A⁺, Alltech, Nicholasville, KY, USA) was composed of internal yeast cell walls (*Saccharomyces cerevisiae*) and algae. At the beginning of the study, the ADS was added to all mineral mixes of animals receiving this treatment. The dietary treatments included (1) a diet with natural contamination (NC); (2) a diet with natural contamination + the adsorbent (NC + ADS); (3) a diet with exogenous contamination (EC) and (4) a diet with exogenous contamination + the adsorbent (EC + ADS).

Mycotoxin production and evaluation in the diets

The identity and dose of mycotoxins used in this study were based on a survey previously conducted by Custodio *et al.* (2019). This initial survey aimed to identify the types of mycotoxins that mainly occurred in feedlots from 30 different production ranches and to establish the mycotoxin levels present in feedstuffs used in beef cattle feedlot diets in Brazil.

The mycotoxins aflatoxins B1 + B2, fumonisins B1 + B2 + B3, trichothecenes A (T-2 toxin) and B (deoxynivalenol (DON)) were produced individually at College of Agriculture Luiz de Queiroz, University of São Paulo. The production of mycotoxins was conducted via natural fermentation of corn or wheat by mycotoxin-producing fungal species. A strain of *Aspergillus flavus* was used for aflatoxin B1 and B2 production, a strain of *Fusarium verticillioides* for fumonisins, a strain of *Fusarium graminearum* for trichothecene B and a strain of *Fusarium sporotrichioides* for trichothecene A. The concentration of each mycotoxin in the diet was standardized throughout the experimental period, and these mycotoxins were weighed and added to the diets daily during the mixing of feedstuffs (only in the exogenously contaminated diets). The mycotoxins were stored in a sterilized room until use. Every day, 100 g of contaminated corn produced in the laboratory was added to achieve exogenous contamination.

Once a week, a sample of each feedstuff used in the diets was collected, and a composite sample was analyzed at the end of the experiment. The composite samples were sent to the Alltech 37[®] + Analytical Services Laboratory (Lexington, KY, USA), where mycotoxin evaluation was performed and comprised two distinct steps. In the first step, the absolute quantification of 38 different mycotoxins was performed using a validated and ISO/IEC 17025:2005 accredited method by means of ultra-performance liquid chromatography electrospray ionization tandem MS (Waters Acquity Tqd; Waters Corp., Milford, MA, USA), involving an isotopic dilution step and a data normalization process. In the second step, the mycotoxin concentrations were further interpreted according to known species-specific sensitivities and normalized according to the principles of toxic equivalent factors, determining the risk equivalent quantities (REQs) expressed in $\mu\text{g}/\text{kg}$ of aflatoxin B1 equivalent (Yiannikouris, 2015; Table 1).

Feed management

Bulls were acclimated to the assigned finishing diet during a 28-day acclimation period. During the first 21 days, the animals received the acclimation diet (Table 2); during the subsequent 7 days, the bulls received a mixture of the acclimation and finishing diets (50 : 50) and after day 28,

the animals received the finishing diet alone. Diets were formulated to meet the nutrient requirements of Nellore bulls gaining 1.5 kg daily (National Academies of Sciences, Engineering, and Medicine, 2016).

The diet was provided daily at 0800 h, using a mixer wagon (RX-40 E; Casale, São Carlos, SP, Brazil) equipped with a scale. The diets were provided *ad libitum* (Table 2), while the orts were weighed daily, and the amount of feed offered was adjusted daily to maintain 1% to 3% orts and to measure DM intake (DMI).

Chemical analyses

Chemical analyses of ingredient samples were carried out at the Laboratory (APTA, Colina, SP, Brazil). Samples were partially dried at 55°C in a forced draft oven for 72 h, ground in a knife mill (Thomas Model 4 Wiley; Thomas Scientific, Swedesboro, NJ, USA) using a 1-mm mesh sieve, and then stored for further chemical analysis.

The contents of DM (method 934.01), ash (method 942.05), CP (method 978.04) and ether extract (method 920.39) were measured according to recommendations of the Association of Official Analytical Chemists (AOAC; 1995). The contents of NDF and ADF were determined by sequential analysis as described by Mertens (2002). Cellulose was solubilized using 72% sulfuric acid, whereby the lignin content was obtained by the difference from the ADF. The samples were subjected to nitric acid digestion and inductively coupled plasma spectroscopy analyses for minerals (Ca, P, Na and K) (method 975.03; AOAC, 1995).

Blood sampling

On days 0, 14, 28 and 97, all animals were fasted for 8 h, then blood was collected via jugular venipuncture. Serum blood samples were collected into tubes without avoiding hemolysis, placed on ice and centrifuged (3000 \times g for 20 min at 4°C) within 1 h after collection. The samples were then placed in labeled Eppendorf tubes and stored at -20°C until subsequent analysis for the following serum enzymes: aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT). Enzyme analyses were performed colorimetrically using commercial kits (LabQuest, Campinas, SP, Brazil).

The contents of total protein, urea, creatinine, total cholesterol and triglycerides were analyzed using an automatic analyzer with high performance for biochemical and turbidimetric tests (Labmax Pleno, Nasu-Gun, Tochigi, Japan).

Slaughter and animal performance

At the beginning of the experiment, after an 8 h fast, four animals (randomly selected) were transported to the slaughterhouse (Minerva Foods, Barretos, SP, Brazil) located 20 km from the research facility. After arrival at the slaughterhouse, animals were kept in resting pens for 18 h (free access to water) and then submitted to humanitarian slaughter under Brazilian Federal Inspection, and the hot carcass weight (HCW) was obtained. After slaughter, carcasses were placed at cold chamber for 24 h at a temperature of 2°C.

Table 2 Ingredients used in the diets and chemical composition of the diets of Nellore cattle finished in feedlot

Items	Content	
	Acclimation diet	Finishing diet
Ingredients (% of DM)		
Sugarcane bagasse	22.0	12.0
Corn	39.8	53.0
Citrus pulp	20.0	20.0
Cottonseed meal	15.0	11.8
Minerals ¹	3.20	3.20
Chemical composition (% of DM basis)		
DM ²	76.9	82.4
CP	13.8	13.7
Ether extract	2.54	3.14
Ash	3.31	2.87
NDF	32.9	24.5
ADF	22.8	15.9
Lignin	6.07	4.30
Non-protein nitrogen (% of total N)	21.3	20.9
P	0.21	0.22
Ca	0.57	0.59
Na	0.07	0.07
K	0.60	0.60
Total digestible nutrients	68.0	74.2
Metabolizable energy (MJ/kg)	10.3	11.2

¹Mineral = Na: 43 g/kg; Ca: 106 g/kg; P: 12.6 g/kg; S: 34 g/kg; Mg: 14.1 g/kg; P: 76 g/kg; Mn: 382 mg/kg; Zn: 1231 mg/kg; Fe: 373 mg/kg; Cu: 373 mg/kg; Co: 49 mg/kg; I: 36 mg/kg; Se: 5 mg/kg; F: 106 mg/kg; monensin: 800 mg/kg; vitamin A: 89 992 IU/kg; non-protein nitrogen: 88.8%; CP: 91.1%.

²The DM content was adjusted with water to 65%.

Carcass gain was calculated using a linear equation to predict the initial HCW of the remaining animals. The equation was applied to the initial BW to determine a predicted initial HCW, as follows (equation (1)):

$$\text{HCW}_{\text{initial}} = -38.9 \pm 30.4 + 0.613 \pm 0.065 \\ \times \text{BW}_{\text{initial}} (R^2 = 0.978; \text{RMSE} = 9.08) \quad (1)$$

where $\text{HCW}_{\text{initial}}$ is the initial HCW (kg) and $\text{BW}_{\text{initial}}$ is the initial BW (kg).

After 97 days of the experiment and 8 h of fasting, all remaining animals were slaughtered following the same procedure as that of the baseline groups, and the final HCW was obtained. Carcass-adjusted gain was determined by subtracting the final HCW from the initial HCW. Carcass-adjusted feed efficiency was calculated by dividing carcass gain (kg/day) by DMI.

Carcass-adjusted final BW was calculated from HCW divided by the average dressing percent of all treatments. Carcass-adjusted average daily gain (ADG) ((final BW – initial BW)/97) and feed efficiency (ADG/DMI) were calculated.

Organs and histopathology

After animals were harvested, the liver and kidneys of all animals were weighed, and samples for color and histopathology examination were collected. For histopathology, kidney samples were obtained from the cortical, medullar and pelvic layers (renal papilla). Liver samples were obtained from the caudate lobe, diaphragmatic surface and left lobe. The kidney and liver samples had a maximum thickness of 0.5 cm and were fixed in buffered formalin solution. After fixation, the samples were stained with hematoxylin and eosin. The assessment of lesions/tissue changes was based on the methodology described by Kraieski *et al.* (2017). The liver and kidney colors (L^* = lightness; a^* = redness; b^* = yellowness) were also analyzed.

Statistical analyses

The experiment was conducted in a randomized complete block design with a 2×2 factorial arrangement of treatments. The pen was considered the experimental unit, and the block was considered the random effect (eight blocks; one replication per block; eight pens per treatment; three animals per pen). For all statistical analyses, the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) was used with the fixed effects of contamination, ADS and the contamination \times ADS interaction.

The analysis of blood and DMI was submitted to ANOVA as repeated measures over time using mixed models, using the REPEATED statement of SAS (SAS Institute Inc.). The day (or week for DMI) was considered the repeated measure, and the subject was considered the pen. For blood variables, the initial collection was considered a covariate. Different residual covariance structures were tested (15 covariance structures) to determine the structure that best fits each variable. The covariance structure was chosen using the

Bayesian information criteria, and the lowest value was considered to be the best fit.

Differences were considered significant when $P < 0.05$, while a trend was considered when $0.05 \leq P \leq 0.10$.

Results

Dry matter intake, performance and carcass traits

There was no interaction ($P = 0.92$) between contaminated diets and the use of ADS on DMI (Table 3). However, there was a tendency ($P = 0.09$) of 5.14% lower DMI in animals fed the diets with exogenous contamination compared to that in animals fed the natural diets. When analyzed as repeated measures (weekly), there was an interaction between diet and period ($P = 0.01$). The DMI of animals fed the EC diets was lower ($P = 0.01$) than that of animals fed NC diets during 6 weeks of the whole study period (Figure 1).

The final BW was affected by the contaminated diets ($P = 0.04$). Animals fed the NC diets were 14.5 kg heavier than animals fed the EC diets (Table 3). Similarly, the ADG was affected by the contaminated diets ($P = 0.04$). Animals fed the natural contamination diets had 1.71 kg/day of ADG compared to the 1.57 kg/day of animals fed the exogenous contamination diets.

There was an interaction between contaminated diets and ADS for dressing percentage ($P = 0.04$) and carcass feed efficiency ($P = 0.02$), and there was a tendency of interaction among factors for final HCW ($P = 0.10$) and carcass gain ($P = 0.08$; Table 3). Animals fed the NC diets had a 1.78-fold greater dressing percentage than that of animals fed the EC diets. However, animals fed the NC + ADS and EC + ADS diets had similar dressing percentages compared to those of animals fed the NC and EC diets. Animals fed the NC and EC + ADS diets had greater carcass feed efficiency than those fed EC. Animals fed the NC + ADS diet had a similar carcass feed efficiency compared to that of animals fed the NC, EC + ADS and EC diets. The HCW of animals fed the NC was 14 kg greater than that of animals fed the EC diets. Finally, the highest carcass gain was observed for animals fed the NC diets, and the lowest was observed for those fed the EC diets. Moreover, the NC + ADS and EC + ADS treatments presented intermediate values, and they did not differ significantly from those of the NC and EC treatments.

Metabolic variables

The serum enzymes AST and GGT were not different among factors ($P \geq 0.19$; Table 4). However, these enzymes were affected by the day of collection ($P < 0.01$). The two enzymes presented higher concentrations in the serum at the end of the experiment ($P < 0.01$). On average, AST and GGT were present at 74.6 and 30.7 U/l, respectively, at the end of the experiment compared with 60.0 and 23.3 U/l, respectively, at the beginning of the study.

Serum concentrations of total protein, total cholesterol and triglycerides did not present interactions among factors ($P \geq 0.17$; Table 4). However, the concentrations of urea and

Table 3 Performance and efficiency of Nellore cattle finished in feedlot fed diets with natural mycotoxin contamination and exogenous contamination with or without adsorbent

Items	Treatments				SEM	P-values		
	Natural		Exogenous			Myc	ADS	Myc × ADS
	Without	With	Without	With				
Mycotoxin contamination diet (Myc)								
Adsorbent (ADS; 1 g/kg of DM)								
Performance: live basis								
Initial BW (kg)	431	430	430	429	4.81	–	–	–
Final BW (kg) ¹	602	590	576	587	8.03	0.04	0.94	0.11
DM intake (kg/day)	12.7	12.6	12.0	12.0	0.38	0.09	0.88	0.92
DM intake (% of BW)	2.46	2.47	2.39	2.36	0.07	0.16	0.90	0.78
ADG (kg)	1.77 ^a	1.65 ^{ab}	1.51 ^b	1.63 ^{ab}	0.06	0.04	0.95	0.08
Feed efficiency (kg/kg)	0.139 ^a	0.131 ^{ab}	0.126 ^b	0.136 ^a	0.004	0.26	0.72	0.02
Performance: carcass basis								
Initial HCW (kg)	225	225	225	224	–	–	–	–
Final HCW (kg)	341 ^a	335 ^{ab}	327 ^b	333 ^{ab}	4.55	0.04	0.94	0.10
Dressing (%)	57.2 ^a	56.6 ^{ab}	56.2 ^b	56.8 ^{ab}	0.30	0.17	0.93	0.04
Carcass gain (kg/day)	1.20 ^a	1.14 ^{ab}	1.05 ^b	1.12 ^{ab}	0.04	0.04	0.94	0.08
Carcass feed efficiency (kg/kg)	0.094 ^a	0.090 ^{ab}	0.088 ^b	0.094 ^a	0.002	0.50	0.67	0.02

Myc = effect of the diet; ADS = effect of presence or absence of adsorbent; Myc × ADS = interaction between diet and adsorbent; ADG = average daily gain; HCW = hot carcass weight.

¹FinalBW is calculated from hot carcass weight divided by the average dressing percent of all treatments; DM intake in % of BW, ADG and feed efficiency were calculated through the adjusted final BW.

^{a,b}Values within a row with different letters differ significantly at $P < 0.05$.

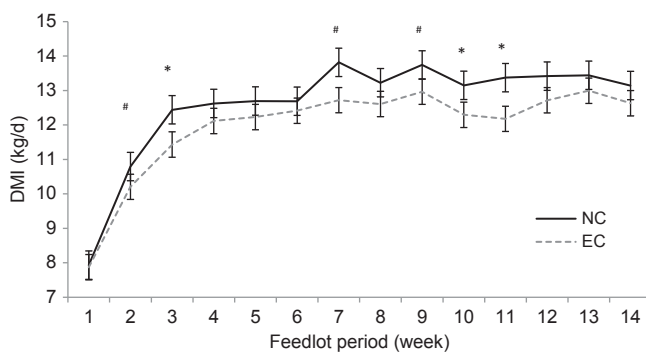


Figure 1 Dry matter intake of Nellore cattle fed diets with natural mycotoxin contamination and exogenous contamination, with or without yeast-derivate adsorbent (ADS), measured weekly during feedlot. Mycotoxin $P = 0.06$, ADS $P = 0.86$, mycotoxin × ADS interactions $P = 0.91$, period $P < 0.01$, mycotoxin × period interactions $P = 0.01$, ADS × period interactions $P = 0.94$, mycotoxin × ADS × period interactions $P = 0.78$. DMI = DM intake; NC = diet with natural contamination; EC = diet with exogenous contamination; *Indicated significant difference among diets ($P < 0.05$). #Indicated a tendency of significant difference among diets ($0.05 \leq P \leq 0.10$).

creatinine tended to interact among factors ($P = 0.08$; Figure 2). Urea was greater for the NC + ADS and EC diets than for the NC and EC + ADS diets. In contrast, creatinine was greater for the NC diets than for the other treatments. There was an interaction between diet contamination and experimental period for urea and creatinine ($P \leq 0.04$). The values of urea increased by 9.10 mg/dl for the NC diets and 1.25 mg/dl for the EC diets at the end of the experiment. Creatinine concentrations of animals fed the NC diets increased from 1.85 mg/dl on day 14 to 1.88 mg/dl on day 96; however, animals fed the EC diets had similar values over time (1.85 mg/dl).

There was no interaction between contaminated diets and ADS for liver weight ($P = 0.78$) and kidney weight ($P = 0.11$; Table 5). However, kidney weight tended to be lower with the use of ADS in the diets ($P = 0.09$). There was no effect of contamination in the diets, ADS usage or an interaction among factors on the histopathology of the liver ($P \geq 0.33$) and kidneys ($P \geq 0.14$).

The results for organ color (Table 5) did not present interactions ($P \geq 0.10$) between diets and ADS. However, the L^* color value of the kidneys was greater for animals fed diets without the ADS than for those fed diets with ADS supplementation. In addition, the b^* color value of the kidneys decreased for animals fed the contaminated diets and for those administered the adsorbent ($P < 0.01$).

Discussion

This study shows the effects of mycotoxins and a yeast-based adsorbent on beef cattle performance. There are few studies examining the effects of mycotoxins on beef cattle (Merrill *et al.*, 2007; Tagaki *et al.*, 2011); however, these previous studies evaluated isolated mycotoxins. Multiple toxins are normally found in the diet (Zain, 2011; Custodio *et al.*, 2019), and mycotoxins can act synergistically in animals (Yiannikouris and Jouany, 2002).

In the present study, it was observed that exogenously contaminated diets affected the DMI during almost the entire experimental period. Although not measured, it is hypothesized that this may be due to metabolic disruptions in the rumen and intestine of cattle fed the EC diet. According to Santos and Fink-Gremmels (2014), mycotoxins can impair rumen function and impair the metabolism of carbohydrates.

Table 4 Blood parameters of Nellore cattle finished in feedlot fed diets with natural mycotoxin contamination and exogenous contamination with or without adsorbent

Items	Day	Treatments				SEM	P-values					
		Natural		Exogenous			Myco	ADS	Myco × ADS	D	Myco × D	ADS × D
AST (U/l)	14	58.6	64.6	56.1	60.8	1.93	0.34	0.67	0.29	<0.01	0.82	0.15
	28	63.5	62.2	59.4	62.8	2.32						
	96	80.5	71.0	71.9	75.0	4.63						
GGT (U/l)	14	23.3	24.1	22.2	23.5	1.28	0.59	0.81	0.19	<0.01	0.26	0.45
	28	24.3	23.0	23.2	25.0	0.53						
	96	30.9	29.0	31.4	31.7	1.11						
TP (g/dl)	14	7.37	7.22	7.32	7.40	0.18	0.72	0.62	0.79	0.03	0.93	0.71
	28	7.10	7.26	7.30	7.19	0.18						
	96	6.89	7.11	6.93	7.04	0.18						
Urea (mg/dl)	14	32.7	32.9	36.9	29.1	2.61	0.30	0.78	0.08	<0.01	0.03	0.14
	28	27.5	31.3	31.5	32.0	1.89						
	96	38.7	45.1	34.4	34.1	3.31						
CR (mg/dl)	14	1.85	1.85	1.85	1.86	0.01	0.73	0.89	0.08	0.05	0.04	0.94
	28	1.87	1.85	1.85	1.87	0.01						
	96	1.88	1.87	1.85	1.86	0.01						
TC (mg/dl)	14	114	88.7	89.9	89.7	13.0	0.35	0.61	0.38	0.79	0.74	0.20
	28	88.6	94.5	85.4	95.2	5.39						
	96	92.1	91.0	92.5	88.6	4.52						
TR (mg/dl)	14	6.71	7.80	8.02	9.95	0.95	0.97	0.22	0.17	0.43	0.20	0.77
	28	8.48	9.62	7.35	7.95	1.50						
	96	10.5	7.99	7.31	10.7	1.19						

Myco = effect of the diet; ADS = effect of presence or absence of adsorbent; Myco × ADS = interaction between diet and adsorbent; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; TP = total protein; CR = creatinine; TC = total cholesterol; TR = triglycerides; D = effect of the day; Myco × D = interaction between diet and day; ADS × D = interaction between adsorbent and day. Three-way interaction (diet, adsorbent and day) was not significant ($P \geq 0.18$).

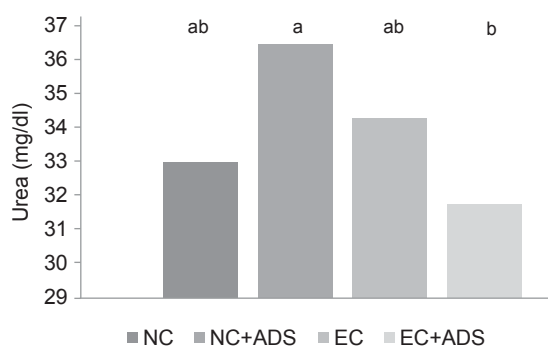


Figure 2 Urea concentration (mg/dl) in blood of Nellore cattle fed diets with natural mycotoxin contamination and exogenous contamination, with or without yeast-derivate adsorbent. Mycotoxin $P = 0.30$, ADS $P = 0.78$, mycotoxin × ADS interaction $P = 0.08$. NC = diet with natural contamination; EC = diet with exogenous contamination; ADS = presence of adsorbent (1 g/kg of DM). ^{a,b}Values differ significantly at $P < 0.05$.

Additionally, Gallo *et al.* (2015) declared that some mycotoxins have antimicrobial activity and are able to reduce microbial activity in the rumen and intestine, which can decrease the degradation rate, NDF digestibility, rumen mobility and microbial protein and affect the DMI. However, these data still scarce (Gallo *et al.*, 2015). Wang *et al.* (2019) declared that aflatoxins can affect the ruminal concentrations of

acetate, propionate, butyrate, valerate, isovalerate and isobutyrate in dairy cows. Nevertheless, such effects can cause economic losses. Moreover, mycotoxins can affect the function of vital organs and cause immunosuppression (Tagaki *et al.*, 2011); however, in the present study, the relatively chronic mycotoxin challenge did not impact metabolic organs, perhaps due to the low exogenous contamination diet.

Dry matter intake strongly influences animal performance. Thus, the final BW and ADG were affected by different DMI values and by impairments in the digestive tract. According to Santos and Fink-Gremmels (2014), if animals are exposed long term to mycotoxins, an impairment of the rumen microbiota is likely to occur, reducing feed efficiency, which may be explained by the antimicrobial activity of some mycotoxins. Additionally, mycotoxins can impact intake or feed refusal and nutrient absorption and impair metabolism (Rodrigues, 2014). In this way, mycotoxin contamination of the diet reduced the growth rate and, consequently, feed efficiency, and the ADS was able to recover part of this damage. Likewise, Merrill *et al.* (2007) studied the use of ADS and observed that increasing the amount of yeast cell wall in the diet increased milk production of Angus × Hereford cows consuming high-ergot-alkaloid tall fescue.

In this study, the lower DMI values arguably explain part of the reduction in performance of animals fed the EC diet

Table 5 Weight, histopathology, and colors of liver and kidney of Nelore cattle finished in feedlot fed diets with natural mycotoxin contamination and exogenous contamination with or without adsorbent

Items ¹		Treatments				SEM	P-values		
Mycotoxin contamination diet (Myco)	Color ²	Natural		Exogenous			Myco	ADS	Myco × ADS
Adsorbent (ADS; 1 g/kg of DM)		Without	With	Without	With				
Liver/100 kg of carcass kg		2.17	2.17	2.13	2.15	0.04	0.40	0.82	0.78
Kidneys/100 kg of carcass kg		0.31	0.31	0.32	0.29	0.01	0.92	0.09	0.11
Total tissue scores of liver		45.6	47.1	47.0	53.5	40.5	0.34	0.33	0.54
Total tissue scores of kidneys		51.3	62.3	61.2	58.9	43.2	0.46	0.32	0.14
Liver	<i>L</i> *	26.6	27.0	26.3	27.0	0.69	0.84	0.37	0.83
	<i>a</i> *	14.4	14.5	13.9	14.3	0.36	0.20	0.36	0.47
	<i>b</i> *	11.3	11.9	11.2	11.7	0.44	0.69	0.16	0.97
Kidney	<i>L</i> *	28.0	26.3	27.9	25.4	0.70	0.40	<0.01	0.49
	<i>a</i> *	14.6	14.2	14.6	13.6	0.33	0.41	0.05	0.40
	<i>b</i> *	12.3	10.9	11.7	9.09	0.36	<0.01	<0.01	0.10

Myco = effect of the diet; ADS = effect of presence or absence of adsorbent; Myco × ADS = interaction between diet and adsorbent; liver/100 kg of carcass = liver for 100 kg of carcass; kidneys/100 kg of carcass = kidneys for 100 kg of carcass.

¹Total tissue scores of liver and total tissue scores of kidneys = stained with hematoxylin and eosin (HE), magnification ×200 of an optical microscope.

²*L** = lightness; *a** = redness; *b** = yellowness.

compared to that of those fed the NC diet, and maybe the other part was caused by damage to animal metabolism (Wang *et al.*, 2019). Analyzing the beef cattle requirements (BR-CORTE; Valadares Filho *et al.*, 2016) and DMI to reach the observed final BW suggests that the different DMI in treatments explains approximately 60% of the difference in growth rate, and the other 40% is probably due to other factors such as rumen motility, NDF digestibility and degradation rate (Gallo *et al.*, 2015). Moreover, some mycotoxins have antimicrobial activity, as explained above, and this can damage the digestibility and metabolism of nutrients impairing animal performance.

The ADS did not increase the DMI, which may be the reason it recovered only part of the loss in growth rate caused by the toxins. However, the ADS, by chemically interacting with the mycotoxins, prevented these toxins from damaging the metabolism, for example, causing subacute rumen acidosis, thereby allowing animals to recover some of the lost performance caused by the EC diet.

Yeast cell walls and their constituents have been shown to adsorb a large spectrum of mycotoxins *in vitro* (Yiannikouris *et al.*, 2006) and *in vivo* to alleviate the effects of dietary mycotoxin exposure in several species (Diaz *et al.*, 2004; Meissonnier *et al.*, 2009). However, the efficacy of this adsorbent depends on the type of mycotoxin (Patience *et al.*, 2014). Kong *et al.* (2014) studied yeast cell wall activity *in vitro* and observed adsorption of 92.7% aflatoxin but only 22.9% adsorption of DON. Other factors can influence the adsorbent effects *in vivo*, including the level of diet contamination, type of diet and adsorption rate (Kong *et al.*, 2016).

The partial performance recovery of ADS was reflected in ADG and carcass gain. No data were found in the literature related to the carcass weight of beef cattle fed mycotoxin-contaminated diets and adsorbents. However, Rossi *et al.* (2010) observed a lower dressing percentage and breast weight for chickens fed diets contaminated with aflatoxin

B1 than those of chickens fed uncontaminated diets. However, Rossi *et al.* (2010) detected an improvement in dressing percentage when a similar ADS to the one used in the present study was added to the diet. This can be explained by the ability of the ADS to interact with mycotoxins, inactivating them.

The ADS (β -d-glucan fraction of yeast cell wall) is involved in the mycotoxin binding, and its structure modulates the attachment strength (Yiannikouris *et al.*, 2004a). Hydrogen and van der Waals bonds have been evidenced in glucan-mycotoxin complexes, and they are stable at the pH level of the digesta in the entire gastrointestinal tract and could contribute to proving the reliability of these adsorbents against mycotoxins (Yiannikouris *et al.*, 2004b; Jouany, 2007).

Regarding metabolic variables, ruminants fed mycotoxin-contaminated diets normally have elevated serum metabolites, including urea, protein, AST and GGT (Chaiyotwittayakun, 2010). In addition, enzyme tests are widely used as indicators of the effect of chemical or toxicological substances in the liver. Elevated serum enzyme activity of diagnostic liver enzymes (AST and GGT) can be a sign of intoxication, suggesting hepatocellular injury (Fink-Gremmels, 2008). The fact that the levels of AST and GGT were unaltered among treatments in this study may indicate that there was no liver damage in these animals.

The non-alteration of enzymes linked to hepatic metabolism may be related to the low contamination of diets, even in the EC diet, and a relatively short exposure. Osweiler *et al.* (1993) observed that doses above 150 ppm of fumonisin B1 caused hepatic alterations in cattle. The authors reported that the liver is only affected in the case of high contamination levels in cattle. In this study, the higher dose of this toxin was 5754 ppb or 5.754 ppm. In another study with beef cattle, Garrett *et al.* (1968) observed liver alteration with doses above 100 ppb aflatoxin B1, which was higher than the doses observed in this study (10 ppb).

Thus, mycotoxins are normally metabolized in the liver and kidneys, especially aflatoxins, which are powerful hepatotoxins and are reported to cause liver necrosis (Ashiq, 2015). In this sense, it is interesting to verify the integrity of these organs through weight, color and histopathology.

In contrast to data found in the literature, the organ weight in this study did not show interactions between diet contamination and ADS usage. As mentioned before, this can probably be explained by the low level of contamination implemented in this study. On the other hand, Ledoux *et al.* (1998), studying chicks fed aflatoxin B1, observed heavier liver, heart, kidney, proventriculus and pancreas weights, whereas chicks fed the combination aflatoxin B1 + ADS diet had similar organ weights to those fed negative control diets. Similarly, Fernandez *et al.* (1997) observed lambs fed 2.5 ppm aflatoxins for 21 days and saw that they presented hepatic and nephritic injuries and increased weight and size of liver and kidney.


According to Ledoux *et al.* (1998), aflatoxins cause a variety of effects in poultry organs, such as liver pathology with pale characteristics. In a study conducted by Kumar and Balachandran (2009), the livers from broilers fed 1 ppm aflatoxin for 28 days showed enlargement, pallor or yellowish discoloration, and their kidneys were enlarged and pale. In our study, the *b** color value of the kidneys was affected by both the contaminated diet and ADS factors, but we have no explanation for this. The *L** value was also affected by the ADS. This result indicates that the kidneys of animals fed the ADS were pale compared to those of animals fed without the ADS. These differences may be due to the role of the kidneys in the metabolism. Kidneys are responsible for filtering the blood. However, we highlight that further studies are needed.

Regarding organ histopathology, some authors have documented that contaminated diets can cause liver lesions and multiorgan damage, including necrosis and hemorrhage in pigs (Chen *et al.*, 2008). Kumar and Balachandran (2009) observed histopathological degenerative and necrotic changes in the liver and kidneys of broilers fed 1 ppm aflatoxin. In this study, the less-severely contaminated diets and ADS did not affect these variables. We highlight that monogastric animals are more susceptible to mycotoxins than ruminants.

In conclusion, under the conditions of this experiment, mycotoxins affect beef cattle performance, and the inclusion of an ADS in the diet allows for the partial recovery of performance when animals are fed exogenously contaminated diets.

Acknowledgements

The authors would like to thank Luiz Fernando Costa e Silva, Anne Koontz and Rebecca Delles for their assistance and all contributions. This study was made possible by grants from the São Paulo Research Foundation (FAPESP) no. 2015/21416-6 (first author), no. 2018/20176-0 (second author) and no. 2017/50339-5 (PDIP). The work was conducted as part of the first author's dissertation (Leticia Custodio).

 L. F. Prados 0000-0001-5142-6819

Declaration of interest

The authors declare no conflicts of interest.

Ethics statement

The experiment was conducted at the Agência Paulista de Tecnologia dos Agronegócios (APTA) in Colina, SP, Brazil, following the guidelines for animal well-being provided by the São Paulo state in Brazil. All procedures and protocols involving the use of animals were approved by the ethics committee on animal use of the Universidade Estadual Paulista (protocol number 15473/15).

Software and data repository resources

None of the data were deposited in an official repository.

References

- Association of Official Analytical Chemists (AOAC) 1995. Official methods of analysis, 16th edition AOAC, Arlington, VA, USA.
- Ashiq S 2015. Natural occurrence of mycotoxins in food and feed: Pakistan perspective. *Comprehensive Reviews in Food Science and Food Safety* 14, 159–175.
- Chaiyotwittayakun A 2010. Mycotoxins and health in dairy cattle. In Proceedings of the 13th Association of Institutions for Tropical Veterinary Medicine (AITVM) Conference, 23–26 August 2010, Bangkok, Thailand, pp. 221–223.
- Chen F, Ma Y, Xue C, Ma J, Xie Q, Wang G, Bi Y and Cao Y 2008. The combination of deoxynivalenol and zearalenone at permitted feed concentrations causes serious physiological effects in young pigs. *Journal of Veterinary Science* 9, 39–44.
- Custodio L, Prados LF, Yiannikouris A, Holder VB, Pettigrew JE, Kuritz LN, Resende FD and Siqueira GR 2019. Mycotoxin contamination of diets for beef cattle finishing in feedlot. *Revista Brasileira de Zootecnia* 48, 1–12.
- Diaz DE, Hagler Junior WM, Blackwelder JT, Eve JA, Hopkins BA, Anderson KL, Jones FT and Whitlow LW 2004. Aflatoxin binders. II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia* 157, 233–241.
- Fernandez A, Belio R, Ramos JJ, Sanz MC and Saez T 1997. Aflatoxins and their metabolites in the tissues, faeces and urine from lambs feeding on an aflatoxin-contaminated diet. *Journal of the Science Food and Agriculture* 74, 161–168.
- Fink-Gremmels J 2008. Mycotoxins in cattle feeds and carry-over to dairy milk: a review. *Food Additives and Contaminants Part A* 25, 172–180.
- Gallo A, Giuberti G, Frisvad JC, Bertuzzi T and Nielsen KF 2015. Review on mycotoxin issues in ruminants: occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. *Toxins* 7, 3057–3111.
- Garrett WN, Heitman H and Booth AN 1968. Aflatoxin toxicity in beef cattle. *Proceedings of the Society for Experimental Biology and Medicine* 127, 188–190.
- Jouany JP 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. *Animal Feed Science and Technology* 137, 342–362.
- Kong C, Park CS and Kim BG 2016. Evaluation of a mycotoxin adsorbent in swine diets containing barley naturally contaminated with *Fusarium* mycotoxins. *Revista Colombiana de Ciências Pecuária* 29, 169–177.
- Kong C, Shin SY and Kim BG 2014. Evaluation of mycotoxin sequestering agents for aflatoxin and deoxynivalenol: an in vitro approach. *SpringerPlus* 3, 346.
- Kraieski AL, Hayashi RM, Sanches A, Almeida GC and Santin E 2017. Effect of aflatoxin experimental ingestion and Eimeria vaccine challenges on intestinal histopathology and immune cellular dynamic of broilers: applying an Intestinal Health Index. *Poultry Science* 96, 1078–1087.
- Kumar R and Balachandran C 2009. Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid. *Veterinarski Arhiv* 79, 31–40.
- Ledoux DR, Rottinghaus GE, Bermudez AJ and Alonso-Debolt M 1998. Efficacy of hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chickens. *Poultry Science* 77, 204–210.

- Meissonnier GM, Raymond I, Laffitte J, Cossalter AM, Pinton P, Benoit E, Bertin G, Galtier P and Oswald IP 2009. Dietary glucomannan improves the vaccinal response in pigs exposed to aflatoxin B1 or T-2 toxin. *World Mycotoxin Journal* 2, 161–172.
- Merrill ML, Bohnert DW, Harmon DL, Craig AM and Schrick FN 2007. The ability of a yeast-derived cell wall preparation to minimize the toxic effects of high-ergot alkaloid tall fescue straw in beef cattle. *Journal of Animal Science* 85, 2596–2605.
- Mertens DR 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. *Journal of AOAC International* 85, 1217–1240.
- National Academies of Sciences, Engineering, and Medicine (NASEM) 2016. Nutrient requirements of beef cattle model, 8th revised edition. National Academy Press, Washington, DC, USA.
- Osweiler GD, Kehrl ME, Stabel JR, Thurston JR, Ross PF and Wilson TM 1993. Effects of fumonisin-contaminated corn screenings on growth and health of feeder calves. *Journal of Animal Science* 71, 459–466.
- Patience JF, Myers AJ, Ensley S, Jacobs BM and Madson D 2014. Evaluation of two mycotoxin mitigation strategies in grow-finish swine diets containing corn dried distillers grains with solubles naturally contaminated with deoxynivalenol. *Journal of Animal Science* 92, 620–626.
- Rodrigues I 2014. A review on the effects of mycotoxins in dairy ruminants. *Animal Production Science* 54, 1155–1165.
- Rossi P, Rutz F, Lima GJMM, Nunes JK, Anciuti MA, Moraes PVD, Silva JGC, Silveira MHD and Maier JC 2010. Efeito do adsorvente a base de glucomamano esterificado no desempenho e caracterização visceral de frangos de corte. *Revista Brasileira de Agrociência* 16, 91–100.
- Santos RR and Fink-Gremmels J 2014. Mycotoxin syndrome in dairy cattle: characterisation and intervention results. *World Mycotoxin Journal* 7, 357–366.
- Tagaki M, Uno S, Kokushi E, Shiga S, Mukai S, Kuriyagawa T, Takagaki K, Hasunuma H, Matsumoto D, Okamoto K, Shahada F, Chenga T, Deguchi E and Fink-Gremmels J 2011. Measurement of urinary zearalenone concentrations for monitoring natural feed contamination in cattle herds: on farm trials. *Journal of Animal Science* 89, 287–296.
- Upadhaya SD, Park MA and Ha JK 2010. Mycotoxins and their biotransformation in the rumen: a review. *Asian-Australasian Journal of Animal Science* 23, 1250–1260.
- Valadares Filho SC, Costa e Silva LF, Gionbelli MP, Rotta PP, Marcondes MI, Chizzotti ML and Prados LF 2016. Nutrient requirements of Zebu and Crossbred cattle (BR-CORTE), 3rd edition. Suprema gráfica e editora, Visconde do Rio Branco, MG, Brazil.
- Wang Q, Zhang Y, Zheng N, Guo L, Song X, Zhao S and Wang J 2019. Biological system responses of dairy cows to aflatoxin b1 exposure revealed with metabolomic changes in multiple biofluids. *Toxins* 11, 77–99.
- Yiannikouris A 2015. Holistic approach for the identification, risk assessment and mitigation of mycotoxins' impact in ruminant. *Journal of Animal Science* 59, 306–307. (Abstr.)
- Yiannikouris A, Andre G, Poughon L, Françoes J, Dussap CG, Jeninet G, Bertin G and Jouany JP 2006. Chemical and conformational study of the interactions involved in mycotoxin complexation with β -d-glucans. *Biomacromolecules* 7, 1147–1155.
- Yiannikouris A, Francois JEAN, Poughon L, Dussap CG, Bertin G, Jeminet G and Jouany JP 2004a. Alkali extraction of β -D-glucans from *Saccharomyces cerevisiae* cell wall and study of their adsorptive properties toward zearalenone. *Journal of Agricultural and Food Chemistry* 52, 3666–3673.
- Yiannikouris A, Andre G, Buleon A, Jeminet G, Canet I, Francois J, Bertin G and Jouany JP 2004b. Comprehensive conformational study of key interactions involved in zearalenone complexation with β -d-glucans. *Biomacromolecules* 5, 2176–2185.
- Yiannikouris A and Jouany JP 2002. Mycotoxins in feeds and their fate in animals: a review. *Animal Research* 51, 81–99.
- Zain ME 2011. Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society* 15, 129–144.