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Effect of aflatoxin B_1 on digestibility and blood parameters in horses

Digestibilidade e efeitos hematológicos da aflatoxina B_1 em equinos

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Abstract
This study aimed to evaluate the effect of aflatoxin B_1 (AFB_1) on the digestibility of nutrients and the hematological profile of horses. The assay lasted 40 days, with 12 days for adaptation and 28 days for experimentation. In the experimental stage, the horses were distributed in a completely randomized design, including three treatment groups with four animals in each group. The treatments used included 0 µg/kg (control), 50 µg/kg, and 100 µg/kg of AFB_1 added to a concentrate in the basal diet. The basal diet contained mycotoxins from naturally contaminated feed. A digestibility essay was carried out at the end of the experimental period through partial feces collection, with LIPE® as an indicator. Blood samples were collected once a week during the assay for hematological and biochemical evaluations. The results of the hematological and biochemical parameters were submitted to analysis of variance (ANOVA) and compared by the Tukey test at 5% significance. The aflatoxins in the diet increased the leukocyte count, especially that of mature neutrophils. Creatine kinase and alkaline phosphatase ($P < 0.05$) activities were higher in horses fed more toxic diets. The digestibility of nutrients was unaffected by the level of mycotoxins in the diet ($P > 0.05$).
Keywords: digestibility, equine, hematology, mycotoxin

Resumo
Objetivou-se com esta pesquisa avaliar o efeito da Aflatoxina B_1 (AFB_1) na digestibilidade dos nutrientes e no perfil hematológico de equinos. O ensaio durou 40 dias, sendo 12 dias para adaptação e 28 dias para experimentação. No período experimental, os cavalos foram distribuídos em delineamento inteiramente casualizado, com três tratamentos, com quatro animais cada. Os tratamentos utilizados foram 0 µg/kg (controle), 50 µg/kg e 100 µg/kg de AFB_1 adicionados ao concentrado da dieta basal. A dieta basal continha alimentos naturalmente contaminados com micotoxinas. Um ensaio de digestibilidade foi realizado no final do período experimental, pelo método de coleta parcial de fezes utilizando o LIPE® como indicador. Amostras de sangue foram coletadas uma vez por semana, durante o ensaio para avaliações hematológicas e bioquímicas. Os resultados dos

parâmetros hematológicos, bioquímicos e do ensaio de digestibilidade foram submetidos à análise de variância (ANOVA) e comparados pelo teste de Tukey a 5% de significância. As aflatoxinas da dieta aumentaram a contagem de leucócitos, principalmente os neutrófilos maduros. A creatina quinase e a fosfatase alcalina (P<0,05) apresentaram maior atividade nos equinos alimentados com dietas com maior toxicidade. A digestibilidade dos nutrientes não foi alterada pelos níveis de micotoxinas presentes nas dietas (P>0,05).
Palavras-chave: digestibilidade, equinos, hematologia, micotoxina

Introduction

Horse diets comprise pasture, hay, and cereals in a nutritional concentrate. These feeds are naturally contaminated by mycotoxin-producing fungi, before, during, or after harvest and during storage. Mycotoxins are secondary metabolites of low molecular weight, produced mainly by fungi belonging to the genus Fusarium and Aspergillus. Mycotoxins, especially aflatoxins, are potentially dangerous genotoxins that damage the DNA damage and lead to the development of diseases in animals and humans[1].

Horses are susceptible to mycotoxin intoxication, especially to fumonisins. Among herbivores, equines are more susceptible than ruminants whose ruminal microbiota acts as a barrier against the toxic metabolites. In horses, the small intestine is the primary site of absorption of xenobiotic compounds prior to being fermented in the large intestine, making them more susceptible to mycotoxins than ruminants[2].

Keller et al.[3] analyzed samples of different commercial concentrates, oats, and feed produced on farms in Rio de Janeiro and reported that Aspergillus (43%), Penicillium (26%), and Fusarium (11%) were the most frequently isolated fungal genera. These authors recommended establishing maximum limits of fungal counts for potential aflatoxin and fumonisin producing species in diets and feeds for horses.

Although mycotoxins have an adverse effect human and animal health, there are no uniform limits or regulations for mycotoxins in different countries, including the MERCOSUR (Brazil, Uruguay, and Argentina)[4]. This reinforces the need to study the toxicological effects of mycotoxins in animal species. Notably, only Brazilian has legislated a maximum limit of 50 μg kg^{-1} for aflatoxins in animal feed; however no limits have been established for other mycotoxins[5]. This study evaluated the effect of aflatoxin B_1 **(**AFB_1) on the digestibility of nutrients and the hematological profile of horses.

Materials and methods

The research was carried out at the Army Sergeant School in Três Corações, MG, Brazil, and was approved by the Committee on Ethics in Animal Experimentation (Protocol No. 1212031017).

The 40-day experimental period was divided into two stages: the pre-experimental or adaptation stage of 12 days and the experimental stage of 28 days. A total of 12 adult

Brazilian sport horses (9 females and 3 males), aged between 5 and 8 years old, with an average body weight of 458.02 ± 22.09 kg were enrolled in the study, which was conducted in a completely randomized design, including three treatments with four animals each. The animal was the experimental unit for all comparison. The experimental treatments used were: treatment I = control diet (0 µg/kg of AFB_1); treatment II = diet with 50 µg/kg of AFB_1; and treatment III = diet with 100 µg/kg AFB_1. AFB_1 was added to the basal diet (Table 1).

Table 1. Composition of experimental diets containing different concentrations of AFB_1 (g kg^{-1} as feed)

Diet components	Treatments		
	T I	T II	T III
Tifton/alfalfa hay (g)	7.400	6.900	6.600
Commercial concentrate (g)	4.000	4.000	4.000
Concentration of mycotoxins in the diet (µg)			
Aflatoxin B_1	0	50	100
Zearalenone	2.758,1	2.687,5	2.655,2
Desoxynivalenol	21.564,7	20.689,9	20.289,0
Fumonisins	2.240,0	2.240,0	2.240,0
Total of daily diet (g)	11.400	10.900	10.600

The experimental basal diet was composed of tifton-85 hay (*Cynodon* spp.) and alfalfa (*Medicago sativa*), commercial concentrate (S Line -280, Royal Horse®), mineral salt, and water ad libitum, elaborated according to the NRC[6] for mature horses. The horses remained free in their paddocks during the day and were housed in individual stalls at night. The animals were fed according to the schedule of the Military Unit, with 2 kg of concentrate meal at 0500 h and 2 kg at 1700 h, alfalfa hay at 1000 h, and tifton-85 hay at 1300 h and 2000 h. During the experimental period, 1 kg of concentrate feed with AFB_1 was prepared and given to the animals at 1700 h, according to the treatment group. All feeds were analyzed for mycotoxins in the basal diet before the adaptation period and were found to contain 5.620 µg/kg of deoxynivalenol (DON), 708 µg/kg of zearalenone (ZEA), and 560 µg/kg of FB_1. The mycotoxins were quantified through high performance liquid chromatography (HPLC), by the Mycology Laboratory of Federal University of Minas Gerais.

AFB_1 was produced by the Mycology Laboratory of Federal University Rural of Rio de Janeiro by fermenting rice with a spore suspension of *Aspergillus parasiticus* (NRRL 2999), according to Shotwell et al.[7]. AFB_1 was extracted and purified through a Mycosep® Aflazon column (Romer Labs Diagnostic GmbH., Tulln, Austria), as per the manufacturer's instructions, and an aliquot (200 µL) was derivatized with 700 µL trifluoroacetic acid:acetic acid:water (20:10:70, v/v). The derivatized aflatoxin was quantified by HPLC, at the Mycology and Mycotoxins Laboratory of Federal University of Minas Gerais, according to Trucksess et al.[8].

Aflatoxins were quantified by injecting 20 µL of the extract from each vial into an HPLC system (JASCO model LC-2000 pump, Tokyo, Japan) connected to a JASCO model FP-2020 programmable fluorescence detector and a data module JASCO model 6937-J041A (ChromNAV). Chromatographic separations were performed on a stainless steel, C18 reversed-phase column (Supelcosil™ LC-ABZ, 150 × 4.6 mm^2, 5 µm particle size) connected to a precolumn (Supelguard™ LC-ABZ, 20 × 4.6 mm^2, 5 µm particle size). The mobile phase was water:methanol:acetonitrile (4:1:1, v/v/v), at a flow rate of 1.0 mL min^{-1}. The fluorescence of aflatoxin derivatives was recorded at the excitation and emission wavelengths of 360 nm and 460 nm, respectively. Standard curves were constructed using different concentrations of AFB_1, which was quantified by correlating peak heights of sample extracts with those of standard curves. The detection limit of the analytical method was 0.4 ng g^{-1}.

AFB_1 yield was 4.25 mg/kg and it was diluted daily in the concentrate feed to obtain the desired concentration, according to the treatment group.

At the end of the experimental period, the animals were submitted to the digestion test by the partial fecal collection method, using LIPE® as an external indicator, according to the methodology described by Saliba[9] and Saliba et al.[10] **.** For six days, from the 21st to the 26th day of the experimental period, a 500 mg capsule of LIPE® was administered at 1700 h.

After 48 hours, feces was collected directly from the rectal ampulla, at the same time for five consecutive days. At the end of the collection period, the daily samples per animal formed a composite sample, which was frozen at −18ºC. The concentration of LIPE in the feces was determined using infrared spectroscopy and fecal production was estimated by using the following equation:

$$\text{Fecal Production (PF, g/day) } += \frac{\text{(Amount of marker ingested by the animal,g)}}{\text{(Concentration of LIPE in feces, g/\%MS)}}$$

The samples of feces and feed were analyzed for dry matter, nitrogen, and ether extract, according to methodology described by Silva and Queiroz[11] at the Animal Nutrition Laboratory of UFMG. Acid detergent fiber and neutral detergent fiber analyses were carried out according to the methodology described by Van Soest et al.[12]. The digestibility of the nutrients was estimated using the formula:

$$\text{Apparent Digestibility (\%)} = \frac{\text{(Nutrient ingested - Nutrient excretedin feces)}}{\text{(Nutrient ingested)}} \text{ x } 100$$

Before, during, and after the experimental period, the clinical, hematological, and biochemical analyses were carried out to check the horses' health, especially for liver damage. Throughout the experimental period, the clinical conditions of the animals were monitored daily by a veterinarian.

Over a four-week period, blood samples were collected at 0800 h once a week, starting on the first day of the experiment, with the control sample being the first. The other samples were collected on the 7th, 14th, 21st, and 28th days and were used to evaluate the health of the animals through hematology and for monitoring their renal and hepatic functions. For the hematological evaluation, the blood samples were collected in a vacuum tube with

EDTA. The globular volume (GV) was evaluated using the microhematocrit method and blood count was done using an automatic counter (Horiba ABX Micros 60®). The following analyses were performed: global leukocyte count; red blood cell count, determination of hemoglobin levels; calculation of mean corpuscular volume (MCV); calculation of the corpuscular hemoglobin concentration mean; and concentration of lymphocytes, monocytes, and mature neutrophils.

For the blood chemistry evaluations, the samples were collected in vacuum tubes containing sodium fluoride for glucose analysis and in vacuum tubes without anticoagulant for the analysis of cholesterol, urea, creatinine, γ-glutamyl transferase (GGT), creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP). After collection, the samples were centrifuged at 7,000 rpm for 10 min to separate the plasma from serum. Thereafter, 1.0 mL aliquots of both plasma and serum were automatically pipetted and stored at −20 °C in 1.5 mL polypropylene tubes, until further analysis. These analyses were performed using the enzymatic or colorimetric method using an automated spectrophotometric analyzer (Labmax Progress, Labtest Diagnostica SA, Lagoa Santa, Brazil). Diagnostic kits (Labtest®) were used for all analyses that were performed at the Biochemistry Laboratory of the Army Sergeant School.

The results were evaluated for normality and homogeneity using the Lilliefors test and the Cochran–Bartlett test, respectively. Data for nutrient digestibility and blood variables were evaluated using analysis of variance (ANOVA) in a split-plot design and the averages were compared using the Tukey test at 0.05 significance level, using the System of Statistical and Genetic Analysis - SAEG[13].

Results

The aflatoxin levels in the diets did not influence dry matter intake and nutrient digestibility ($P > 0.05$) (Table 2).

Table 2. Intake and average coefficients of apparent digestibility of nutrients in horses fed different concentrations of AFB_1 (on dry matter basis)

Variables	Treatments			Mean	SD	CV (%)
	Basal	50 µg kg^{-1}	100 µg kg^{-1}			
Dry matter intake[1]	2.5	2.3	2.4	2.4	0.2	6.8
Dry matter digestibility	74.0	74.7	75.0	74.6	0.7	0.8
Crude protein digestibility	73.9	73.7	74.2	73.9	2.0	3.0
Ether extract digestibility	81.5	81.8	82.6	81.9	1.1	1.3
Neutral detergent fiber digestibility	73.3	73.6	73,7	73.5	0.3	0.4
Acid detergent fiber digestibility	69.0	68.0	67.1	68.0	2.0	2.9

[1]% of BW

Abbreviations: BW, body weight; SD, standard deviation; CV, coefficient of variation

Table 3. Mean hematology values for horses fed different concentrations of AFB_1 for 28 days

Variables	Average	CV (%)	P-value			Reference values
			AFB_1	Time	AFB_1 x Time	
Globular Volume (%)	33.8	5.9	0.334	0.001	NS	38–42[1]
Erythrocytes (10^6 μL^{-1})	7.5	5.9	NS	0.001	NS	7–11[1]
Hemoglobin (g dL^{-1})	11.4	5.5	0.253	0.000	NS	11–17[1]
MCV (fL)	45.4	0.9	NS	0.003	NS	42–47[1]
MCHC (%)	33.7	1.2	NS	0.000	NS	31–38.6[2]
Total leukocytes (10^3 μL^{-1})	7.9	8.5	0.078	0.000	0.325	6–11[1]
Lymphocytes (10^3 μL^{-1})	3.5	16.3	NS	0.003	0.124	2–5.5[1]
Monocytes (10^3 μL^{-1})	1.4	17.7	NS	0.028	0.025	0.2–0.8[1]
Mature neutrophils (10^3 μL^{-1})	2.9	12.3	0.000	0.001	0.027	2.3–8.6[3]

[1]Hodgson et al.[14]; [2]Carlson[15]; [3]Robinson and Sprayberry[16]

Abbreviations: CV: coefficient of variation; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration

Table 4. Mean concentration of monocytes and mature neutrophils in horses fed different concentrations of AFB_1 for 28 days

Time (days)	Treatments		
	Control	50 µg kg^{-1}	100 µg kg^{-1}
Monocytes [1]			
Zero	1.47^{Aab}	1.42^{Aa}	1.55^{Aab}
7	1.08^{Ab}	1.52^{Aa}	1.40^{Aab}
14	1.62^{Aa}	1.40^{Aa}	1.65^{Aa}
21	1.10^{Aa}	1.27^{Aa}	1.38^{Aab}
28	1.52^{Aab}	1.50^{Aa}	1.07^{Ab}
Mature neutrophils[2]			
Zero	2.3^{Bab}	3.25^{Aab}	3.62^{Aa}
7	1.8^{Cab}	2.8^{Bb}	4.2^{Aa}
14	2.7^{Ba}	2.85^{ABab}	3.67^{Aa}
21	2.35^{Ba}	3.55^{Aa}	4.02^{Aa}
28	1.57^{Cb}	2.52^{Bb}	3.70^{Aa}
P-value			
	AFB_1	Time	AFB_1 x Time
Monocytes	NS	0.028	0.025
Mature neutrophils	0.000	0.001	0.027

Averages in the rows followed by different uppercase letters differed in the Tukey test ($P < 0.05$)

Averages in the column followed by different lowercase letters differed in the Tukey test ($P < 0.05$)

[1]Coefficient of variation (CV): 17.6 %

[2]CV: 12.3 %

The hematological results obtained in the weekly blood samples showed an interaction with time for all variables evaluated ($P < 0.05$) (Table 3). AFB_1 had a direct effect on the concentration of mature neutrophils. Moreover, AFB_1 affected the concentration of monocytes and mature neutrophils as a function of time ($P < 0.05$). The other hematological variables were not affected by AFB_1 $P > 0.05$).

The mean concentration of total leukocytes and lymphocytes was not affected by AFB1 in the diet. However, AFB1 influenced the concentrations of monocytes and mature neutrophils (P < 0.05) (Table 4). The monocyte concentration was influenced by the collection times and by the AFB1 concentration, over time. (P < 0.05). However, the presence of AFB1 alone was not sufficient to alter this concentration (P > 0.05).

Irregular changes in monocyte concentration were observed for horses in treatment groups I and III. However, the interaction of AFB_1 with time was clearer in treatment group III, with a reduction in monocyte concentration during the experimental period.

The concentration of mature neutrophils was directly influenced by the presence of AFB_1 in the diet, by the collection times alone and also by the AFB_1 interaction during that time ($P < 0.05$).

The horses belonging to treatment group III had a higher mean concentration of mature neutrophils than the other groups; however, the response was not linear, with the concentration of mature neutrophils at 28 days less than that after 21 days of treatment.

The serum biochemistry results (Table 5) demonstrated that there was effect of time on all variables ($P < 0.05$). AFB_1 over time did not affect the variables ($P > 0.05$) and except for the activity of CK and ALP, there was no direct effect of AFB_1 for the other variables ($P > 0.05$).

Table 5. Mean values of serum biochemistry for horses fed different concentrations of AFB_1 for 28 days

Variables	Average	CV (%)[5]	P-value			Reference values
			AFB_1	Time	AFB_1 x Time	
Total protein (g dL^{-1})	5.33	22.39	0.073	0.000	0.133	5.2–7.9[2]
Albumins (g dL^{-1})	2.11	22.98	NS	0.000	0.144	2.6–3.7[2]
Globulins (g dL^{-1})	3.21	22.44	0.092	0.000	0.126	2.6–4.0[2]
Cholesterol (mg dL^{-1})	86.18	20.25	0.211	0.000	0.090	75–150[1]
Urea (mg dL^{-1})	29.46	20.40	0.243	0.000	NS	22–42[4]
Creatinine (mg dL^{-1})	0.95	14.65	0.245	0.000	NS	1.2–1.8[4]
GGT (U L^{-1})	7.73	21.81	0.268	0.182	0.331	9–26[4]
AST (U L^{-1})	288.73	24.01	0.058	0.208	NS	197–454[4]
CK (U L^{-1})	295.42	32.41	0.023	0.000	0.440	116–290[4]
LDH (U L^{-1})	845.17	37.97	0.090	0.319	NS	162–412[2]
ALP (U L^{-1})	67.68	20.93	0.012	0.026	NS	100–500[3]

[1]Robinson and Sprayberry[16]; [2]Gonzalez and Silva[17]; [3]Kerr[18]; [4]Padilha et al[19]; [5]Coefficient of variation

Serum activity of CK and ALP were influenced by the AFB_1 concentration, which was higher in the treatment group III (Table 6).

Table 6. Mean values of creatine kinase (UI/L) and alkaline phosphatase (UI/L) for horses fed different concentrations of AFB_1 for 28 days

Time (days)	Treatments			Mean
	Control	50 µg kg^{-1}	100 µg kg^{-1}	
Creatine Kinase (UI/L)[1]				
Zero	176.5	291.5	223.5	230.5^{CD}
7	215.5	296.0	357.0	289.5^{BC}
14	136.5	164.2	258.5	186.4^{D}
21	316.2	331.5	337.5	328.4^{B}
28	349.8	498.8	478.2	442.3^{A}
Average	238.90^{B}	316.4^{AB}	330.9^{A}	-
Alkaline phosphatase (UI/L)[2]				
Zero	52.7	66.5	71.0	63.4^{AB}
7	58.3	67.8	94.7	73.6^{AB}
14	43.3	50.3	84.3	59.3^{B}
21	65.2	76.0	91.3	77.5^{A}
28	56.2	60.2	77.5	64.6^{AB}
Average	55.2^{B}	64.2^{AB}	83.7^{A}	-
P-value				
	AFB_1	Time	AFB_1 × Time	
Creatine Kinase	0.023	0.000	0.440	
Alkaline phosphatase	0.012	0.026	NS	

Averages in the column or row followed by the same letters, do not differ in the Tukey test ($P > 0.05$);
[1] Coefficient of Variation (CV): 32.4%; [2] CV: 20.9%

Discussion

The average dry matter intake was approximately 24.0 g kg^{-1}d^{-1} of the body weight, which is suitable for horses with an average weight of 450 kg, according to the NRC[6]. The presence of mycotoxins in the experimental diets, and the possible interactions between them were not enough to alter the intake and the apparent digestibility coefficients of the studied nutrients. Feed refusal was not observed, indicating that under the conditions of this study there were no significant changes in the analyzed variables.

These results are similar to those of Khol-Parisini et al.[20], who observed a delay in

the intake of contaminated feed, with no impact on voluntary foraging. The horses consumed 2 kg of oats/d, containing 20.2 mg/kg of DON, which corresponded with a daily intake between 6.9 to 9.5 mg/100 kg of weight over 14 days. Similarly, Schulz et al.[21] did not observe adverse effects on the health of horses fed hay that was naturally contaminated with DON at concentrations ranging from 9.37 to 18.6 mg/kg in the DM over a 21-day period, which corresponded to a daily intake of < 5–75 mg/kg of body weight. In this study, the mean daily intake of mycotoxins in µg per kg/body weight ranged from 0 to 0.2 for AFB_1, 4.8 to 5.0 for FB_1, 5.7 to 6.1 for ZEA, and 43.9 to 47.6 for DON. Although the levels of mycotoxins per kg/body weight used in this study were low, the length of the experimental period may not have been enough to affect the metabolism of the animals, considering the variables studied.

AFB_1 did not affect the GV, erythrocyte concentration, hemoglobin levels, and MCV and MCHC values, and only affected the collection times for these variables ($P < 0.05$). The values were within the physiological limits for the equine species[16-18].

The immunogenic effect of AFB_1 and trichothecenes, such as DON, have been investigated alone or in combination on white blood cells[22,23]. The higher concentration of mature neutrophils in treatment groups II and III suggests that the immunological response to AFB_1 is concentration dependent. However, the weekly average between treatment groups I and III showed no significant difference. A reduction in the concentration of mature neutrophils was observed for all three groups at the end of 28 days, and it was significant in treatment groups I and II.

DON can induce liver damage and decrease the immune response[24,25], this response may be greater in animals receiving a diet with higher AFB_1 concentrations due to a possible additive or synergistic effect. However, horses fed higher doses of mycotoxin presented a high concentration of mature neutrophils, suggesting that the horses were tolerant to the adverse effects of mycotoxin. This finding is consistent with that of Caloni and Cortinovis[26] and Cortinovis et al.[27], who verified that horses fed with barley naturally contaminated with 36–44 mg kg^{-1} DON for 40 days did not show refusal of feed or alterations of biochemistry and blood hematology parameters. Caloni and Cortinovis[26] reviewed experimental AFB_1 intoxication in horses and reported that although inconclusive, aflatoxicosis can occur at concentrations between 0.5 and 1.0 mg/kg (dry matter). These concentrations induce clinical changes and hepatic damage depending on the length of exposure.

Higher levels of AFB_1 were expected to have some effect on hepatic and renal function[1,28]. However, the maximum amount of AFB_1 ingested by horses was 9.4 µg/kg DM for 28 days, suggesting that under the conditions studied the amount ingested by the animals may not have been sufficient to cause any changes in the parameters evaluated, not even when other mycotoxins, such as FB, were present in the diet[29]. The concentrations of urea and creatinine underwent changes over time ($P < 0.05$). The highest concentrations of urea and creatinine were observed after 21 days. Although significantly higher than the samples collected at other times, the concentrations remained within the reference parameters for horses[19].

AFB_1 was not expected to influence CK activity. Muscle injury is indicated by elevated

levels of AST and CK[19,30]. Notably, other mycotoxins, especially FB_1, were also present in the diet and could have interacted. FB_1 affects sphingolipid metabolism and can alter cell permeability[31,32], which could explain the elevated CK activity. On the other hand, alterations between collections may be due to variations in the collection and processing of samples, resulting in higher values for day 28[33]. However, the averages remained within the physiological reference values for horses[18].

ALP is widely distributed in the body, with a predominant hepatic isoform in the serum[34]. The higher ALP activity observed in treatment group III may be related to the hepatotoxic action of mycotoxins in the diet[22,35,36]. The mean ALP values in the weekly collections were not as expected. The highest mean value was observed at the end of 21 days. Trueman et al.[37] reported that an increase in ALP activity is often unexplained and that variations in results may be influenced by sample collection and processing. Our results are within the reference range (0–599 IU/L) for horses. During the experimental period no pathological alterations in the bone, liver, or gastrointestinal system were observed.

Conclusions

The mycotoxins did not affect nutrient digestibility under the conditions used. AFB_1 increased leukocytes concentrations, especially that of mature neutrophils, suggesting a greater immunological response to toxic diets. Higher serum CK and ALP activity was observed in horses fed a 100 μg kg^{-1} AFB_1 diet, possibly due to the hepatotoxicity of mycotoxins in the feed.

Mycotoxins are a silent threat to animals, especially horses. Thus, that appropriate precautions should be taken to protect these animals.

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Conflict of interests

The authors declare no conflict of interest.

References

1. Buszewska-Forajta M. Mycotoxins, invisible danger of feedstuff with toxic effect on animals. Toxicon (Oxford), 2020;182:34–53.

2. Fink-Gremmels J, Spronk L. Hygienic quality of feed: implications of feed contamination with moulds and mycotoxins on equine health and performance. In: European Equine Health and Nutrition Congress. Belgium: European Equine Health and Nutrition Congress.; 2013. p. 63–7.

3. Keller KM, Queiroz BD, Keller LAM, Ribeiro JMM, Cavaglieri LR, González-Pereyra ML, Dalcero AM, Rosa CAR. The mycobiota and toxicity of equines feeds. Vet Res Commun. 2007;(31):1037–45.

4. Anvisa/MS. Resolução de Diretoria Colegiada – RDC No 07, de 18 de fevereiro de 2011. Ministério da Saúde - MS Agência Nac Vigilância Sanitária - ANVISA. 2011;2011:8.

5. Ministério da Agricultura Pecuária e Abastecimento (MAPA). Portaria MAPA/SNAD/SFA no 7, de 09 de novembro de 1988. Diário Of da União. 1988;09 nov.(Seção 1):21.968.

6. National Research Council - NRC. Nutrient requirements of horses. Washington, D.C: National Academies Press, 6a ed Rev; 2007. p. 341.

7.Shotwell OL, Hesseltine CV, Stubblefield RD, Sorenson WG. Production of aflatoxin on rice. Appl Microbiol. 1966;(14):425–9.

8.Trucksess MW, Stack ME, Nesheim S, Albert R, Romer T. Multifunctional column coupled with liquid chromatography for determination of aflatoxins B1, B2, G1 and G2 in corn, almonds. Brazil nuts, peanuts, and pistachio nuts: Collaborative study. J AOAC Int. 1994;77:1512–21.

9. Saliba EOS. Uso de indicadores: Passado, presente e futuro. Proceedings of Conference of Markers on Animal Nutrition.2005. Belo Horizonte, Brazil, pp. 4–22.

10. Saliba EOS, Faria EP, Rodriguez NM, Moreira GR, Sampaio, I.B.M.; Saliba ,J.S.; Gonçalves, L.C.; Borges, I.; Borges, A.L.C. Use of Infrared Spectroscopy to Estimate Fecal Output with Marker Lipe® Case Study. Int J Food Sci Nutr Diet.2015. S4:001, 1-10.

11.Silva DJ, Queiroz A. Análise de alimentos: métodos químicos e biológicos. 3a. Universidade Federal de Viçosa, editor. UFV-Viçosa-MG; 2002. 235 p.

12.Van Soest PJ, Robertson JB, Lewis B. Development of a comprrehensive sytem of feed analyses and its application to forages. J Anim Sci. 1991;74:3583–97.

13. Sistema de análises estatísticas e genéticas - SAEG. Viçosa -MG: Universidade Federal de Viçosa; 2007. p. 301.

14. Hodgson DR, McKeever KH and McGowan CM. The athletic horse: principles and practice of equine sports medicine. In: Hodgson, D.R.; Rose RJ, editor. Hematology and Biochemistry. 2nd ed. Philadelpia: Elsevier Saunders; 2014. p. 63–79.

15. Carlson GP. Coleta de amostras e interpretação dos exames laboratoriais. In: Smith B., editor. Tratado de medicina interna de grandes animais. São Paulo: Manole; 1993. p. 395–424.

16. Robinson NE, Sprayberry KA. Current Therapy in Equine Medicine. 6a. St Louis (USA): Saunders; 2009.

17. Gonzalez FHD, Silva SC. Introdução a Bioquímica Clínica Veterinária. 2a. Porto Alegre - RS: UFRGS; 2006. 364 p, il.

18. Kerr MG. Veterinary Laboratory Medicine: Clinical Biochemistry and Haematology. 2a. Oxford-England: Blackwell Science; 2002. 392 p.

19. Padilha FGF, Dimache LAG, Almeida FQ, Ferreira AMR. Blood biochemical parameters of Brazilian sport horses under training in tropical climate. R Bras Zootec. 2017;46(8):678–82.

20. Khol-Parisini A, Hellweg P, Razzazi-Fazeli E, Saalmüller A, Strasser A, Tichy A, Zentek J. Highly

deoxynivalenol contaminated oats and immune function in horses. Arch Anim Nutr. 2012;66(2):149–61.

21. Schulz AK, Kersten S, Dänicke S, Coenen M, Vervuert I. Effects of deoxynivalenol in naturally contaminated wheat on feed intake and health status of horses. Mycotoxin Res. 2015;31(4):209–16.

22. Bryden WL. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Anim Feed Sci Technol. 2012;173:134–58. Available from: http://dx.doi.org/10.1016/j.anifeedsci.2011.12.014

23. Pierron A, Alassane-Kpembi I, Oswald IP. Impact of mycotoxin on immune response and consequences for pig health. Anim Nutr. 2016;2:63–8.

24. Placinta CM, D'mello CP, Macdonald AM. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Anim Feed Sci Technol. 1999;78:21–37.

25. Peng Z, Chen L, Nüssler AK, Liu L, Yang W. Current sights for mechanisms of deoxynivalenol□induced hepatotoxicity and prospective views for future scientific research: A mini review. J Appl Toxicol. 2016; Available from: https://doi.org/10.1002/jat.3428

26. Caloni F, Cortinovis C. Toxicological effects of aflatoxins in horses. Vet J. 2011;188:270–3. Available from: http://dx.doi.org/10.1016/j.tvjl.2010.06.002

27. Cortinovis C, Battini M, Caloni F. Deoxynivalenol and T-2 Toxin in Raw Feeds for Horses. J Equine Vet Sci. 2012;32:72–4. Available from: http://dx.doi.org/10.1016/j.jevs.2011.08.002

28. Riet-Correa F, Rivero R, Odriozola E, Adrien ML, Medeiros RMT, Schild AL. Mycotoxicoses of ruminants and horses. J Vet Diagnostic Investig. 2013;25:692–708.

29. Maziero MT, Bersot LS. Micotoxinas em alimentos produzidos no Brasil. Rev Bras Prod Agroindustriais. 2015;12(1):89–99.

30. Fazio F, Assenza A, Tosto F, Casella S, Piccione G, Caola G. Training and haematochemical profile in Thoroughbreds and Standardbreds: A longitudinal study. Livest Sci. 2011;141:221–6. Available from: http://dx.doi.org/10.1016/j.livsci.2011.06.005

31. Ramasamy S, Wang E, Hennig B, Merrill AH. Fumonisin B1alters sphingolipid metabolism and disrupts the barrier function of endothelial cells in culture. Toxicology and Applied Pharmacology. 1995;133:343–8.

32. Abdul NS, Marnewick J.L. Fumonisin B1□induced mitochondrial toxicity and hepatoprotective potential of rooibos: An update. J Appl Toxicol. 2020; Available from: https://doi.org/10.1002/jat.4036

33. Sloet van Oldruitenborgh-Oosterbaan MM. Appropriate blood variables helpful in diagnosing (sub) clinical disease in the horse. In: Lindner A, editor. Conference on Equine Sports Medicine and Science. Cordoba, Spain: Wageningen Academic Publishers; 1998. p. 14–33.

34. Zaher DM, Mohammed IG, Omar HA, Aljareh SN, Al□Shamma SA, Ali AJ, Zaib Z, Iqba J. Recent advances with alkaline phosphatase isoenzymes and their inhibitors. Arch Pharm. 2020;353. Available from: https://doi.org/10.1002/ardp.202000011

35. Voss KA, Smith GW, Haschek WM. Fumonisins: Toxicokinetics, mechanism of action and toxicity. Anim Feed Sci Technol. 2007;137:299–325.

36. Kamle M, Mahato D K, Devi S, Lee KE, Kang SG, Kumar P. Human Health and their Management Strategies. Toxins (Basel). 2019;1–23.

37. Trueman KF, Lumsden JH, Mcsherry BJ. Examination of the origin of increased equine serum alkaline phosphatase concentrations. Can Vet J.1983;24:108–11.