UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

IDENTIFICATION AND CONTROL EFFECTS OF MYCOTOXINS IN NELLORE BULLS FINISHED IN FEEDLOT

M.Sc. Letícia Custódio

Animal Scientist

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TÍTULO DA TESE: IDENTIFICATION AND CONTROL EFFECTS OF MYCOTOXINS IN NELLORE BULLS FINISHED IN FEEDLOT

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DADOS CURRICULARES DA AUTORA

Letícia Custódio, nascida em 23 de março de 1988, em Barretos, São Paulo. Filha de Jaime José Custódio e Sueli Aparecida Ferreira Custódio. Graduada em Zootecnia pelo Centro Universitário da Fundação Educacional de Barretos, em Barretos - SP (2006-2010) sob a orientação da Professora Doutora Marcella de Toledo Piza Roth. Desenvolveu estágio extracurricular durante a graduação na Agência Paulista de Tecnologia dos Agronegócios – APTA, em Colina – SP (2007-2010), sob a orientação do professor Gustavo Rezende Siqueira. Em 2010 desenvolveu o estágio curricular na University of Florida em Ona – FL, EUA, sob a orientação do professor João Vendramini. Mestre em Ciência Animal e Pastagens pela Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo, em Piracicaba - SP (2011-2013) na área de Conservação de forragens e bovinos leiteiros, sob a orientação do Professor Doutor Luiz Gustavo Nussio. Em fevereiro de 2015 ingressou no Curso de Doutorado em Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista, em Jaboticabal – SP, sob a orientação do Professor Doutor Gustavo Rezende Siqueira.

“O destino não é uma questão de sorte, é uma questão de escolha. Não é algo para se esperar, é algo para se conquistar”.

William Jennings Bryan

A Deus, à Nossa senhora Aparecida

e à minha família,

Dedico!!!

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SUMMARY

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"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Projeto intitulado "**Caracterização e controle de micotoxinas na alimentação de bovinos de corte confinados",** protocolo nº 15473/15, sob a responsabilidade do Prof. Dr. Gustavo Rezende Siqueira, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de junho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL -SP, em reunião ordinária de 14 de setembro de 2015.

Vigência do Projeto	Maio 2016 a Janeiro/2017
Espécie / Linhagem	*Bos indicus* / Nelore
Nº de animais	196
Peso / Idade	340 Kg / 20 meses
Sexo	Machos
Origem	Agropecuária Imperial

Jaboticabal, 14 de setembro de 2015.

Profª Drª Paola Castro Moraes
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
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IDENTIFICATION AND CONTROL EFFECTS OF MYCOTOXINS IN NELLORE BULLS FINISHED IN FEEDLOT

ABSTRACT: The study was divided into two phases. The first phase was designed to identify which mycotoxin was present in Brazillian feedlot diets, and the second phase was designed to evaluate the performance of Nellore bulls finished in feedlot fed mycotoxins, and the effects of mycotoxin adsorbent (ADS). Thus, the objective of the first phase was to identify which mycotoxins were present in ingredients used in diets fed to feedlot cattle and its concentrations. The survey covered 30 Brazillian feedlots located in the 5 largest beef-producing states. Samples of total mixed ration (TMR) and ingredients were collected on-site and sent to the 37+® Analytical Services Laboratory (KY, USA) for analysis of mycotoxins. The quantification of 38 different mycotoxins was performed using ultra-performance liquid chromatography coupled to tandem mass spectrometry. 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 (REQ) expressed in µg/kg of aflatoxin B1-equivalent. Descriptive statistics were obtained using the UNIVARIATE procedure of SAS and multivariate statistics were obtained using STATISTICA. The toxins identified in TMR were: fumonisins, trichothecenes A, trichothecenes B, fusaric acid, aflatoxins and ergot (means of 2,330, 104.3, 79.5, 105, 10.5, and 5.5 µg/kg, respectively). Fumonisins were the primary mycotoxins found and at highest concentrations in TMR samples. Peanut meal was the most contaminated ingredient. The objective of the second phase was to evaluate the effect of mycotoxins and ADS on performance and meat quality of Nellore cattle finished in feedlot. One-hundred 24-mo-old Nellore bulls (430 ± 13 kg of body weight (BW)) were used in a randomized block design with a 2 × 2 factorial arrangement of treatments. The factors consisted of two diets (Factor 1) with natural contamination (NC) or exogenous contamination (EC) and presence (10g/d/animal; ADS) or absence of ADS (Factor 2). The NC and EC diets had, respectively, the following contaminations: aflatoxin 0 and 10 µg/kg, fumonisin 5114 and 5754 µg/kg, trichothecenes A 0 and 22.1 µg/kg, trichotecenes B 0 and 42.1 µg/kg and fusaric acid 42.9 and 42.9 µg/kg. At the beginning of the experiment, all

animals were weighed, and 4 were randomly selected to be slaughtered to evaluate initial carcass weight. After 97 days of experiment, all animals were weighed and slaughtered. Steaks from *Longissumus thoracics* harvested between 11th to 13th ribs, in which three steaks were randomly assigned to aging times of 7, 14 and 28 days at 4°C. The meat quality was analyzed. There was no interaction among factors for DMI (P = 0.92), however there was a tendency for EC- diets decrease DMI by 650 g/d (P = 0.09). The ADG was greater for NC- when compared to EC- fed cattle (P = 0.04) and there was a tendency for interaction among factors (P = 0.08) being 1.77, 1.65, 1.51 and 1.63 kg for NC-, NC+ADS, EC- and EC+ADS, respectively. There was a tendency for interaction among factors for carcass gain (P = 0.08). Daily carcass gain was 1.20, 1.14, 1.05 and 1.12 kg/d, respectively, for cattle receiving NC-, NC+ADS, EC- and EC+ADS. Then, the NC had greater carcass gain compared to EC- and the addition of ADS recovered part of the gain when used in EC diets. The chemical composition, color, cooking loss and shear force of meat were not affected ($P \geq 0.38$) by the factors. In conclusion, mycotoxin affects the performance of beef cattle, and the ADS may alleviate part of this damage when animals were fed diets containing higher contamination. However, these factors did not negatively affect meat quality.

Key words: adsorbent, carcass gain, meat quality, performance, survey

CARACTERIZAÇÃO E CONTROLE DE MICOTOXINAS NA ALIMENTAÇÃO DE BOVINOS CONFINADOS

RESUMO: O experimento foi dividido em duas fases. A primeira fase foi desenvolvida para identificar quais micotoxinas estavam presentes nas dietas de confinamentos brasileiros e a segunda fase foi para verificar o desempenho de bovinos confinados alimentados com micotoxinas e efeito do Mycosorb A^{+} ADS. Assim, o objetivo da primeira fase foi identificar quais micotoxinas e em quais concentrações estavam presentes em ingredientes utilizados em dietas de bovinos de cortes confinados. A pesquisa abrangeu 30 confinamentos brasileiros localizados em 5 diferentes estados. Amostras de ração total (RT) e ingredientes foram coletadas no local e enviadas para o 37+® Analytical Services Laboratory (KY, USA) para análise de micotoxinas. A quantificação de 38 micotoxinas diferentes foi realizada utilizando cromatografia líquida de ultra-desempenho acoplada a espectrometria de massa. As concentrações de micotoxinas foram interpretadas de acordo com sensibilidades específicas de espécies conhecidas e normalizadas de acordo com os princípios de fatores equivalentes tóxicos, determinando o Risk Equivalency Quantities (REQ) expressas em µg/kg equivalente de aflatoxina B1. Estatísticas descritivas foram obtidas utilizando o procedimento UNIVARIATE do SAS e estatísticas multivariadas foram obtidas utilizando o STATISTICA. As toxinas identificadas nas RT foram: fumonisinas, tricotecenos A, tricotecenos B, ácido fusárico, aflatoxinas e ergot (média de 2330; 104,3; 79,5; 105; 10,5 e 5,5 µg/kg). As fumonisinas foram as micotoxinas mais encontradas e em maiores concentrações nas amostras de RT. O amendoim foi o ingrediente mais contaminado. O objetivo da segunda fase foi avaliar o efeito de micotoxinas e ADS sobre o desempenho e a qualidade da carne de bovinos confinados. Foram utilizados 100 bovinos (430 ± 13 kg de peso corporal (PC) e 24 meses). O delineamento foi em blocos casualizados, em esquema fatorial 2 × 2 de tratamentos. Os tratamentos consistiram de dois fatores: Fator 1: RT com contaminação natural (CN) ou contaminação exógena (CE) e Fator 2 presença (10g/d/animal; ADS) ou ausência do ADS. As dietas CN e CE apresentaram, respectivamente, as seguintes contaminações: aflatoxina 0 e 10 µg/kg, fumonisina 5114 e 5754 µg/kg, tricotecenos A 0 e 22,1 µg/kg, tricotecenos B 0

e 42,1 µg/kg e ácido fusárico 42,9 e 42,9 µg/kg. No início do experimento, todos os animais foram pesados e 4 animais selecionados aleatoriamente foram abatidos para avaliar o peso inicial da carcaça. Após 97 dias de experimento, todos os animais foram pesados e abatidos. Bifes do *Longissumus thoracics* foram retirados entre a 11ª e a 13ª costelas, sendo três bifes aleatoriamente designados para tempos de maturação de 7, 14 e 28 dias a 4°C. A qualidade da carne foi analisada. Não houve interação entre os fatores para o consumo de matéria seca (CMS); (*P* = 0,92), porém houve tendência de redução para dietas CE- em 650 g/dia (*P* = 0,09). O ganho médio diário (GMD) foi maior para CN- em relação ao CE- (*P* = 0,04) e houve tendência de interação entre fatores (*P*=0,08) sendo 1,77, 1,65, 1,51 e 1,63kg para CN-, CN+ADS, CE-, CE+ADS, respectivamente. Os animais da CN- apresentaram maior PC final (596 kg) do que CE- (582 kg, *P* = 0,04). Houve tendência de interação entre os fatores para ganho de carcaça (*P* = 0,08). O ganho médio diário de carcaça foi de 1,20, 1,14, 1,05 e 1,12 kg, respectivamente, para CN-, CN+ADS, CE-, CE+ADS. Assim, o CN- apresentou maior ganho de carcaça em relação ao CE- e, além disso, o ADS recuperou parte do ganho quando usado em dietas CE. A composição química, cor, perda por cocção e maciez da carne não foram afetadas (*P* ≥ 0,38) pelos fatores. Em conclusão, a micotoxina afeta o desempenho de bovinos de corte, e o ADS pode recuperar parte desse dano quando os animais consomem dietas com uma contaminação mais alta. Porém, esses fatores não afetam a qualidade da carne.

Palavras-chave: adsorvente, desempenho, ganho de carcaça, levantamento de dados qualidade de carne

CHAPTER 1 - GENERAL CONSIDERATIONS

1. INTRODUCTION

Health issues related to the production system intensification may frequently occur, and these problems can cause disorders to the animals and impact the immune system, performance and consequently increase production costs. Some feedstuff contaminants can cause serious damage to animals, such as mycotoxins (Friend et al., 1992; Merril et al., 1996; Blank et al., 2003; Iqbal et al., 2013). Fungi and mycotoxins can occur in feedstuffs used in beef cattle, such as grains, silages, hay and by-products (Mallmann et al., 2009) and, this issue becomes important regarding feedlot diets. If losses related to mycotoxins in animal feeds were scaled, the range of economic losses would be huge in Brazil (Trail et al.,1995; Jobim et al., 2001).

Mycotoxins are substances naturally produced by fungi and usually a form of microorganisms defense (Jouany, 2001). Most natural feedstufs are susceptible to contamination. The fungi growth is typically stimulated by environmental factors, as high temperature and humidity, both pre and post-harvest (Binder et al., 2007). However, toxin production is dependent on factors such as microbial competition, nutrient availability and substrate structure, water activity, pH, temperature, relative humidity, presence of bugs, and application of fungicides and pesticides (Hameed et al., 2013; Anfossi et al., 2016). However, the occurrence of fungal growth does not indicate the presence of mycotoxins (Cheeke and Shull, 1985).

Late harvest may aggravate mycotoxin production in the crop, because the longer grain stays in the field, the more susceptible to stress factors it becomes (Duncan et al., 1994). In addition, no-proper storage of grains or forages may also allow fungi growth and mycotoxins (Motta et al., 2015). According to Santos and Fink-Gremmels (2014), the mycotoxin problem may be related to preharvest infestation of cereals and grains by toxinogenic *Fusarium* species, as well as post-harvest contamination of stored/ensiled materials by *Penicillium (P. roqueforti* and *P. carnosum a.o.)* and *Aspergillus* species. Thus, contamination can be avoided through good management practices, but it is difficult to ensure that all material coming from the field is contamination free.

The effects generated by mycotoxins on animals depend on the amount, time of exposure and synergistic action (Smith and Korosteleva, 2010). These effects may be reproductive, immunological and performance disorders (Mallmann et al., 2009).

Most of mycotoxin studies evaluated its effects on monogastric animals, since these animals are more susceptible to the toxic effects of mycotoxins compared to ruminant (Di´az-Llano and Smith, 2014; Kong et al., 2016). In ruminants, the harmful effects of mycotoxin may be less aggressive, because ruminal microorganisms can inactivate some of these compounds (Upadhaya et al.,2010). However, it is not all mycotoxins that are inactivated in the rumen and, in addition, they may affect ruminal microorganisms due to their antibiotic effect (Fink-Gremmels, 2008) and others can be transformed in products more dangerous than the mycotoxin. According to Marczuk et al. (2014), some mycotoxins have antibacterial properties, they modify the ruminal microflora and minimize detoxicating effects of ruminal digesta.

A serious consequence of the mycotoxins may be related to animal products, such as meat, milk and eggs, which may contain residues of these compounds, and harm human health (Bruerton, 2001). Thus, it is important to study the hygienic and sanitary quality of the feedstuffs used in cattle diets and how these impact health, performance and quality of the final product.

First of all, to avoid the contamination of feedstuffs used in animal diets, it is necessary to identify which is the most frequently contaminated ingredient and which mycotoxins are often found in these materials. After these identifications, it becomes possible to seek strategies to minimize the harmful effects to animals.

As mentioned above, the best way to avoid contamination would be through proper management of the crop, forages or by-products used to feed animals. However, since this is not always possible, there are other strategies that can be adopted when feed is already contamination, such as the use of adsorbents in the diets. The use of adsorbents in feeds is an strategy that may reduce the absorption of mycotoxins by the animals, since these compounds contain substances that complex with toxins, preventing them to be absorbed in the gastrointestinal tract, as they form an adsorbent-mycotoxin complex and they are eliminated in feces (Yiannikouris and Jouany, 2002).

We hypothesized that mycotoxins could decrease performance of Nellore bulls finished in feedlot, but the use of yeast cell wall adsorbent can attenuate this damage. In addition, mycotoxins may negatively impact the meat quality of Nellore cattle. Thus, the objective of this study was to identify contaminated ingredients, measuring which type of mycotoxins, as well as the level of contamination of diets used in feedlots in Brazil. Further, the effects of these mycotoxins and the use of adsorbent (Mycosorb A^{+}) in performance and meat quality of Nellore cattle finished in feedlot were evaluated as well.

2. LITERATURE REVIEW

Main mycotoxins and their effects on animals

Aflatoxins

Aflatoxin is the most aggressive mycotoxin for animal health and is produced by fungi *Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus nomius* (Battacone et al., 2012). These fungi invade plant tissue, in particular when damaged and it is produced in warm climates (Cotty and Jaime-Garcia, 2007). The contamination can occur before or after harvest mainly on starch cereal, cottonseeds, and peanuts crops (Richard, 2007).

These toxins can be identified as aflatoxin B1, B2, G1 and G2 and mainly affect the liver, forming abscesses, which are represented by primary biochemical lesions (Foster et al., 1983). The aflatoxin B1 (AFB1) is the most important toxin in this group, acting as mutagen and carcinogen (McLean and Dutton, 1995). These are associated with the worldwide incidence of liver cancer, which is one of the most lethal cancers (Liu et al., 2012).

Aflatoxins are not altered by rumen microorganisms, but, when it occurs, only 10% of the ingested aflatoxin can be transformed in aflatoxicol and this substance maintains the same toxic power as the original molecule (Upadhaya et al., 2010). Thus, the rumen does not provide protection against aflatoxins.

Studies have indicated that values between 0.3% and 6.2% of AFB1 in animal feedstuffs are metabolized, biotransformed, and secreted in milk in the form of aflatoxin M1 (AFM1) (Creepy, 2002; Unusan, 2006; Iqbal et al., 2013; Duarte et al., 2013). The AFM1 may be considered more dangerous than AFB1, because AFB1 is

dependent a metabolic activation to result in its carcinogenicity acute (Neal et al., 1998). However, the AFM1 has toxic effects without metabolic activation (Caloni et al., 2006). Furthermore, this toxin is included in the category of carcinogenic agents to humans (Iarc, 2002).

In ruminants, chronic exposure to aflatoxins reduces appetite, and leads to poor feeding efficiency and low milk production (Rossi et al., 2009; Whitlow, 2010). Furthermore, death due to aflatoxicosis in calves has been reported (Lynch, 1972).

Ochratoxin A (OTA A)

Ochratoxin A is produced by *Aspergillus and Penicillium* fungi (Halasz et al., 2009). *Aspergillus* species predominate in warm and temperate regions while *Penicillium* is frequently found in colder areas (Futagami et al., 2011). This toxin seriously affects monogastric animals, because it can cause inhibition of protein synthesis, lipid peroxidation, DNA changes, respiratory chain inhibition, cellular apoptosis, and inhibition of enzymes involved in kidney and liver metabolism (Kruger, 2006).

However, in ruminant animals, due mainly to the activity of protozoa (Mobashar et al., 2012), the OTA A is almost all degraded by ruminal microorganisms in a less toxic compound: ochratoxin α (Battacone et al.,2010). However, the detoxification capacity of the rumen can be exceeded in situations of high contamination (Ribelin et al., 1978). These authors indicated that the lethal single oral dose of OTA A in cattle is probably higher than 13 mg/kg of body weight (BW). In addition, when animals are receiving ingredients that are able to maintain low rumen pH, which is common in feedlot diets, the inactivation capacity of the microorganisms on OTA A may be reduced, which facilitates the direct uptake of toxin from the rumen into the blood (Marquardt and Frohlich, 1992).

Blank et al. (2003) observed increased of ochratoxin α in serum of sheep when they fed increased doses of OTA (0, 9.5, 19.0 and 28.5 µg OTA/kg of BW). Besides this, small amounts of these toxins were detected in plasma, suggesting OTA A could bypass the rumen (Denli and Perez, 2010). Höhler et al. (1999) observed that when sheep were fed 14 mg of OTA/kg of diet, animals reduced dry matter intake (DMI) compared to sheep fed no contaminated diet. In addition, the

authors reported residues of OTA in milk, which suggests a ruminal escape of this toxin.

Fumonisins

Fumonisins are mainly produced by *Fusarium verticillioides and Fusarium proliferatum* fungi (Tiemann and Danicke, 2007). These fungi are important cereal pathogens, mainly corn, at various stages of development, including post-harvest periods, when the grains are stored (Diaz and Boermans, 1994). Fumonisins are diesters of tricarballylic acid and polyhydric alcohols which are very similar to sphingosine structure (Norred, et al., 1992). The variations of the hydroxyl group of the fumonisin molecules determine their different types, which can be B1, B2, and B3 (Visconti et al., 1995).

Sphingolipids are strongly influenced by fumonisins, since this mycotoxin may block their synthesis, due to the inhibition of ceramide synthetase and prolonged inhibition that can promote cell death induced by free sphingoid bases (Riley et al., 1998). These toxins affect more monogastric animals. The symptoms in horses are necrotic brain lesions (Kellerman et al., 1990), in swine and chickens occurs cellular apoptosis, affecting part of normal organ development and tissue maintenance (Merril et al., 1996).

Although this mycotoxin is less toxic to ruminants, it normally passes by the rumen, and if it is ingested in high amounts, can affect different organs, but liver and kidneys are the most affected. According to Fink-Gremmels (2008), one of the signs of intoxication in cattle are: high enzymatic activity of serum hepatic enzymes (aspartate aminotransferase and gammaglutamyl transferase). Furthermore, the toxicity of fumonisin B1 may induce the initiation of carcinogenic tumors in the liver (Gelderblom et al., 2001).

Zearalenone (ZEA)

Zearalenone is a lactone produced by *Fusarium graminearum, Fusarium culmorum, Fusarium equiseti* and *Fusarium crookwellense* fungi (Kummar et al., 2008). The ZEA contamination has been reported in cereal grains, mainly in temperate climates (Hagler et al., 2001). Typically, this toxin is found at low

concentrations in contaminated grains in the field but, it increases under high moisture storage conditions (30 to 40%); (Gupta, 2007). This toxin is frequently detected in grains with another mycotoxin (deoxynivalenol), and despite it is heat stable, it may be partially destroyed during extrusion of cereals (Castells et al., 2005).

The zearalenone can cause reproductive and estrogenic problems in animals (Minervini and Dell'aquila, 2008) In monogastric, it is rapidly absorbed and distributed to the ovary, uterus, adipose tissue and interstitial tissue (Kuipergoodman et al., 1987). Kurtz and Mirocha (1978) observed that dietary concentrations of ZEA as low as 1.0 mg/kg may lead to hyperestrogenic syndromes in swines, and higher concentrations can lead to disrupted conception, abortion, and other reproductive problems.

In ruminants, about 90% of ingested ZEA is converted into α-zearalenol and β-zearalenol by the ruminal microorganisms (Cruz, 2012). The α-zearalenol has higher estrogenic potency than ZEA because it can be converted to zeranol, which acts as a growth promoter, but its toxic effect causes less damage because it is less absorbed, and when absorbed it is converted to β-zearalenol in the liver. The β-zearalenol has toxic activity on endometrial cells, but its affinity for estrogen receptors is smaller (Bottalico et al., 1985).

In this way, ruminants are less sensitive to ZEA exposure than nonruminant animals, because of the metabolization of this toxin in the rumen (Seeling et al., 2005; Fink-Gremmels and Malekinejad, 2007). However, if rumen acidosis occurs in cattle, it is expected that the microflora will fail to eliminate ZEA (Takagi et al., 2011). This fact occurs because one of the consequencies of rumen acidosis are destruction of a large percentage of the normal rumen microflora, so less chance to toxins metabotization.

Trichothecenes

Trichothecenes are produced by *Fusarium* sporotrichioides, *Fusarium graminearum*, *Fusarium poae*, and *Fusarium culmorum* (Upadhaya et al., 2010) and they can also be produced by *Trichothecium* (Jones and Lowe, 1960). This toxin can be classified in type A, which are: T-2, HT-2, neosolaniol, 15-monoacetoxiscirpenol (15-MAS) and diacetoxiscirpenol (DAS); and in type B, which are: desoxinilvalenol

(DON or vomitoxin), 15-acetildesoxinivalenol (15aDON), fusarenona X (FX) and the nivalenol (NIV) (Santin et al., 2001). These toxins may be present in most of the cereals during harvest and storage (Yiannikouris and Jouany, 2002). However, DON is considered the most usual mycotoxin in silages and other forages (Storm et al., 2008)

Swines and chickens have been shown to be very sensitive to T-2 toxin and DON (Friend et al., 1992). The toxic effects may reach nervous, immune and digestive systems (Lazzari, 1997).

According to Upadhaya et al. (2010), ruminants are less susceptible to these mycotoxins. However, Swanson et al. (1987) demonstrated that when DAS, DON, and T-2 toxin were incubated with rumen fluid, all three were rapidly metabolized by the microflora producing monoacetoxyscirpenol (MAS) that is also a toxic compound.

The effect of these mycotoxins is to inhibit the initiation, elongation and termination of protein synthesis in microrganisms cells, making them more potent than other mycotoxins (Ehrliche and Daigle, 1987). Therefore, they cause mucosal lesions, weight loss, interference in motor coordination and cutaneous ulcers (Cavan et al., 1988).

Others mycotoxins

Toxins from *Penicillium* are produced by *Penicillium roqueforti* and *Penicillium paneum* (Storm et al., 2008). These metabolites can cause immunosuppressive and antibacterial effects in animals (Fink-Gremmel et al., 2008). Moreover, animals consuming forages contaminated by *Penicillium* strains may present loss of appetite, influence on nutrient efficiency, ketosis, abomasal ulcer, gastroenteritis, laminitis, paralysis and abortion (Nout et al., 1993; Nielsen et al., 2006; Fink-Gremmel et al., 2008; Pereyra et al., 2008). These effects are probably due to the production of their toxic metabolites. For ruminants, however, no adverse effects on blood parameters were detected when sheep were fed 300 mg/kg/day of mycotoxins from *Penicillium* (Mohret al., 2007). µg/kg

Fusaric acid is a toxin produced by *Fusarium* species (Bacon et al., 1995). This toxin is often present in cereals (Yiannikouris and Jouany, 2002) and cause loss of appetite, lethargy, and loss of muscle coordination in swine (Smith and McDonald,

1991). Fusaric acid is highly recognized for its synergistic power with other mycotoxins. This toxin increases the toxicity of trichothecenes (Smith et al., 1997), fumonisin (D'Mello and McDonald, 1996) and ZEA (Porter et al., 1996).

Tapia et al. (2005) observed that patulin, produced by *Penicillium paneum,* interfered on rumen activity. Sabater-Vilar et al. (2004) reported severe cases of neurotoxicosis, comprising tremors, ataxia, paresis, recumbence and death concomitantly occurred in beef cattle because of this mycotoxin.

The citrinin are produced by species of the genus *Aspergillus* and *Penicillium* and often occur concomitantly with OTA in feedstuffs (Bouslimi et al., 2008). Stec et al. (2008), evaluating this mycotoxin *in vitro* studies, reported immunotoxic effects of citrinin only at very high doses. Furthermore, Griffiths and Done (1991) conducting *in vivo* studies, observed that dairy cows fed citrinin and OTA contaminated diets presented signs of pruritus, pyrexia, hemorrhagic syndrome, fever, diarrhea and uremia.

Alternaria derived toxins are alternariol and alternariol monomethyl ether. They are toxic for bacteria and mammalian cells *in vitro*, whereas altertoxins are mutagenic for bacteria and induce cell transformation (Wang et al., 1996; Ostry, 2008). Studies about these mycotoxins are limited.

Tall fescue straw is a source of forage widely used in USA for ruminants (Hovermale and Craig, 2001). However, the use is limited because of ergot alkaloids contaminations (Morgan-Jones and Gams, 1982). Cattle consuming high-ergot alkaloid in tall fescue straw have presented lower feed intake, excitability, increased rectal temperature and respiration rate, decreased reproductive efficiency and lighter body weight (Mizinga et al., 1992; Aldrich et al., 1993; Zain, 2011).

Mycotoxins Synergism

Synergistic effect may occur among different mycotoxins. Feeds are frequently contaminated simultaneously by several fungi that can produce several toxins and there may be synergetic effects (Yiannikouris and Jouany, 2002). Mycotoxins severely affect health and performance of animals as discussed above; however, there are few studies that cover the effects of more than one mycotoxins and the

complementary effect of one over the other (D'Mello and McDonald, 1996; Porter et al., 1996; Smith et al., 1997; Bouslimi et al., 2008).

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In turkeys, a combination of fumonisin B1 and DAS reduced 46% of body weight and a combination of fumonsin B1 and OTA reduced 37% (Kubena et al., 1997). Other combinations, involving DON and DAS, DAS and aflatoxins and aflatoxins and fumonisin B1 may also have synergetic effects (Harvey et al., 1995a; Harvey et al., 1995b). There are no studies in the literature reporting the combined and synergistic effect of mycotoxins for beef cattle.

Mycotoxin Adsorbents

Prevention through preharvest and harvest management is the best method for controlling mycotoxin contamination. There are some strategies that can be applied to minimize the contamination of mycotoxins in feedstuffs used in animal diets, such as: crop rotation, time of irrigation, planting and harvesting, plant breeding for resistance to toxigenic fungi, genetically modified crops resistant to insect penetration, and competitive exclusion by using of non-toxigenic strains in the field (Duncan et al., 1994). When the material are stocked, there are some strategies as additives composed by either organic acids or bacteria that produced organic acids, to prevent fungi growth and in this way, mycotoxin production can be avoid.

However, when feedstuffs are already contaminated, it is possible to add adsorbents in diets prior to feeding animals avoiding the mycotoxin absorption. The adsorbents can be divided into two main groups: inorganics and organics. The inorganic adsorbents may be carbon, zeolites, bentonites, clays, calcium hydrated sodium aluminosilicates and diatomaceous soil (Wyatt, 1991; Piva et al., 1995). Organic adsorbents may be oat bark, wheat bran, alfalfa fiber, yeast cell wall, cellulose, hemicellulose and pectin (Dilkin and Mallmann, 2004). A good adsorbent may have a broad spectrum of adsorption acting on a large amount of mycotoxins (Mallmann et al., 2006) and it can not react with other substances.

Inorganic additives as zeolites, bentonites and montmorillonites adsorb more aflatoxin, which has a strong positive charge (Buragas, 2005). Adsorbents based on sodium and calcium aluminosilicate are derived from zeolites, so they are effective in reducing the effects of aflatoxin. Phillips et al. (1988) observed an increase in the weight gain of broilers fed aflatoxin-contaminated feed containing sodium and calcium aluminosilicate.

Inorganic materials were used for reducing the toxic effect of aflatoxins (Galvano et al., 2001; Huwig et al., 2001; Lemke et al., 2001). However, it has limited efficacy when there are other mycotoxins in the diet (Jouany et al., 2005). The use of yeast cell wall as organic adsorbents has the advantages of surface area for adsorption of a large number of mycotoxins (Yiannikouris et al., 2004; Shetty and Jespersen, 2006).

The addition of yeast cell wall as adsorbent in contaminated diets has been used as dietary approach to reduce effects of mycotoxins (Raymond et al., 2003; Diaz et al., 2004; Diaz-Llano and Smith, 2006; Firmin et al., 2011; Marson, 2014). Raymond et al. (2003) observed an increase in intake and reduction of the gammaglutamyl transferase activity in horses fed grain contaminated with DON, 15aDON, fusaric acid and ZEA containing yeast cell wall as organic adsorbent. Diaz-Llano and Smith (2006) reported reduction of stillborn piglets when gilts were fed yeast cell wall in diets contaminated with *Fusarium* mycotoxins.

In studies with ruminants Diaz et al. (2004) observed a decrease of 58.5% of aflatoxin M1 in milk with the use of yeast cell wall. Firmin et al. (2011) concluded that feed supplementation with yeast cell wall reduced the absorption of AFB1 and increased the elimination of AFB1 and AFM1 in ewe feces. Takagi et al. (2014) confirm the significant reduction of urinary ZEA concentrations after a period of adsorbent supplementation for dairy cattle. Finally, Marson (2014) observed that the use of yeast cell wall adsorbent was economically feasible for beef cattle finishing in feedlot.

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CHAPTER 2

The following article is in accordance with Toxins Journal's publication guidelines.

Article

Survey of mycotoxin contamination of diets for beef cattle finishing in feedlot

Abstract: The objective of this survey was to identify which mycotoxins were present in ingredients used in diets fed to beef cattle in feedlots and their concentrations. The survey covered 30 Brazilian feedlots located into the 5 largest beef-producing states. Samples of total mixed ration (TMR) and ingredients were collected on-site and sent to the 37+® Analytical Services Laboratory (KY, USA) for analysis of mycotoxins. The quantification of 38 different mycotoxins was performed using ultra-performance liquid chromatography coupled to tandem mass spectrometry. 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 (REQ) expressed in μg/kg of aflatoxin B1 (AFB1)-equivalent. Descriptive statistics were obtained using the UNIVARIATE procedure of SAS and multivariate statistics were obtained using STATISTICA. The toxins identified in TMR were: fumonisins, trichothecenes A, trichothecenes B, fusaric acid, aflatoxins and ergot (mean values: 2,330; 104.3; 79.5; 105; 10.5; 5.5 μg/kg, respectively). All samples presented at least one mycotoxin contamination, and 65.5% of the samples were classified as low contamination, 27.6% medium contamination and 6.90% high contamination. In conclusion, fumonisins were the mycotoxin most frequently found and at highest concentrations in TMR samples, and peanut meal was the most contaminated ingredient.

Keywords: aflatoxin; beef cattle; feedlots; fumonisin; ingredients; REQ

Key Contribution: The study evaluated the occurrence of mycotoxins in diets of beef cattle. These findings are of importance in the monitoring and management of mycotoxins in beef cattle systems since mycotoxins can limit the optimal performance of animals.

1. Introduction

Mycotoxin contamination occurs in many materials, including animal feed, animal products and soil. As these toxins affect animal production and health, they can cause substantial economic losses. Factors that can affect the mycotoxins production by fungi include abiotic factors, such as temperature and humidity, and biotic factors, such as fungal load at the time of transportation and storage. Besides that, there are two types of fungi, those that acts before harvest, commonly called field fungi, and those that occur only after harvest, called storage fungi [1].

The most important genus of mycotoxigenic fungi are *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium and Stachybotrys* [2]; while the most common mycotoxins investigated and found in ingredients of animal diets worldwide are: aflatoxin, fumonisin, zearalenone, ochratoxin and trichothecenes [3]. However, as the presence of these mycotoxins relates to specific environmental conditions and type of material, the characteristics of contamination could vary regionally. Nevertheless, the trading of ingredients among regions, countries and continents could also play a role in the contamination dynamic and change mycotoxins distribution patterns.

Thus, the objectives of this survey were to identify the mycotoxins and their concentrations present in ingredients used in typical total mixed rations (TMR) fed at Brazilian feedlots, and to relate these results to characteristics of the feedlot.

2. Results

2.1. Information about the visited feedlots

Eighteen feedlots (60%) had less than 5,000 animals, whereas 7 (23%) had from 5,000 to 10,000 animals and 5 feedlots (17%) had more than 10,000 animals on feeding. The observed average daily gain (ADG) ranged from 0.70 kg to 1.85 kg in these feedlots, however most of feedlots (63%) had animals with ADG of about 1.53 kg; (Table 1).

Table 1. General characteristics of the feedlots surveyed.

Item	Mean	Min[1]	Max[1]
Number of animals	7,085	324	50,000
ADG, kg	1.53	0.70	1.85
Days on feed	107	85	155
Feeding frequency (times/day)	5	3	8

[1]Min = Minimum; Max = maximum.

Most of the feedlots (83%) stored the ingredients in a common barn, and 57% of the feedlots cleaned up the storage barns (Table 2). Forty percent of the visited feedlots had apparent fungi in TMR, however only one feedlot (3%) used a mycotoxin adsorbent (Table 2). Besides that, from the twelve diets with apparent fungi, six showed moderate mycotoxin contamination, while other six had low contamination. On the other hand, diets with high contamination (6.9%) presented no apparent fungi.

Table 2. Information about barns, apparent fungi in TMR and the use of mycotoxin adsorbents of feedlots.

Item	N of responses	% of responses
Type of barn for feed storage		
Common	25	83.3
Double side	3	10.0
Silo	1	3.33
No barn	1	3.33
Barn cleaning		
Yes	17	56.7
No	13	43.3
Apparent fungi in TMR		
Yes	12	40.0
No	18	60.0
Use mycotoxin adsorbent		
Yes	1	3.33
No	29	96.7

2.2. Characterization of TMR samples of visited feedlots

Almost all TMR samples (93.3%) presented fumonisin (B1+B2), 80% had fusaric acid and 66.7% had trichothecenes A (T-2, H-T2, diacetoxiscirpenol, and neosolaniol); whereas aflatoxin (B1+B2+G1+G2), trichothecenes B (DON, 15-acetyl DON, 3-acetyl DON, fusarenol X, nivalenol, and DON 3-glicoside), ergot and others mycotoxins produced by *Penicillium* (patulin, penicillic acid, roquefortine C, mycophenolic acid, and wortmannin) were present in fewer samples (6.67, 20.0, 33.3, and 6.67, respectively). Ochratoxin A, zearalenone and other mycotoxins produced by *Aspergillus* (Gliotoxin, Sterigmatocystin, Verruculogen) were not found (Figure 1). The levels of samples contamination are presented in the Figure 2.

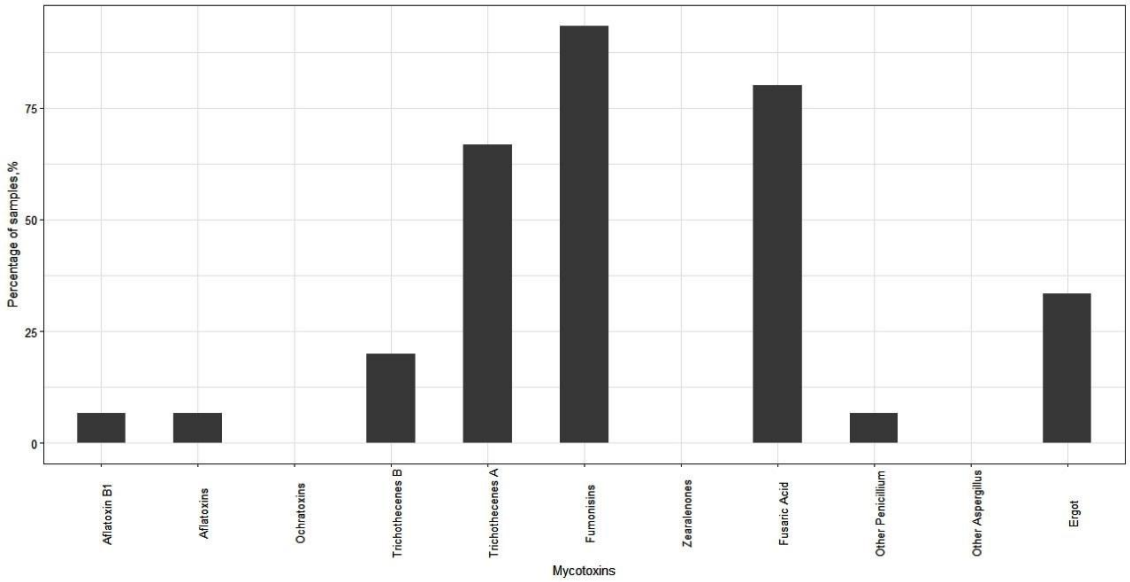

Figure 1. Mycotoxin occurrence (%) in total mixed ration (TMR) samples of 30 feedlots collected in five Brazilian states (Aflatoxins = Aflatoxin B1+B2+G1+G2; Ochratoxin = OTA; Trichothecenes B = DON; Trichothecenes A = Toxin T-2 + HT-2; Fumonisins = Fumonisins B1+B2; Others *Penicillium* = Patulin, Penicillic Acid, Roquefortine C, Mycophenolic Acid, Wortmannin; Others *Aspergillus* = Gliotoxin, Sterigmatocystin, Verruculogen.).

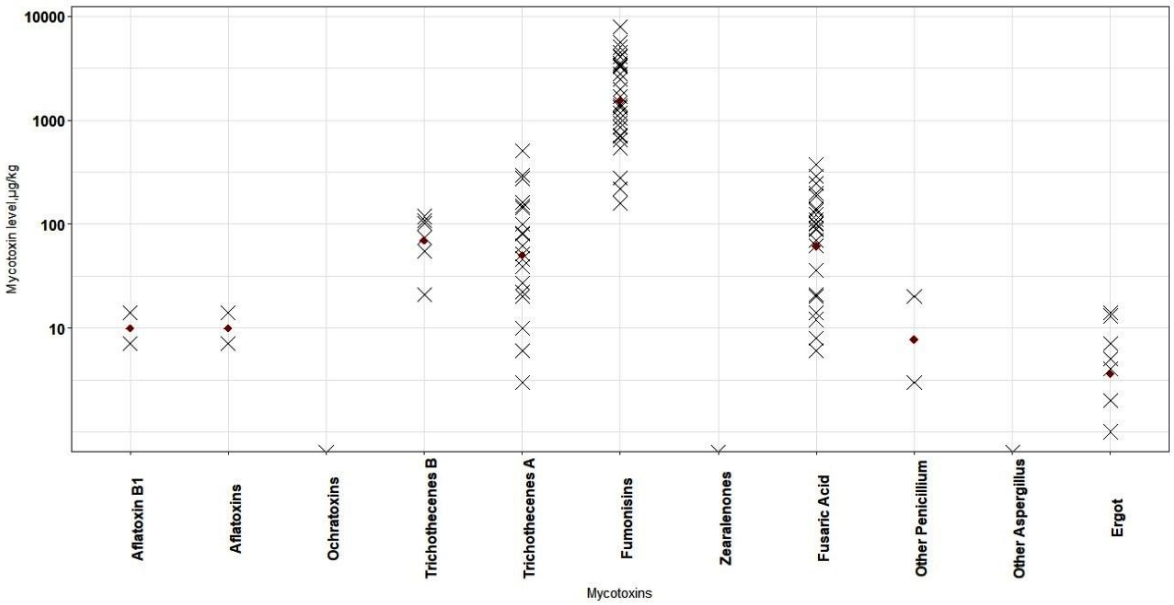

Figure 2. Mycotoxin concentration (µg/kg) in total mixed ration (TMR) samples of 30 feedlots collected in five Brazilian states (Aflatoxins = Aflatoxin B1+B2+G1+G2; Ochratoxin = OTA; Trichothecenes B = DON; Trichothecenes A = Toxin T-2 + HT-2; Fumonisins = Fumonisins B1+B2; Others *Penicillium* = Patulin, Penicillic Acid, Roquefortine C, Mycophenolic Acid, Wortmannin; Others *Aspergillus* = Gliotoxin, Sterigmatocystin, Verruculogen.).

In this study, 65.5% of TMR samples were classified as having low Risk Equivalent Quantities (REQ), 27.6% were classified as intermediate REQ and 6.9% were classified as high REQ (Figure 3). The maximum REQ found in TMR used in the feedlots evaluated in this survey was 230 µg/kg AFB1-equivalent and the minimum REQ was 1 µg/kg AFB1-equivalent.

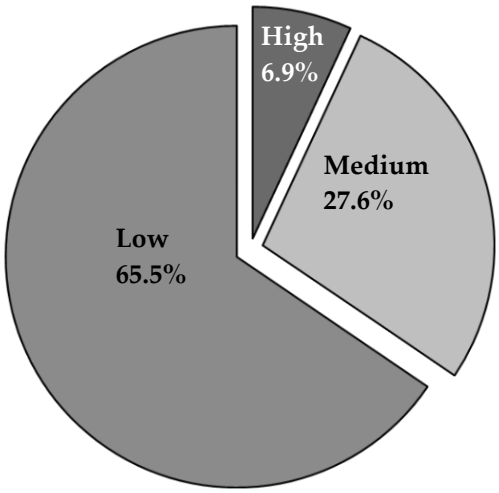

Figure 3. Percent of total mixed ration (TMR) samples of 30 feedlots in five Brazilian states with Risk Equivalent Quantities (REQ) at Low (0 – 50 µg/kg), Medium (51 -100 µg/kg) and High (>101 µg/kg) risk to beef cattle.

The analysis of principal components allowed the construction of the two-dimensional biplot formed by the first two major components (Factor 1 and Factor 2); (Figure 4). In the Figure 4, the samples contaminated with aflatoxins were separated from the other samples and the REQ. As the REQ computes a multi-contamination complex situation pertaining to feedstuffs into one single value [4]. It is possible to observe that the combination of other mycotoxins can be more dangerous than aflatoxin by itself.

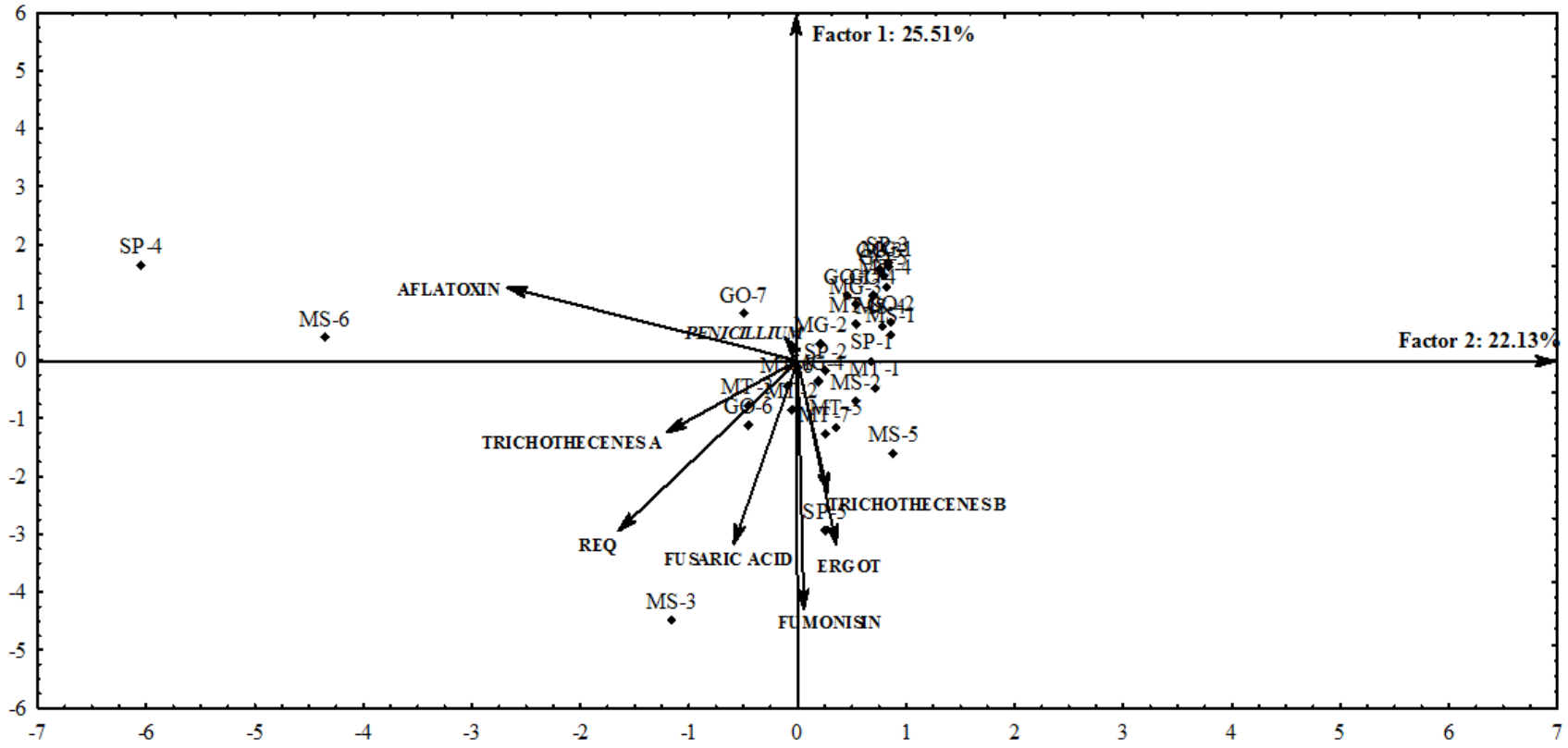

Figure 4. Principal components of Risk Equivalent Quantity (REQ) of total mixed ration (TMR) samples of 30 feedlots collected in five Brazilian states.

It is possible to observe, according to the contamination of the TMR samples per region, that Mato Grosso do Sul state presented the highest contaminations in some of the TMR (above 200 μg/kg; Figure 5).

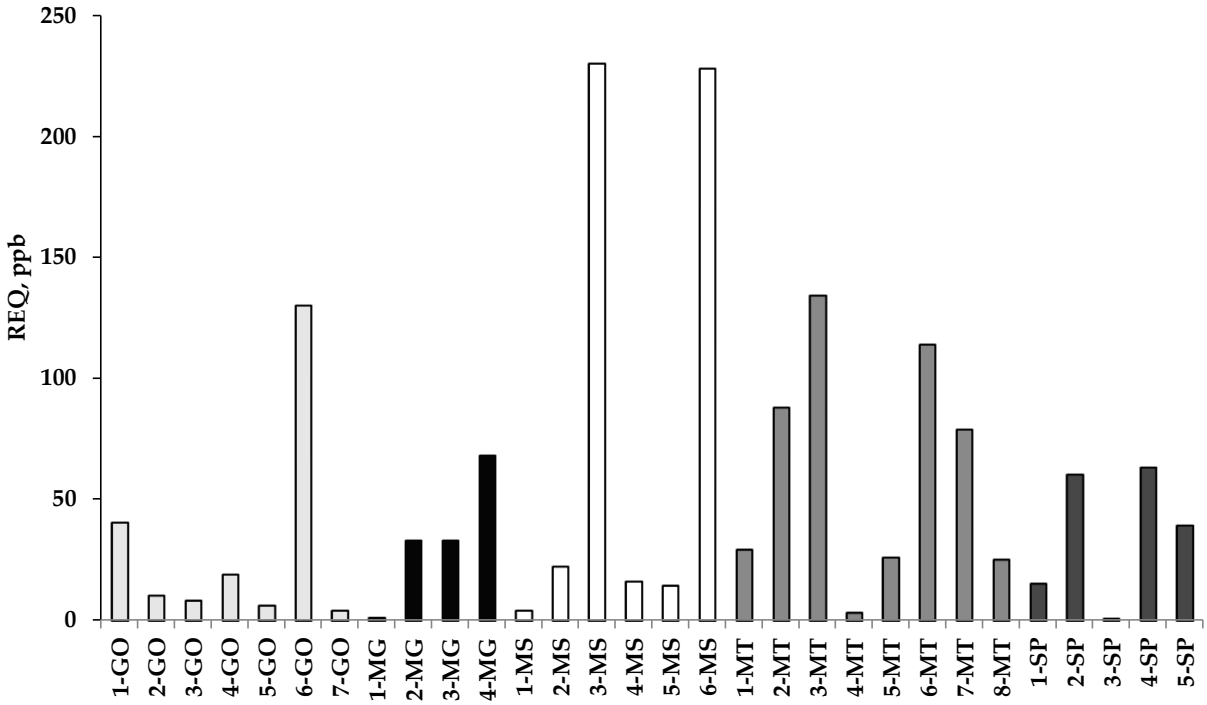

Figure 5. Contamination per state of total mixed ration (TMR) samples of 30 feedlots in five Brazilian states (GO = Goiás; MG = Minas Gerais; MS = Mato Grosso do Sul; MT = Mato Grosso; SP = São Paulo).

2.3. Characterization of ingredients of ten most contaminated TMR samples of visited feedlots

The ingredients from the 10 most contaminated diets were analyzed to determine which ingredients were responsible for the high contamination and which mycotoxins were associated with each ingredient. It was possible to observe that the source of roughage most often used in these feedlots was corn silage and the most common sources of concentrates were corn and cottonseed (Table 3). The Table 4 shows the mean levels of contamination of ingredients from the 10 most contaminated diets, indicating which ingredients were most responsible for TMR contamination and which mycotoxin stood out in each source.

Table 3. Roughage and concentrate sources used in diets of visited feedlots.

Item	N of responses	% of responses
Roughage source		
Corn silage	13	43.3
Corn residue	2	6.67
Grass silage	2	6.67
Sugarcane straw + Tifton hay	2	6.67
Others[1]	11	36.7
Concentrate source		
Corn grain	23	76.7
Whole Cottonseed	16	53.3
Cottonseed cake	11	36.7
Citrus pulp	7	23.3
Soybean meal	7	23.3
Soybean hulls	6	20.0
Corn germ	5	16.7
High moisture corn	5	16.7
Peanut meal	4	13.3
Sorghum grain	4	13.3
Corn gluten feed	3	10.0
Soybean residue	3	10.0
Others[2]	12	40.0

[1]Other roughages source: 3,33% each: *Brachiaria* hay, Corn silage + Ear corn silage, Corn silage + Grass silage, Corn silage + Sugarcane bagasse, Cotton residue, Ear corn silage + Sugarcane bagasse, Cotton residue, Ear corn silage + Sugarcane bagasse, Grass, millet and sorghum silage, Sorghum silage, Sugarcane bagasse, Sugarcane bagasse + *Brachiaria* hay, Sugarcane silage.

[2]Others concentrate sources: 3,33% each: Dehydrated cottonseed, Crambe meal, Corn DDGS, Micro algae, Orange Bagasse, Peanut starch, Rice meal, Rice residue, Rehydrated sorghum, Sunflower cake, Sunflower meal, Tomato + corn residue.

Table 4. Mean mycotoxin contamination (μg/kg) of ingredients of ten most contaminated total mixed ration (TMR).

Item[1]	n	REQ	AFB1	Other aflatoxins	Tricho B	Tricho A	Fumo	ZEA	F. Acid	Penic	Asper	Ergot
Roughages												
Corn silage	1	50.0	-	-	-	-	7,116	-	619	-	-	22.0
Sugarcane bagasse	2	6.00	-	-	25.0	-	1,774	-	13.0	-	-	-
Sugarcane straw	1	134	-	-	2,276	21.0	-	-	87.0	-	-	-
Tifton hay	1	12.5	-	-	-	-	270	-	25.5	13.0	1.50	0.50
Concentrates and by-products												
Citrus pulp	4	14.0	-	-	-	-	-	-	88.3	-	-	125
Corn	9	58.4	-	-	12.1	2,536	18,402	5.60	104	-	-	-
Corn germ	1	103	-	-	613	-	25,801	-	152	-	-	-
Cottonseed cake	2	21.0	-	-	-	-	-	-	409	-	0.50	-
Cottonseed	3	4.67	-	-	-	-	1,042	-	39.7	-	-	-
Crambe meal	1	6.00	-	-	-	-	1,192	-	54.0	-	-	2.00
DDGS by corn	1	118	6.00	6.00	150	-	12,184	206	693	11.0	-	3.00
Peanut meal	2	1,018	471	578	-	-	-	-	483	-	555	-
Peanut starch	1	58.0	-	-	-	-	3,362	-	676	-	21.0	-
Corn gluten feed	1	277	-	-	-	-	48,828	-	2,347	30.0	-	201
Soybean hulls	2	8.50	-	-	-	8.50	217	-	15.0	-	1.00	-
Soybean meal	3	63.7	-	-	-	84.3	28.3	-	13.0	-	-	4.70
Soybean residue	1	239	-	-	-	306	243	-	-	-	-	3.00

[1]DDGS by corn: Dry destilled grain plus solubles; REQ = Risk equivalent quantities; AFB1 = Aflatoxin B1; Other Aflatoxins = B1+B2+G1+G2; Tricho B = DON; Tricho A = Toxin T-2 + HT-2; Fumonisins = Fumonisins B1+B2; ZEA = Zearalenone; F. Acid = Fusaric Acid; Penic = Patulin, Penicillic Acid, Roquefortine C, Mycophenolic Acid, Wortmannin; Asper = Gliotoxin, Sterigmatocystin, Verruculogen.

The cluster analysis of all contaminated ingredients allowed the construction of a dendrogram resulting from hierarchical grouping analysis (a) and non-hierarchical method (b) (Figure 6). In both Figures "a" and "b", three different groups could be characterized. In Figure 6(a), groups were characterized by contamination levels while in Figure 6(b), groups were clustered according to the contamination levels and to the mycotoxin type.

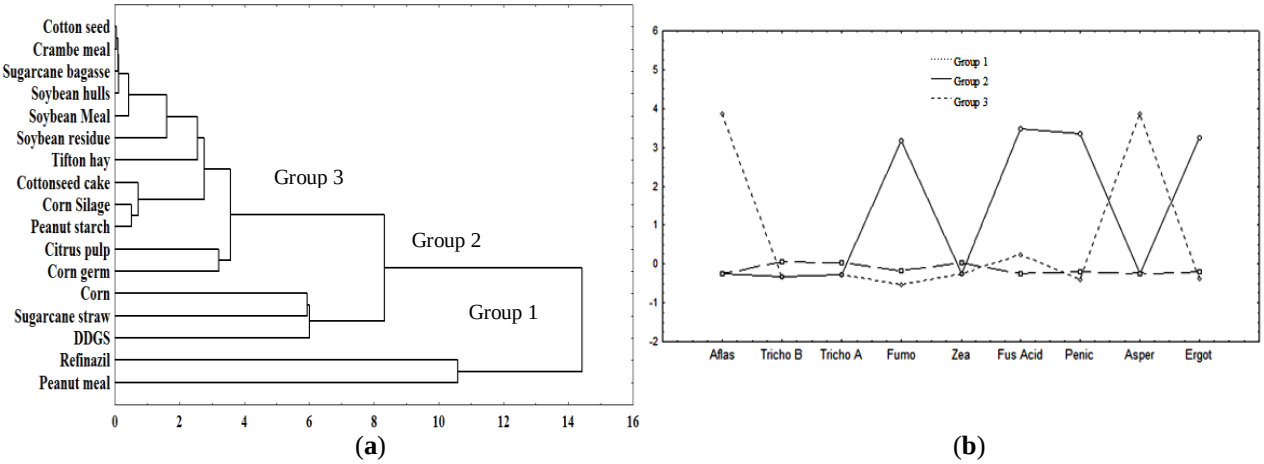

Figure 6. Dendrogram of the cluster analysis by the hierarchical method and non-hierarchical method of groups profile 1, 2 and 3 constructed by the K-means algorithm of the ingredients from the 10 most contaminated diets. (a): Group 1: Most contaminated ingredients, Group 2 Intermediate contaminated ingredients and Group 3: Less contaminated ingredients.
(b): Aflas: Aflatoxins B1+B2+G1+G2, Tricho B = DON; Tricho A = Toxin T-2 + HT-2; Fumo = Fumonisins B1+B2; ZEA = Zearalenone; Fus Acid = Fusaric Acid; Penic = Patulin, Penicillic Acid, Roquefortine C, Mycophenolic Acid, Wortmannin; Asper = Gliotoxin, Sterigmatocystin, Verruculogen.

3. Discussion

Mycotoxins are an important issue, because they can affect production cost and could also impact health of animals and humans [5]. This study assessed what types and amount of mycotoxins are present in the diets of beef cattle at Brazilian feedlots. The diets of visited feedlots showed different levels of contamination, but all TMR samples had some sort of contamination. The type of mycotoxins varied largely by the type of diet component, but the level of contamination varied within ingredient. One example is a sample of peanut meal and one of corn that were extremely contaminated, whereas other samples of the same materials collected from other feedlots had low contamination, indicating the dependency of more than one factor.

The high prevalence of fumonisin, trichothecene A and fusaric acid in the samples can be attributed to the type of feedstuffs used in the diets. Fumonisin is produced by species of Fusarium and these toxins occur very often in corn [6]. In this survey 76.7% of the feedlots used corn, 43.4% used corn silage, 16.7% used corn germ, 10% used refinazil and 3.33% used corn dry destilled grain plus solubles (DDGS), which is consistent with the high frequency of fumonisin in TMR samples of this survey. More broadly, corn is the primary source of grain used in feedlot diets [7,8], so this toxin can be very important for cattle in feedlots, since the ingestion of highly contaminated diets can cause lower intake, the variable that most influences performance, [9] besides, affect metabolic organs, such as liver and kidneys [10].

Trichothecenes A and B are also produced by species of Fusarium and they are present in corn and forages [11] widely used in TMR samples analyzed in this survey. These toxins are not very toxic for ruminants [12], however, [13] reported that ruminants ingesting feed contaminated by DON

presented mycotoxin biotransformation and excretion in fluids, such as blood and milk, which classifies this mycotoxin as a risk to human health.

Fusaric acid also can be present in cereals and forages, and it was present in almost all TMR samples. Moreover, this toxin increases the toxicity of trichothecenes through a synergistic mechanism [14]. The synergism is common because mycotoxins are seldom found in isolation, and when multiple mycotoxins are consumed, they may have strong interactions that increase the risk to animal performance and health. As a result, the animal may have a stronger response than what would be expected if it was consumed only a single mycotoxin.

Other mycotoxins, including aflatoxins, ergot and mycotoxins produced by *Penicillium*, were found. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* [2]. Peanut is one substrate that is most commonly contaminated with aflatoxin, because it is one of the most susceptible foods to contamination by fungi that produce this toxin [15]. And although aflatoxin was present in only 6.7% of the TMR samples, this mycotoxin is very important for animal health [16], because it is hepatotoxic, carcinogenic, and immunosuppressive [17], besides it is not metabolized in the rumen, which negatively affects the ruminant.

Ergot and mycotoxins produced by *Penicillium* can also be dangerous for ruminants. According to [18], ergot alkaloids are produced by a group of fungi of the genus *Claviceps* and frequently found in cereal grains. When ingested by ruminants this toxin can decrease feed intake, elevate body temperature, lead to excessive salivation, increase respiration rate and decrease peripheral circulation [19]. Mycotoxins produced by *Penicillium* are mostly found in materials stored in bad conditions, such as silages and hays, and can reduce appetite, impact nutrient efficiency, and increase abomasal ulcers, laminitis, gastroenteritis and paralysis [20].

These toxins are not always present in materials with obvious fungal growth, because the microorganisms may produce mycotoxins only if they suffer some type of stress [21]. This occurred in this study, since diets with high contamination of mycotoxin did not presented apparent fungi and diets with apparent fungi, had low or moderate contamination.

When the general information about the feedlots and the contamination levels of the samples were contrasted, it was observed that the contamination had no relationship with the performance of the animals. The mean of ADG of the animals in the feedlots with high or moderate risk diets was 1.56 kg, whereas animals in feedlots with low risk diets were 1.52 kg. The animals in feedlots with the highest ADG (1.85 kg) consumed moderate risk diet, whereas the animals in feedlots with the lowest ADG (0.70 kg), consumed low risk diet. This difference can be explained, probably because the variability of the genetic potential, management of the animals and the different diets. Studies to confirm the impact of mycotoxins on beef cattle performance need to be conducted.

An important issue in this study, which is related to what was stated above, is the real risk of each mycotoxin in the feed. The concentration of each mycotoxins does not always demonstrate the real risk of the sample, since some mycotoxins are present in small concentrations, but may present high risks for ruminants and the reverse, or the combination of different mycotoxins could be more dangerous than one single mycotoxin [14].

In this way, besides identifying which mycotoxins were present in TMR and which levels, it is also important to estimate the risks associated with the presence of different types and concentration of mycotoxins. It was possible to estimate the equivalent risk of the feed through the REQ (Risk Equivalent Quantity), created by [4] based on the concepts of chemistry, which generates a real risk of the mycotoxin. The risk assessment calculates a risk equivalency quantity (REQ) expressed in μg/kg of AFB1-equivalent, which computes a multi-contamination complex situation pertaining to feedstuffs into one single value [4]. According to [4], the REQ, expressed as μg/kg of AFB1- equivalent can be classified and the range between 0 to 50 μg/kg is considered low, 51 to 100 μg/kg is considered intermediate and above 100 μg/kg is considered high for beef cattle. In this study, the maximum REQ

found in TMR used in the feedlots evaluated was 230 μg/kg and the minimum REQ was 1 μg/kg AFB1-equivalent.

Besides that, through the detection of multiple mycotoxins, it is possible to estimate the risk of all mycotoxins together [14]. The principal components showed part of this combination (Figure 4), since while aflatoxin is the most aggressive single mycotoxin, REQ is along with the other mycotoxins, probably because the combination of mycotoxins and their high concentrations could be more dangerous than aflatoxin by itself.

So, these data demonstrated that it is important to consider the combined occurrence of different types of mycotoxins in ingredients and TMR samples. However, this is often neglected in other analytical approaches. Co-occurrences are important since mycotoxins could have an additive effect, potentially further increasing their negative impact in animal.

Other important issue to observe about mycotoxins contamination is that contamination by fungi is environmentally dependent, as these microorganisms overcome in more humid and warmer environments. Furthermore, the production of mycotoxins depends on environmental factors that are able to cause fungi stress, allowing them to produce the toxins. In this sense, aflatoxin contamination occurs most often in the Southern United States [3].

The Mato Grosso do Sul (MS) state in Brazil is characterized by a tropical climate, high temperature, rain in summer and dry and cold winter. High temperatures and humid it may provide condition for development of fungi, and dry and cold winter can cause some stress, which stimulates mycotoxin production. Because of these factors, the MS state presented the greatest contaminations in TMR samples.

After all analyzes of TMR samples, it was possible to further investigate into diets through the analyses performed in the ingredients used in the TMR composition. Through this, we observed that from all ingredients, the most used source of roughage was corn silage and the most used sources of concentrate was corn grain and cottonseed. These ingredients are very common in Brazilian feedlots. In a survey conducted by [7], about management practices and nutritional recommendations used by feedlot nutritionists in Brazil, they observed that corn silage was used by 28.5% of the respondents as roughage source. They also observed that corn was the primary source of grain, whereas cottonseed was the primary by-product used in feedlot diets.

Through the results of ingredient contamination from the 10 most contaminated diets, it was possible to observe more specifically which ingredient was responsible for TMR contaminations, and which mycotoxin stood out in each source. As aflatoxin is a dangerous toxin for beef cattle, the ingredient that showed high concentrations of this mycotoxin, was peanut meal, which presented the highest REQ as well.

Aflatoxin is commonly found in peanut samples. In a study conducted by [15], aflatoxin was found in 40.4% of peanuts analyzed. The occurrence of aflatoxins is higher in peanuts because it is the preferred product for the fungi that produces this toxin, and there are also delays and rains in the post-harvest drying period. Another form of contamination is when the peanut is stored at high humidity [22].

In addition to peanut, aflatoxin can be found in many other products widely used in Brazil, such as corn and oilseeds [13]. These ingredients presented considerable contamination with high REQ, like corn and its by-products (refinazil, DDGS), as well as soybean and soybean residues. However, only peanut and DDGS by corn presented aflatoxin contamination.

Sugarcane straw also presented high REQ values. This contamination was due trichothecenes B, trichothecenes A and fusaric acid. These contaminations occurred because this material is the residue of the sugarcane that stays in the field after the harvest and is more subject to contamination.

The cluster analysis of all contaminated ingredients allowed the construction of a dendrogram resulting from hierarchical grouping analysis. In the dendrogram it is possible to observe the

formation of three groups: group 1 was characterized by the most contaminated ingredients, group 2 by medium contaminated ingredients and group 3 the less contaminated ingredients.

Through the results of the cluster analysis by non-hierarchical method it was possible to observe the formation of three different groups. Group 1 was that had high contamination of aflatoxin and other mycotoxins produced by *Aspergillus*, group 2 was the group that has high contamination of fumonisin, fusaric acid, mycotoxins produced by *Penicillium* and ergot while group 3 were the samples that had low contamination for all mycotoxins.

The clustering factors found in these analyses were the level of ingredients contamination. However, it was interesting that most of mycotoxins of the same group are produced by the same fungal genus, as in group 1, where the mycotoxins were produced by fungi of *Aspergillus* genus. In group 2, we observed fumonisin and fusaric acid, which are produced by Fusarium genus, although there is also the presence of ergot mycotoxins produced by *Penicillium*. In the group 3, we verified represents low contaminated samples for all mycotoxins.

In this way, we can define through these analyses is that the groups characterize the different ingredients, since each ingredient has a higher concentration of each mycotoxin. As example, corn and its by-products present concentrations of fumonisin and fusaric acid, whereas feeds, such as peanuts, have high concentrations of aflatoxin and mycotoxins by *Aspergillus.*

4. Conclusion

In conclusion, current data obtained in this study evidence that 100% of TMR are contaminated and some strategies need to be implement to minimize the risk for beef cattle. In addition, fumonisins were the mycotoxins found most frequently and at highest concentrations in TMR fed at Brazilian feedlots. Peanut meal was the most contaminated ingredient and more aggressive for beef cattle. Moreover, the greatest risk of contamination is in the combination of different mycotoxins instead of an isolated one.

5. Materials and methods

The survey was applied in the years 2015/2016. Thirty Brazilian feedlots located in five different Brazillian states: eight in Mato Grosso (MT), six in Mato Grosso do Sul (MS), seven in Goiás (GO), four in Minas Gerais (MG) and five in São Paulo (SP) (Table 5) were surveyed. These states are the 5 largest beef-producing states, responsible for 81.3% of all animals finished in feedlot in Brazil [23], and therefore were they chosen for the evaluation. Each feedlot was visited by the authors, where samples of ingredients and TMR were collected and a questionnaire about concerning aspects of the feedlot and its management was completed.

Table 5. Visited Brazilian states and their number of animals at feedlot.

Brazilian State	**Number of Animals**[1]	**% Total**
Mato Grosso	977,131	24.4
Goiás	817,442	20.4
Mato Grosso do Sul	636,395	15.9
São Paulo	628,940	15.7
Minas Gerais	197,906	4.90
Total	**4,008,764**	**100**

[1] [23]

The questionnaire contained 61 questions that were categorized into the following topics: general information about the facilities (12 questions), general cattle management (21 questions), diets (18 questions), concentrates and co-products used (4 questions), and roughage used (6 questions).

Samples of total mixed ration (TMR) (n=30) and ingredients were sent to the research facility (Colina, SP, BRA). These samples were lyophilized and ground in the laboratory. Subsequently, TMR samples were vacuum packed and sent to the 37+® Analytical Services Laboratory (Lexington, KY, USA) for mycotoxin analysis.

The evaluation of mycotoxins comprised two distinct steps: in a 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 (UPLC) electrospray ionization tandem mass spectrometry (ESI-MSMS) involving an isotopic dilution step and data normalization process. In a 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 (REQ) expressed in μg/kg of AFB1-equivalent [4].

After the evaluation of TMR samples, the ingredients of the 10 most contaminated TMR (n=41) were sent to the 37+® Analytical Services Laboratory (Lexington, KY, USA) for analysis of mycotoxins. This procedure was done to evaluate which ingredients were most responsible for TMR contamination.

The responses generated from the questionnaire and the analytical data were submitted to descriptive analysis using the UNIVARIATE procedure of SAS (SAS Institute, Inc., Cary, NC, USA) and multivariate statistics using STATISTICA (STATSOFT, Inc, Tulsa, OK, USA). The multivariate analyses were performed using cluster analysis, which allows grouping of the variables using the Ward method and considers the Euclidean distance for group establishment. Starting of the number of groups adopted in the cluster analysis by hierarchical method, the analysis of grouping has developed using a non-hierarchical method using the k-means algorithm, which complements the results of the previous analysis. Finally, through the analysis of principal components it was possible to evaluate the importance of each component and the discriminatory power of each variable.

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CHAPTER 3

The following article is in accordance with Journal of Animal Science publication guidelines.

Running head: Mycotoxins and adsorbent on finishing cattle

Mycotoxin contaminated diets and adsorbent affect performance of Nellore bulls finished in feedlot[1]

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ABSTRACT: Mycotoxins are present in almost all feedstuffs used in animal nutrition, but are often ignored in beef cattle systems, although they can affect animal performance. The objective of this study was to evaluate the effects of mycotoxins and mycotoxin adsorbent on performance of Nellore cattle finished in feedlot. One hundred Nellore cattle (430 ± 13 kg and 24 months) were used in a randomized block design with a 2 × 2 factorial arrangement of treatments. The factors consisted of two diets (Factor 1) with either natural contamination (**NC**) or exogenous contamination (**EC**) and presence (10 g/animal/d; **ADS**) or absence of mycotoxin adsorbent (Factor 2). The NC and EC diets had, respectively, the following contaminations: aflatoxins 0.00 and 10.0 µg/kg, fumonisins 5,114 and 5,754 µg/kg, trichothecenes B 0.00 and 42.1 µg/kg, trichothecenes A 0.00 and 22.1 µg/kg, and fusaric acid 42.9 µg/kg for both diets. At the beginning of the experiment, all animals were weighed, and 4 randomly selected animals were slaughtered to evaluate initial carcass weight. After 97 days of experiment, all animals were weighed and slaughtered. There was no interaction among factors for dry matter intake (**DMI**; $P = 0.92$). However there was a tendency for exougenous contamination diets (EC) to decrease DMI by 650 g/d ($P = 0.09$). There was a trend for interaction among factors ($P = 0.08$) for ADG, where the highest ADG was observed for natural contamination diets without adsorbent (NC-) (1.77 kg) and the lowest was observed

for EC without adsorbent (1.51 kg). The NC+ADS and EC+ADS treatments presented intermediate values (1.65 and 1.63 kg, respectively) and they did not differ significantly from NC- and EC- treatments. The animals fed NC diet had greater final BW (596 kg) than those at the EC diet (582 kg; $P = 0.04$). There was a tendency for interaction among factors for carcass gain ($P = 0.08$). Similar to ADG, the highest carcass gain was observed for NC without adsorbent (1.20 kg/d) and the lowest was observed for EC without adsorbent (1.05 kg/d). The NC+ADS and EC+ADS treatments presented intermediate values (1.14 and 1.12 kg/d, respectively) but they did not differ significantly from NC- and EC- treatments. So, the NC- had greater carcass gain compared to EC- and the use of ADS recovered part of the gain when used in EC diets. In conclusion, mycotoxin affects the performance of beef cattle and adsorbent may mitigate its impact.

Key words: carcass gain, dressing, fungi, mycotoxin production

INTRODUCTION

The effects of mycotoxins in animals depend of the amount consumed, exposure time, and interaction among different toxins (Upadhaya et al., 2010). These effects may impact reproduction, immune system and performance (Zain, 2011). Since 1968, it is known that mycotoxins affect beef cattle (Garrett et al., 1968), in addition, a recent study of Custodio et al. (2017) reported that at least one type of mycotoxin is presented in the samples of feedlot diets collected in a survey conducted in Brazil. However, studies evaluating the effects of mycotoxin on ruminant were developed mainly with dairy cattle (Fink-Gremmels, 2008; Chaiyotwittayakun, 2010; Santos and Fink-Gremmels, 2014). The main effects involved reduced dry matter intake and consequently reduced performance in dairy cattle (Fink-

Gremmels, 2008). Moreover, Osweiler et al. (1993) observed that the ingestion of mycotoxins can elevate serum enzyme activity of liver enzymes, suggesting impairment of liver function.

The mycotoxin adsorbents may be an effective strategy to reduce or even control the harmfull effects of these toxins (Jouany, 2007). Adsorbents may decrease the mycotoxins absorption by the animals creating stable chemical interactions with the toxins and further limiting their bioavailability in the gastrointestinal tract (Yiannikouris and Jouany, 2002). Merril et al. (2007) studying milk production of beef cattle (Angus × Hereford) fed mycotoxin contaminated diets, observed a linear increase in milk production (9.8 to 14.2 kg/d) as the intake of yeast cell wall adsorbent increased (0 to 60 g/d). However, studies using beef cattle and effects of mycotoxin adsorbents on performance are still scarce.

So, we hypothesized that the mycotoxins contaminated diets could decrease performance of Nellore bulls finished in feedlot and that the yeast cell wall adsorbent could attenuate this damage. Thus, the objective was to evaluate the effects of mycotoxin levels and adsorbent on intake and performance of Nellore cattle finished in feedlot.

MATERIALS AND METHODS

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 State Law No. 11.977 of the São Paulo state (SP) 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*, Jaboticabal campus (protocol number 15473/15).

Experimental animals and treatments

The experimental period lasted 97 days, divided into an adaptation period of 28 days and 69 days for the evaluation period. In total, one hundred 24-mo-old Nellore bulls (430 ± 13

kg of body weight [**BW**]) were used into a randomized block design (blocked by initial BW) in a 2 × 2 factorial arrangement of treatments. Four animals, representative of different BW blocks, were slaughtered at the beginning of the study as baseline group. The remaining animals were allocated into thirty-two pens (3 animals per pen) where each pen was considered an experimental unit. All pens measured 4 × 15 m (totaling area 60 m^2, feed bunk of 4 m) and were equipped with individual water fountain with 100 L capacity, with high flow valve and covered trough.

The treatments were evaluated into a 2 × 2 factorial arrangement to investigate the main effects of diets, adsorbent and their interactions. The factor 1 was the diet while the factor 2 was the presence or the absence of adsorbent (10 g/animal/day). The organic mycotoxin adsorbent (**ADS**) (Mycosorb A^+, Alltech, Nicholasville, KY) was composed of internal yeast cell wall (*Saccharomyces cerevisiae*) and algae. In the beginning of the study, the ADS was added in all mineral mix of animals receiving this treatment. The treatments were as follows: diet with natural contamination without ADS (**NC-**); diet with natural contamination + adsorbent (**NC+ADS**); diet with exogenous contamination without ADS (**EC-**); diet with exogenous contamination + adsorbent (**EC+ADS**).

Mycotoxin production and evaluation in the diets

The identity and the dose of mycotoxins used in this study were based on the survey previously conducted by Custodio et al. (2017). This initial survey aimed to identify the type of mycotoxin mainly occurring 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 in the Mycotoxin and Mycology Laboratory of the Department of Agroindustry, Food and Nutrition of

ESALQ/USP (Piracicaba, SP, BRA). The production of mycotoxins used a natural fermentation of corn or wheat by mycotoxin-producing fungal species. The strain of *Aspergillus flavus* was used for aflatoxins B1 and B2 production, the strain of *Fusarium verticilioides* for fumonisins, the strain of *Fusarium graminearum* for trichothecenes B and the strain of *Fusarium sporotrichioides* for trichothecenes A. The concentration of each mycotoxin in the diet was standardized throughout the experiment period and these mycotoxins were weighed and added in the diets daily during the feedstuffs mixing (only in the exogenous contaminated diets). The mycotoxin was stored in a sterilized room until use.

Once a week, one sample of each feedstuff used in the diets was collected and a composite sample was analyzed at the end of the experiment. Thus, the composite samples were sent to the Alltech 37+® Analytical Services Laboratory (Lexington, KY, USA) where mycotoxin evaluation was performed and comprised into 2 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 (**UPLC**) electrospray ionization tandem mass spectrometry (Waters Acquity Tqd, Waters Corp., Milford, MA, USA), involving isotopic dilution step and a data normalization process. In the second step, the mycotoxin concentrations were further interpreted according to known specie specific sensitivities and normalized according to the principles of toxic equivalent factors, determining the risk equivalent quantities (**REQ**) expressed in µg/kg of aflatoxin B1 (**AFB1**) - equivalent (Yiannikouris, 2015) (Table 1).

Feed management

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

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

The diet was provided once a day at 0800 hours, using a RX-65E Casale mixer wagon (Casale, São Carlos, SP, BRA) equipped with a scale. The diets were provided *ad libitum* (Table 2), while the orts were weighed daily, and the amount offered adjusted to maintain 1 to 3% orts of the amount supplied in order to reduce selection and to measure dry matter intake (**DMI**).

Chemical analyses

Chemical analyses of ingredient samples were carried out at the Laboratory of Analysis of Products of Plant and Animal Origin (**LAPROVA**; APTA, Colina, SP, BRA). Samples were partially dried at 55ºC 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 dry matter (**DM**; method 934.01), ash (method 942.05), crude protein (**CP**; method 978.04), and ether extract (**EE**; method 920.39) were measured according to recommendations of the AOAC (1995). The contents of neutral detergent fiber (**NDF**) and acid detergent fiber (**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 non-protein nitrogen (**NPN**), neutral detergent insoluble nitrogen (**NDIN**), and acid detergent insoluble nitrogen (**ADIN**) were determined according to Licitra et al. (1996). To obtain ash, the samples were incinerated in a muffle furnace, at 550ºC, according to the AOAC (1995) procedure. 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).

The net energy (**NE**) for gain (Mcal/d) and NE for maintenance (Mcal/d) were calculated thought the equations of the NRC (1984) and Lofgreen and Garrett (1968), respectively. These estimates were used to obtain the NE for maintenance of the diet (Mcal/kg DM) (NRC, 1984) and NE for gain of the diet (Mcal/kg DM) (Zinn and Shenn, 1998).

Blood sampling

On the days 0, 14, 28, and 97, all animals were kept solids fasting for 8 h, then blood was collected via jugular venipuncture beginning at 8 h in the morning and completed within 4 h. Serum blood samples were collected into tubes without avoiding hemolysis, placed on ice, and centrifuged (3,000 × g for 20 min at 4°C) within 1 h after collection. They were placed in labelled Eppendorf tubes and stored at -20 °C until subsequent analysis for the following serum enzymes: aspartate aminotransferase (**AST**) and gamma-glutamyl transferase (**GGT**). The enzyme analyses were performed by a colorimetric method by using commercial kits (LabQuest, Campinas, SP, BRA).

The contents of total protein, urea, creatinine, alkaline phosphatase, magnesium, total cholesterol, and triglycerides were analyzed using an automatic analyzer with high performance (**HPLC**) for biochemical and turbidimetric tests (Labmax Plenno, Nasu-Gun, Tochigi, JAP).

Slaughter and animal performance

At the beginning of the experiment, after 8 hours of solid fasting, 4 animals (randomly selected) were transported to the slaughterhouse (Minerva Foods, Barretos, SP, BRA), located 20 km from the research facility. After arrival at the slaughterhouse, the animals were kept in resting pens for 18 h and then submitted to humanitarian slaughter under Brazilian Federal

Inspection and the hot carcass weight (**HCW**) was obtained. After slaughter, the half-carcasses were placed in the cold chamber for 24 h at a temperature of 2˚C.

The initial carcass weight at the remain animals were estimated using a regression equation between BW and HCW from data of the baseline group, as follows:

$Y = 0.613x – 38.9$ ($R^2 = 0.978$);

where: x = initial body weight, kg and Y = initial hot carcass weight, kg.

After 97 days of experiment and 8 hours of solids fasting, all remained animals were slaughtered, following the same procedure of baseline groups, and the final HCW was obtained. Then, the carcass gain was determined by subtracting the final HCW from initial. Carcass feed efficiency were calculated dividing carcass gain (kg/d) by DMI.

Final BW was calculated from HCW divided by the average dressing percent of all treatments. Thus, the ADG ((final BW – initial BW)/97) and feed efficiency (ADG/DMI) were calculated.

Organs and histopathology

During the slaughter, 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 samples of kidney and liver 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 color (L*, a*, and b*), were analyzed according to Müller (1987).

Statistical Analyses

The experiment was conducted in a randomized block design with a 2 × 2 factorial arrangement of treatments, each pen was considered as an experimental unit and initial BW was the criteria adopted to form the blocks (8 blocks; 1 replication/block; 8 pens/treatment; 3 animals/pen). Data were analyzed as a mixed model with the fixed effects of contamination, ADS, and their interactions, whereas block was considered random in the model.

The analysis of blood and DMI data were submitted to analysis of variance as repeated measures over time, using the REPEATED statement of SAS (SAS Inst. Inc., Cary, NC). For blood variables, the initial collection was considered as covariate. Different residual covariance structures were tested to determine the structure that best fit each variable. The covariance structure was chosen using the Bayesian information criteria (BIC), in which the lowest value of BIC was used as a selection.

For all statistical analyses, the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used. Differences were considered significant when $P < 0.05$ while 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 tendency ($P = 0.09$) to decrease DMI by 5.14% when diets with exogenous contamination were fed to Nellore cattle. When analysed weekly, DMI of animals fed EC diets was lower ($P = 0.01$) when compared to NC in 6 weeks of the whole study period (Figure 1).

The final BW was affected by contaminated diets (P = 0.04). Animals fed NC diets were 14.5 kg heavier than animals fed EC diet (Table 3). The ADG had a trend (P = 0.08) of interaction between contaminated diets and the use of ADS. The ADG of animals fed NC- diets was 17.2% greater than EC- diet, while the NC+ADS and EC+ADS diets presented intermediate values, respectively, 9.3 and 7.9% greater compared to EC- diet. Regarding to estimated dietary NE, the NC- and EC+ADS diets presented greater (P = 0.03) NE maintenance and NE gain compared to NC+ADS and EC- diets (Table 3).

There was interaction between contamination diets and ADS for dressing percentage (P = 0.04) and carcass feed efficiency (P = 0.02) and there was tendency of interaction among factors for final HCW (P = 0.10) and carcass gain (P = 0.08; Table 3). Animals fed NC-, NC+ADS and EC+ADS had 1.78, 0.71, and 1.07% more dressing percentage compared to animals fed EC- diets, respectively. Animals fed NC- and EC+ADS had greater carcass feed efficiency than EC- and NC+ADS. The HCW of NC-, NC+ADS and EC+ADS were numerically 14, 8 and 6 kg heavier than animals fed EC- diets. Finally, the highest carcass gain was observed for NC- and the lowest was observed for EC-. Moreover NC+ADS and EC+ADS treatments presented intermediate values and they did not differ significantly from NC- and EC- treatments.

Metabolic Variables

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

Serum concentrations of total protein, alkaline phosphatase, magnesium, total cholesterol, and triglycerides did not present interactions among factors ($P \geq 0.12$; Table 4).

However, concentrations of urea and creatinine tended to have interaction among factors (P = 0.08). Urea was greater for NC+ADS and EC- diets than NC- and EC+ADS diets. In a different way, creatinine was greater for NC- diets compared to the other treatments. There was interaction for diet contaminations and experiment period for urea and creatinine ($P \leq$ 0.04). The values of urea increased 9.10 mg/dL for NC diets and 1.25 mg/dL for EC diets at the end of the experiment. For creatinine, animals fed NC increased from 1.85 to 1.88 mg/dL and animals fed EC diets did not present different values. The mean values of descriptive components of the blood variables are within the normal range for cattle in finishing phase as reported by González (2000).

There was no interaction between 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 use of ADS in diets (P = 0.09). There was no effect of contamination diets, ADS or interaction among factors on the histopathology of liver ($P \geq 0.33$) and kidneys ($P \geq 0.14$).

Results for organ color (Table 5) did not present interactions ($P \geq 0.10$) between diets and ADS. However, the b* color for kidney decreased by the contaminated diets and the use of the adsorbent ($P < 0.01$).

DISCUSSION

This study shows effects of mycotoxins and an adsorbent (Mycosorb A^{+}) on beef cattle performance. There are few studies about the effects of mycotoxins on beef cattle (Merril et al., 2007; Tagaki et al., 2011); however, these previous studies evaluated isolated mycotoxins. Multiple toxins are normally found in the diets (Zain, 2011; Custodio et al., 2017) and, besides this, mycotoxins can act combined and synergistically in animals (Yiannikouris and Jouany, 2002; Oh et al., 2017).

It was observed that exogenously contaminated diets affected DMI during almost the entire experimental period, probably because of metabolic disturbances in the rumen (not measured in this study). According to Santos and Fink-Gremmels (2014), mycotoxins can impair rumen function, causing sub-acute rumen acidosis and impaired metabolism of carbohydrates. Moreover, mycotoxins can affect function of vital organs and cause immunosuppression (Tagaki et al., 2011; Marczuk et al., 2012); however, in the present study the relatively chronic mycotoxin challenge did not impact metabolic organs, maybe due to the REQ of exogenous contamination diet being classified as low for beed cattle (Yiannikouris, 2015).

The DMI strongly influences animal performance (Koknaroglu et al., 2005), thus, the final BW and ADG were affected by the different DMI. In this way, the mycotoxin contamination of the diet reduced the growth rate and the ADS was able to recover part of this damage. Likewise, Merril et al. (2007), studying the use of ADS, 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. On the other hand, Queiroz et al. (2012) did not observe an influence of aflatoxin B1 and montmorillonite-based mycotoxin adsorbent on DMI and milk yield of dairy cows.

In this study, the lower DMI arguably explains part of the reduction in performance for EC diet compared to NC and the other part was probably caused by damage to animal metabolism. Analyzing the beef cattle requirements (BR-CORTE; Valadares Filho et al., 2016) and DMI to reach the observed final BW suggest that the different DMI in treatments explains about 60% of the difference in growth rate, and the other 40% probably is due to metabolic factors.

The ADS did not increase DMI, and this may be the reason that cattle fed ADS it recovered only part of the loss in growth rate caused by the toxins. However, the ADS, by chemically interacting with mycotoxins, prevented these toxins from damaging the metabolism, for example causing a sub-acute rumen acidosis, and thereby allowing animals to recover some of the lost performance caused by EC diets.

Yeast cell walls and their constituents have been shown to adsorb a large spectrum of mycotoxins *in vitro* (Yiannikouris et al., 2006; Yiannikouris et al., 2013) and *in vivo,* to alleviate the effects of dietary mycotoxin exposure in several species (Diaz et al., 2004; Chowdhury et al., 2005; Diaz-Llano and Smith, 2006; Meissonier et al., 2009; Firmin et al., 2011). However, the efficacy of this adsorbent depends on the type of mycotoxins (Patience et al., 2014). Kong et al. (2014), studying yeast cell wall activity *in vitro,* observed an adsorption of 92.7% of aflatoxin but only 22.9% 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 by cattle fed ADS was reflected in ADG and carcass gain. No data were found in the literature related to carcass weight of beef cattle fed mycotoxin contaminated diets and adsorbent. However, Rossi et al. (2010) observed lower dressing percentage and breast weight for chickens fed diets contaminated with aflatoxin B1 compared to uncontaminated diets, and when ADS was added to the diet, the animals showed an improvement in dressing percentage.

The observed values for NE for maintenance and NE for gain provide further evidence of a detrimental effect of the exogenous contamination which, may be reduced by the application of an adsorbent in the diet. The greater NE for gain in NC- and EC+ADS diets is due to the better energy utilization by the animals, improving performance and efficiency in

converting this energy into carcass. So, mycotoxin decreases the NE in the diets and when the ADS is used in EC diet, occurs improvement of this NE for gain.

Regarding metabolic variables, normally ruminants fed mycotoxin contaminated diets have elevated serum metabolites including urea, protein, aspartate aminotransferase, and gamma glutamyl transferase (Chaiyotwittayakun, 2010). Besides this, enzyme tests are widely used as indicators of the effect of chemical or toxicological substances in the liver (Moreira et al., 2012). Elevated serum enzyme activity of diagnostic liver enzymes (AST and GGT), can be signs of intoxication, suggesting hepatocellular injury (Fink-Gremmels, 2008). The fact that levels of AST and GGT were unaltered among treatments in this study, may indicate that there was no liver damage in these animals, because mycotoxins did not reach this organ.

The non-alteration of enzymes linked to hepatic metabolism may be related to the low contamination of diets, even in EC diet. Osweiler et al. (1993) observed that doses above 150 mg/kg of fumonisin B1 caused hepatic alteration 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 5,754 µg/kg or 5.754 mg/kg. In another study with beef cattle, Garrett et al. (1968) observed liver alteration with doses above 100 µg/kg of aflatoxin B1, higher than doses observed in this study (10 µg/kg).

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

Differently from data found in literature, the organ weight in this study did not show interactions between diet contamination and ADS. As mentioned before, probably this can be explained by low level of contamination 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. In the same way, Fernandez et al. (1997) observed lambs fed 2.5 mg/kg of aflatoxin for 21 days presented hepatic and nephritic injuries, 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 mg/kg of aflatoxin for 28 days showed enlargement, pallor or yellowish discoloration, and kidneys were enlarged and pale. In our study, the b* color of kidney was affected by both factors, contaminated diets and ADS, but we have no explanation for this.

Regarding organ histopathology, some authors have documented that contaminated diets can caused liver lesions and multi-organ damages, including necrosis and hemorrhage in pigs, when they were fed diets with at least 3,000 µg/kg of aflatoxin B1/kg or 1,000 µg/kg of DON/kg (Harvey et al., 1989; Cheng et al., 2006; Chen et al., 2008). In this study, the less-severely contaminated diets and ADS did not affect these variables. In the same way, Accensi et al. (2006) showed no damage when pigs were fed diets with less than 840 µg/kg of DON/kg. On the other hand, Kumar and Balachandran (2009) observed histopathologically degenerative and necrotic changes in the liver and kidneys of broilers fed 1 mg/kg of aflatoxin. We highlight that monogastric animals are more susceptible to mycotoxins compared to ruminants.

In conclusion, under the conditions of this experiment, mycotoxins affect beef cattle performance and the inclusion of ADS in the diet allowed the partial recovery of performance when animals were fed exogenous contaminated diets.

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Table 1. The most frequent mycotoxins found in diets used in feedlots and their concentration in the natural and exogenous diets contamination.

Mycotoxin[1]	NC[2]	Exogenous contamination	EC[2]
Aflatoxin, µg/kg	-	10.0	10.0
Fumonisin, µg/kg	5,114	640	5,754
Trichothecenes B, µg/kg	-	42.1	42.1
Trichothecenes A, µg/kg	-	22.1	22.1
Fusaric acid, µg/kg	42.9	-	42.9
REQ, µg/kg	15.0	-	45.0

[1]Aflatoxin: B1+B2+G1+G2; Fumonisin: B1+B2; Trichothecenes B: DON, 15-acetyl DON, 3-acetyl DON, fusarenol X, nivalenol, and DON 3-glicoside; Trichothecenes A: T-2, H-T2, diacetoxiscirpenol, and neosolaniol; REQ: Risk Equivalent Quantities.
[2]NC: Diet with natural contamination, EC: Diet with exogenous contamination.

Table 2. Ingredients used in the diets and chemical composition of the diets used in the feedlot.

Ingredients (%)	Adaptation Diet	Finishing Diet
Sugarcane bagasse	22.0	12.0
Corn	39.8	53.0
Citrus pulp	20.0	20.0
Cottonseed meal	15.0	11.8
Mineral[1]	3.20	3.20
Chemical Composition (% of DM)	**Adaptation Diet**	**Finishing Diet**
Dry Matter[2]	76,9	82.4
Crude Protein	13.8	13.7
Ether Extract	2.54	3.14
Ash	3.31	2.87
Neutral Detergent Fiber	32.9	24.5
Acid Detergent Fiber	22.8	15.9
Lignin	6.07	4.30
Non-Protein Nitrogen, % of Total Nitrogen	21.3	20.9
Soluble Nitrogen, % of Total Nitrogen	25.6	25.8
Neutral Detergent Insoluble Nitrogen, % of Total Nitrogen	28.9	22.1
Acid Detergent Insoluble Nitrogen, % of Total Nitrogen	19.2	13.9
Phosphorus	0.21	0.22
Calcium	0.57	0.59
Sodium	0.07	0.07
Potassium	0.60	0.60
Total Digestible Nutrients	68.0	74.2
Metabolizable Energy, MJ/kg	10.3	11.2

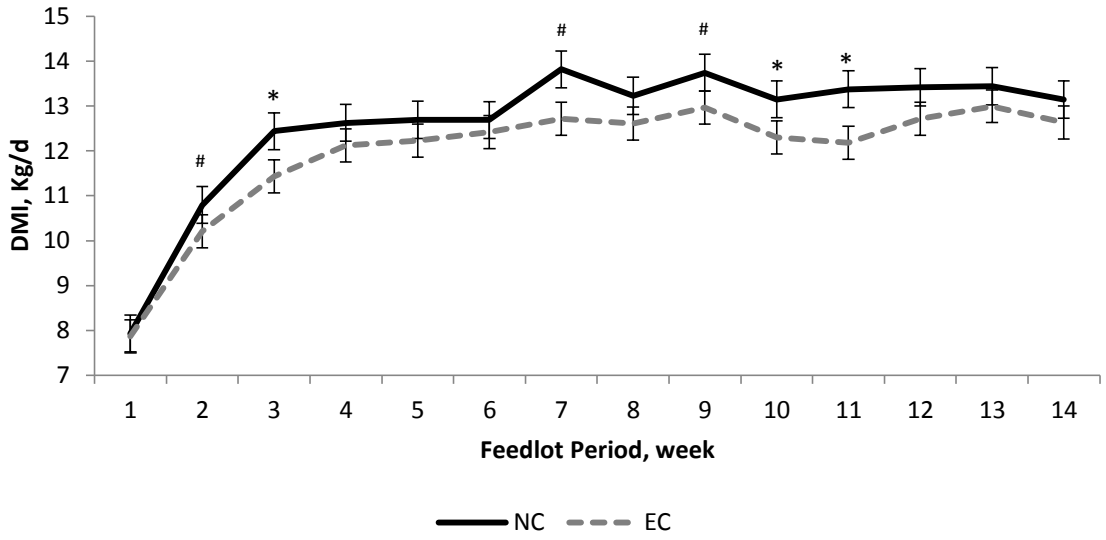
[1] Mineral = Sodium: 43g/kg; Calcium: 106g/kg; Phosphorus: 12.6g/kg; Sulfur: 34g/kg; Magnesium: 14.1g/kg; Potassium: 76g/kg; Manganese: 382mg/kg; Zinc: 1231mg/kg; Iron: 373mg/kg; Copper: 373mg/kg; Cobalt: 49 mg/kg; Iodine: 36mg/kg; Selenium: 5mg/kg; Fluorine: 106mg/kg; Monensin: 800mg/kg; Vitamin A: 89992 IU/kg; NPN: 88.8%; Crude Protein: 91.1%.

[2] Dry Matter: dry matter content of diet was adjusted daily with water to maintain 65%.

Table 3. Feedlot performance of Nellore cattle fed diets containing either natural mycotoxin contamination or exogenous contamination, with or without adsorbent.

	Treatments[2]				SEM[3]	P-value[4]		
Item[1]	NC-	NC+ADS	EC-	EC+ADS		Myco	ADS	Myco×ADS
Performance: Live Basis								
Initial BW, kg	431	430	430	429	-	-	-	-
Final BW, kg	602	590	576	587	8.03	0.04	0.94	0.11
DMI, kg/d	12.7	12.6	12.0	12.0	0.38	0.09	0.88	0.92
DMI, % of BW	2.46	2.47	2.39	2.36	0.07	0.16	0.90	0.78
NE maintenance, Mcal/kg of DM	1.86[a]	1.79[b]	1.75[b]	1.85[ab]	0.04	0.54	0.74	0.03
NE gain, Mcal/kg of DM	1.22[a]	1.16[ab]	1.13[b]	1.21[a]	0.03	0.54	0.76	0.03
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/d	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

[1]Final BW = calculated from hot carcass weight divided by the average dressing percent of all treatments; DMI, % of BW, ADG and Feed efficiency were calculated through the adjusted final BW;
[2]NC- = natural contamination diet without adsorbent; NC+ADS = natural contamination diet with adsorbent; EC- = exogenous contamination diet without adsorbent; EC+ADS = exogenous contamination diet with adsorbent
[3] SEM = standard error of the means;
[4] Myco = effect of the diet; ADS = effect of presence or absence of adsorbent; Myco × ADS = interaction between diet and adsorbent;
Means without a common letter are different based on T test ($P < 0.05$)



NC = natural contamination; EC = exogenous contamination;

Figure 1. Dry matter intake of animals fed diets with natural mycotoxin contamination and exogenous contamination, with or without yeast-derivate adsorbent, in Nellore cattle measured weekly during feedlot period. 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 $P = 0.78$. *Indicated significant difference among diets ($P < 0.05$). #indicated a tendency of significant difference among diets ($0.05 \geq P \leq 0.10$).

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

Item[1]	Treatments[2]					SEM[3]	P-value[4]			
	D	NC-	NC+ADS	EC-	EC+ADS		Myco	ADS	Myco×ADS	D
AST (U/L)	14	58.6	64.6	56.1	60.8	1.93	0.34	0.67	0.29	<0.01
	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
	28	24.3	23.0	23.2	25.0	0.53				
	96	30.9	29.0	31.4	31.7	1.11				
AP (U/L)	14	249	219	242	246	12.9	0.26	0.81	0.45	0.75
	28	229	237	233	249	12.8				
	96	229	229	241	232	12.8				
TP (g/dL)	14	7.37	7.22	7.32	7.40	0.18	0.72	0.62	0.79	0.03
	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
	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
	28	1.87	1.85	1.85	1.87	0.01				
	96	1.88	1.87	1.85	1.86	0.01				
Mg (mg/dL)	14	2.84	2.89	2.81	2.81	0.12	0.15	0.68	0.12	<0.01
	28	2.60	3.04	2.77	2.55	0.12				
	96	2.66	3.04	2.54	2.55	0.12				
TC (mg/dL)	14	114	88.7	89.9	89.7	13.0	0.35	0.61	0.38	0.79
	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
	28	8.48	9.62	7.35	7.95	1.50				
	96	10.5	7.99	7.31	10.7	1.19				

[1] AST = aspartate aminotransferase; GGT = gamma glutamyl transferase; AP = Alkaline phosphatase; TP = Total protein; CR = Creatinine; Mg = Magnesium; TC = Total cholesterol; TR = Triglycerides; D = Day;
[2]NC- = natural contamination diet without adsorbent; NC+ADS = natural contamination diet with adsorbent; EC- = exogenous contamination diet without adsorbent; EC+ADS = exogenous contamination diet with adsorbent
[3] SEM = standard error of the means;
[4]Myco = effect of the diet; ADS = effect of presence or absence of adsorbent;
The interactions of mycotoxin and day were only significant for urea ($P = 0.03$) and creatinine ($P = 0.05$). Interactions between adsorbent and day and triple interactions were included in the model, but these results are not reported in Table ($P \geq 0.20$).

Table 5. Weight, histopathology, and colors of organs of animals fed diets with natural mycotoxin contamination and exogenous contamination. with or without adsorbent in Nellore cattle finished in feedlot.

		Treatments[2]				SEM[3]	P-value[4]		
Item[1]	Color	NC-	NC+ADS	EC-	EC+ADS		Myco	ADS	Myco×ADS
Liver/100 kg of car. kg		2.17	2.17	2.13	2.15	0.04	0.40	0.82	0.78
Kidneys/100 kg of car. kg		0.31	0.31	0.32	0.29	0.01	0.92	0.09	0.11
TTSL		45.6	47.1	47.0	53.5	40.5	0.34	0.33	0.54
TTSK		51.3	62.3	61.2	58.9	43.2	0.46	0.32	0.14
Liver	L*	26.6	27.0	26.3	27.0	0.69	0.84	0.37	0.83
	a*	14.4	14.5	13.9	14.3	0.36	0.20	0.36	0.47
	b*	11.3	11.9	11.2	11.7	0.44	0.69	0.16	0.97
Kidney	L*	28.0	26.3	27.9	25.4	0.70	0.40	<0.01	0.49
	a*	14.6	14.2	14.6	13.6	0.33	0.41	0.05	0.40
	b*	12.3	10.9	11.7	9.09	0.36	<0.01	<0.01	0.10

[1]Liver/100 kg of car = liver for 100 kg of carcass; Kidneys/100 kg of car = kidneys for 100 kg of carcass; TTSL = Total tissue score of liver; TTSK = Total tissue score of kidneys. HE. obj. 200x.
[2] NC- = natural contamination diet without adsorbent; NC+ADS = natural contamination diet with adsorbent; EC- = exogenous contamination diet without adsorbent; EC+ADS = exogenous contamination diet with adsorbent
[3] SEM = standard error means;
[4] Myco = effect of the diet; ADS = effect of presence or absence of adsorbent; Myco × ADS = interaction between diet and adsorbent;

CHAPTER 4

The following short comunication is in accordance with Meat Science Journal's publication guidelines.

Do mycotoxin contaminated diets and yeast cell wall adsorbent affect meat quality of Nellore bulls finished in feedlot? - A short communication

Declarations of interest: none.

ABSTRACT

Ninety-six Nellore bulls (430 ± 13 kg and 24 months) were assigned to a completely randomized block design (2 × 2 factorial arrangement of treatments) to evaluate meat quality. Dietary treatments consisted of natural or exogenous contamination with mycotoxins (Factor 1), with or without adsorbent (10 g/animal/d; Factor 2). The diets were provided during 97 d. The meat chemical composition was unaffected ($P \geq 0.61$) by the factors and the averages of variables were 74.2% moisture, 22.7% protein, 1.04% ether extract, and2.10% ash. The *L**, *a**, *b**, *E**, *C** ($P \geq 0.38$), cooking loss ($P = 0.94$) and Warner-Bratzler shear force ($P = 0.50$) were also similar among factors. Under the condition of this study, mycotoxin-contaminated diets and adsorbent do not affect meat quality of Nellore bulls finished in feedlot.

Keywords: adsorbent, aflatoxin, beef cattle, cooking losses, mycotoxin, WBSF

1. Introduction

Diet affects meat quality (Guerrero, Valero, Campo & Sañudo, 2013); also, contamination of diets with mycotoxins can influence the meat characteristics due to the metabolic effects on animal (Surai et al., 2002). Ruminants may be less affected by mycotoxins than nonruminant animals, because ruminal microorganisms inactivate some of these compounds (Upadhaya, Park & Ha, 2010). However, not all mycotoxins are inactivated in the rumen (Fink-Gremmels, 2008). Then, as different mycotoxins are present in almost all ingredients used for beef cattle (Custodio et al., 2017) and these toxins can affect performance and health of the animals (Gallo, Giuberti, Frisvad, Bertuzzi & Nielsen, 2015), they may also affect meat quality.

Mycotoxin contamination affects negatively meat color and the use of organic adsorbent improved color characteristics of chicken meat (Wang, Fui, Miao, Feng, 2006). According to Surai et al. (2002), mycotoxins, such as T-2 toxin, Deoxynivalenol (DON), and Fumonisin, induced high lipid peroxidation that can result in muscle cell membrane breakage.

However, there are no previous studies about meat characteristics of beef cattle fed mycotoxin-contaminated diets and adsorbents in the literature.

Therefore, based on the evidence that mycotoxins affect beef cattle performance (Custodio et al., 2018), our hypothesis is mycotoxins may decrease meat quality of beef cattle and an adsorbent based on yeast cell wall may attenuate this effect. Thus, the objective of this study was to evaluate the effect of mycotoxins and adsorbent on meat quality of Nellore bulls finished in feedlot.

2. Materials and methods

Ethical clearance was obtained from the Ethics Committee on Animal Use of the *Universidade Estadual Paulista*, Jaboticabal campus (protocol number 15473/15).

Ninety-six contemporary Nellore bulls (430 ± 13 kg and 24 months) were assigned to a completely randomized block design (blocked by initial BW; 8 blocks) in a 2 × 2 factorial arrangement of treatments. The animals were housed into 32 pens (3 animals/pen; 8 pens/treatment) where each pen was considered an experimental unit. All pens measured 4 × 15 m (feed bunk of 4 m and water fountain with 100 L capacity).

Diet treatments consisted of natural contamination (NC) or mycotoxin exogenous contamination (EC), with or without adsorbent (Mycosorb $A^{+®}$ [ADS], Alltech Inc., Nicholasville, KY, USA), performing a 2 × 2 factorial arrangement (two levels of mycotoxin contamination and presence or absence of adsorbent). The adsorbent was provided in the amount of 10 g/animal daily, which is an organic mycotoxin adsorbent composed of internal yeast cell wall (*Saccharomyces cerevisiae*) and algae.

The definition of mycotoxins and the doses used were based on a survey previously conducted (Custodio et al., 2017). The mycotoxins were produced individually in the Mycotoxins and Mycology Laboratory of the Department of Agroindustry, Food and Nutrition at ESALQ/USP (Piracicaba, SP, BRA). The production of mycotoxins was from the natural fermentation of corn or wheat by mycotoxin-producing fungal species. There were used a strain of *Aspergillus flavus* for Aflatoxin B1 (AFB1) and B2 production, a strain of *Fusarium verticilioides* for Fumonisin, a strain of *Fusarium graminearum* for Trichothecenes B, and a strain of *Fusarium sporotrichioides* for Trichothecenes A.

The finishing diet contained sugarcane bagasse (12%), ground corn (53%), citrus pulp (20%), cottonseed meal (11.8%), and mineral mix (3.20%), with 13.7% crude protein and

74.2% total digestible nutrients. The *ad libitum* diet was provided once a day at 8:00 am. The concentration of each mycotoxin was standardized throughout the experiment period in the diet and these mycotoxins were weighed and added in the EC diets daily when the feedstuffs were mixed into each bucket.

Mycotoxin diet evaluation was performed at the Alltech 37+® Analytical Services Laboratory (Lexington, KY, USA). In the first step, the absolute quantification of 38 different mycotoxins was performed by means of ultra-performance liquid chromatography (UPLC) electrospray ionization tandem mass spectrometry (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 specie specific sensitivities and normalized according to the principles of toxic equivalent factors, determining the risk equivalent quantities (REQ) expressed in µg/kg of AFB1-equivalent. Natural contamination diet presented, in µg/kg, Fumonisin (5,114) and Fusaric acid (42.9). Exogenous contamination diet presented, in µg/kg, Fumonisin (5,754), Fusaric acid (42.9), Aflatoxin (B1+B2+G1+G2; 10.0), Trichothecenes A (22.1), and Trichothecenes B (42.1). Therefore, the calculated REQ was 15 and 45 µg/kg, respectively, for NC and EC diets.

After 97 d in the feedlot, the animals were transported to the slaughterhouse (Minerva Foods®, Barretos, SP, BRA) located 20 km from the research facility, were kept in resting pens for 18 h with free access to water and then submitted to humanitarian slaughter under Brazilian Federal Inspection. Hot carcass weight was obtained (average of 334 ± 13.3 kg) and then they were placed in the cold chamber for 24 h at 2˚C.

Twenty-four hours after slaughter, the ultimate pH, ribeye area and backfat thickness were measured on the left side of the carcass, between the 12th and 13th ribs (Cañeque & Sañudo, 2005). Steaks from the *longissimus thoracis et lumborum* were cut and individually vacuum-packaged for analysis at 1 (24 hours after slaughter; two steaks) 7, 14, and 28 d after slaughter (aged steaks, one steak/d). After, they were frozen until posterior the analysis at -20°C.

Meat composition (one steak from day 1) was analyzed using near infrared analysis through the equipment FoodscanTM (FOSS, Hillerod, DEN). One steak, from each day, was used for color, WBSF and cooking losses analyzes. L^* (lightness), a^* (redness), and b^* (yellowness) were measured at three per steak using HunterLab colorimeter (4500L, Petaling-

Jaya, SE, MAL), and *E** (color difference) and *C** (Chroma) were calculated (Cañeque & Sañudo, 2005).

Warner-Bratzler shear force (WBSF) steaks were prepared according methodology proposed by (Belk et al., 2015). After that, six cores per steak with 1.27 cm diameter each were removed parallel to the longitudinal orientation of muscle fibers and sheared perpendicular to the longitudinal orientation of the muscle fibers with a WBSF attachment (1-mm thick) using a Texture Analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY, USA) fitted with a 25 kg load cell. Maximum force measured to cut each core was expressed as N. Cooking losses were calculated as the difference between the weight of the steaks before and after oven broiling.

Data were analyzed as a mixed model with the fixed effects of diet contamination, ADS, and their interactions, using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Block was considered as random effect in the model. Color, cooking loss, and WBSF were submitted to analysis of variance as repeated measures over time using the REPEATED statement of SAS. Different residual covariance structures were tested to determine the structure that best fit each variable. The covariance structure was chosen using the Bayesian information criteria, in which the lowest value was used. Means were considered significantly different when $P < 0.05$ and tendency when $0.05 \leq P \leq 0.10$.

3. Results

There was no interaction among the factors for any variable evaluated ($P \geq 0.18$). The meat composition had on average 74.2% moisture, 22.7% protein, 1.33% total collagen, 1.29% ether extract, and 2.10% ash (Table 1). Color, cooking loss, and WBSF were not affected by treatments ($P \geq 0.49$), however they were affected by the aging times. The *L** and *b** increased over time ($P \leq 0.01$), while *a** and fraction of redness relative to yellowness (*E**) decreased ($P \leq 0.01$). The saturation (*C**) was not altered over time ($P = 0.14$). Regarding cooking loss, steaks lost more juice with passing aging times ($P \leq 0.01$) while they became softer ($P \leq 0.01$; Table 2).

4. Discussion

The main question of this study was if mycotoxin and adsorbent would affect the meat quality of finishing Nellore bulls. Surai et al. (2002) and Smith, Chowdhury & Swamy (2004)

observed mycotoxins effects on animal metabolism that may affect meat quality. However, in this study, the factors did not affect any result of meat quality. Nonruminant animals are more sensitive to mycotoxins contaminations in diets compared to ruminants (Harvey et al., 1989; Patience et al., 2014), so the meat of nonruminant animals may be more affected. Furthermore, in ruminants the meat quality may be affected if the contamination of the diets were more challenging (in this study, maximum REQ = 45 µg/kg). According to Yiannikouris (2015), REQ diets from 0 to 50 µg/kg present low risk for beef cattle. There are no studies about meat quality of beef cattle fed mycotoxin and adsorbent in the literature. However, a very important fact related with mycotoxins in animals' feedstuffs and meat are these toxins can pass to meat (not measured in this study), as it can pass to milk and eggs (Bruerton, 2001), thus becoming a risk to human health. Moreover, in Brazil, the maximum tolerated level of AFB1 in human food is 5 µg/kg (Freire, Vieira, Guedes & Mendes, 2007).

Although mycotoxins did not affect meat characteristics of finishing Nellore bulls, aging times certainly affected them. Examining WBSF, observed that the number of tough steaks decreased from 2 to 21 days aging, with 59.1% of steaks being tough after 2 days aging, 50% after 4 days aging, 20.8% after 8 days aging, 8.3% after 14 days aging and 5.3% after 21 days aging. The decrease in relative fraction of tough meat samples suggested chemical and physical changes during aging process. On the other hand, aging time negatively affected color characteristics. Monsón, Sañudo & Sierra (2005), studying the effect of meat aging time on consumer acceptability, observed that acceptability scores begin to decrease at 14 or 21 days of aging. In this study, color analysis suggested that the steaks generally were lighter and more yellow, but less red, as aging time increased. In general, E^* decreased over time, probably indicating the loss of meat redness and C^* was similar over time.

5. Conclusion

Mycotoxins and yeast cell wall based adsorbent do not affect meat quality of Nellore bulls finished in feedlot under the contamination level and conditions of this study.

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Table 1 Carcass characteristics of animals fed diets with natural mycotoxin contamination and exogenous contamination, without or with yeast cell wall adsorbent in Nellore cattle finished in feedlot

Item[1]	Treatments[2]				SEM[3]	*P*-value[4]		
	NC-	NC+ADS	EC-	EC+ADS		Myco	ADS	Myco×ADS
pH	5.70	5.66	5.65	5.60	0.03	0.083	0.202	0.915
BFT, mm	4.43	4.46	3.78	4.67	0.35	0.487	0.161	0.183
REA, cm^2	80.90	78.87	79.25	78.57	2.39	0.672	0.555	0.768
REA, cm^2/100 kg	23.70	23.61	24.23	23.62	0.63	0.712	0.601	0.754
Meat chemical composition (%)								
Moisture	74.3	74.2	74.2	74.2	0.13	0.835	0.851	0.613
Protein	22.6	22.6	22.7	22.7	0.15	0.373	0.904	0.979
Total collagen	1.39	1.30	1.30	1.33	0.62	0.665	0.607	0.337
Ether extract	1.04	1.12	0.97	1.03	0.13	0.484	0.558	0.937
Ash	2.11	2.11	2.15	2.02	0.19	0.875	0.669	0.656

[1] BFT = backfat thickness; REA = ribeye area.

[2] NC- = natural contamination diet without adsorbent; NC+ADS = natural contamination diet with adsorbent; EC- = exogenous contamination diet without adsorbent; EC+ADS = exogenous contamination diet with adsorbent.

[3] SEM = standard error means.

[4]Myco = effect of the diet; ADS = effect of presence or absence of yeast cell wall adsorbent; Myco × ADS = interaction between diet and yeast cell wall adsorbent.

Table 2 Meat color of animals fed diets with natural mycotoxin contamination and exogenous contamination, without or with yeast cell wall adsorbent in Nellore cattle finished in feedlot

Item[1]	D	Treatments[2]				SEM[3]	*P*-value[4]						
		NC-	NC+ADS	EC-	EC+ADS		Myco	ADS	Myco×ADS	D	Myco×D	ADS×D	Myco× ADS×D
*L**	1	36.71	37.09	38.05	37.49	0.47	0.318	0.522	0.384	<0.01	0.847	0.578	0.986
	7	37.86	38.17	39.32	38.95	0.55							
	14	39.27	39.32	40.47	39.76	0.51							
	28	39.17	38.84	40.64	39.60	0.53							
*a**	1	17.23	17.70	17.99	17.41	0.25	0.551	0.812	0.525	<0.01	0.781	0.419	0.575
	7	17.71	18.00	18.09	18.07	0.25							
	14	17.23	17.21	17.28	17.01	0.25							
	28	17.15	17.14	17.12	17.55	0.25							
*b**	1	13.72	13.67	14.32	13.72	0.27	0.114	0.373	0.454	<0.01	0.389	0.757	0.802
	7	13.83	13.99	14.25	14.04	0.27							
	14	14.16	14.05	14.32	14.02	0.27							
	28	14.17	14.05	14.76	14.64	0.27							
*E**	1	1.78	1.77	1.73	1.74	0.02	0.135	0.398	0.667	<0.01	0.468	0.462	0.787
	7	1.75	1.77	1.73	1.75	0.03							
	14	1.66	1.66	1.64	1.64	0.02							
	28	1.65	1.66	1.58	1.65	0.02							

*C**	1	22.45	22.37	23.03	22.18	0.33	0.127	0.425	0.342	0.140	0.703	0.564	0.679
	7	22.48	22.81	23.03	22.89	0.33							
	14	22.30	22.23	22.45	22.04	0.33							
	28	22.25	22.17	22.61	22.86	0.33							
CL, %	1	32.41	32.51	33.13	30.96	0.66	0.880	0.248	0.943	<0.01	0.303	0.751	0.490
	7	32.00	28.76	29.04	29.09	1.78							
	14	31.04	32.16	33.78	32.98	1.36							
	28	34.61	33.44	33.88	33.19	0.82							
WBSF, N	1	66.49	67.67	66.69	63.74	2.06	0.177	0.697	0.503	<0.01	0.378	0.101	0.956
	7	66.39	66.10	60.31	56.78	4.31							
	14	53.15	60.02	54.13	57.86	2.75							
	28	49.52	51.29	48.25	48.54	2.35							

[1]L* = brightness; a* = red coloration; b* = yellow coloration; E* = fraction of redness relative to yellowness and brightness; C* = saturation; D = days of aging; CL = cooking loss; WBSF = Warner Bratzler Shear Force.

[2] NC- = natural contamination diet without adsorbent; NC+ADS = natural contamination diet with adsorbent; EC- = exogenous contamination diet without adsorbent; EC+ADS = exogenous contamination diet with adsorbent.

[3] SEM = standard error means.

[4]Myco = effect of the diet; ADS = effect of presence or absence of ADS; Myco × ADS = interaction between diet and yeast cell wall adsorbent; D = effect of the day; Myco × Day = interaction between diet and day; ADS × Day = interaction between yeast cell wall adsorbent and day; Myco × ADS × Day = interaction between diet, yeast cell wall adsorbent and day. D = days of aging.

Declaração de Responsabilidade

As opiniões, hipóteses e conclusões ou recomendações expressas nesse material são de responsabilidades da autora e não necessariamente refletem a visão da FAPESP e da CAPES.