Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v44i1.53575

NONRUMINANT NUTRITION

Effect of adsorbents on diets with corn contaminated by mycotoxins on the productive performance and health of broilers

Jovanir Inês Müller Fernandes*, Jessiane Sauer Baldo, Ana Clara Polo Ferreira, Juliana Schulter Schuroff, Anderson Henrique Reuter and Bruna Carolina Druzian Salinas

Laboratório de Experimentação Avícola, Universidade Federal do Paraná-Setor Palotina, Rua Pioneiro, 2153, 85950-000, Palotina, Paraná, Brazil. *Author for correspomdence. E-mail: jovanirfernandes@gmail.com

ABSTRACT. A total of 1,296 broiler chicken were housed, distributed in a completely randomized design with 6 treatments and 6 repetitions. The treatments consisted of a control diet formulated with corn considered adequate and without the addition of adsorbent, a diet formulated with corn naturally contaminated with mycotoxin (CCM) and four diets formulated with CCM and added with different commercial adsorbents. At the end of the first week and at 21 days of age of the birds, it was observed that the control diet resulted in greater ($p < 0.05$) live weight and weight gain in relation to the inclusion of CCM. The relative weight of the liver was lower for the control group compared to the groups receiving a diet with CCM and CCM + ads D. The relative weight of the proventriculus and cloacal bursa was lower for the control group compared to those who received a diet with CCM + ads B and CCM + ads C. There was no significant effect ($p > 0.05$) of the diets on the analysis of serum biochemistry and the occurrence of fatty and hydropic degeneration in the liver of broilers. The use of adsorbents can mitigate the harmful effects of mycotoxins, however, these products have specific binding capacity to the type of mycotoxin present in food.

Keywords: liver degeneration; weight gain; fumonisin; fungi; binders; trichothecenes.

Received on May 7, 2020.
Accepted on September 17, 2020.

Introduction

Corn is one of the main ingredients of broiler feed, present in about 60% of starter animal feed, representing 65% of the metabolizable energy and approximately 22% of the protein in the starter phase (Guerre, 2016). In this way, the quality of corn has a significant impact on the production cost, the zootechnical performance and the quality of the products originated from chicken.

In the field and during storage, corn kernels are exposed to the action of physical, chemical and biological factors, favoring deterioration processes (Almeida, Sabino, Fonseca, & Corrêa, 2005). After harvest, the losses caused by the effects of the metabolism of the grains, by the attacks of insects, mites and microorganisms, which are expressed by the deterioration, in the occurrence of defects such as moldy, burned, fermented grains and damaged by different causes and contaminated by fungi that produce mycotoxins represent about 20% of crop damage (Resanović, Nesic, Nesic, Palić, & Jacevic, 2009; Guerre, 2016).

Mycotoxins are toxic metabolites to animals, recognized for their deleterious effects on human and animal health, being able to induce carcinogenic, immunosuppressive, hepatotoxic and mutagenic effects (Freire, Vieira, Guedes, & Mendes, 2007; Elnabarawya, Madian, Hassan Aly, & Madbouly, 2016; Elnabarawya, Madian, Hassan Aly, & Madbouly, 2016).

Fungi develop, most of the time, in damaged parts of the plants, mechanically or by pests, where the rotting of the plant is observed (Maziero & Bersot, 2010). However, for fungi to develop and produce mycotoxins, favorable conditions are necessary, such as grain moisture content, temperature, physical and sanitary conditions, level of fungal inoculation, oxygen content and presence of insects and mites (Costa & Zanella, 2012; Streit, Naehrer, & Rodrigues, 2013). However, the presence of fungi in the food does not necessarily imply mycotoxin production, just as the toxin may be present in the food even in the absence of fungi (D'Mello & Macdonald, 1997; Guerre, 2016).

Mycotoxins are heat stable and resistant to most processing conditions, persisting and being passed through the food chain and into the final product, such as meat, milk and eggs. In this way, they directly impact the agribusiness of many countries, since they can interfere or even prevent exports. In addition, it also contributes to the reduction of animal and agricultural production and, in some countries, it can also affect human health (Freire et al., 2007).

Thus, the control of fungal growth is the most effective way to prevent the occurrence of mycotoxins, but it requires high investments and greater mobilization in the grain production chain. On the other hand, when mycotoxins are already present, the incorporation of adsorbents to diets has been the best strategy to mitigate the negative impacts of these metabolites on animals' health and productivity (Jacela et al., 2010; Gimneo & Martins, 2011).

Mycotoxin adsorbents are inert materials and have the function of binding to a part of mycotoxins, through chemical or physical adsorption bonds, and preventing part of their absorption in the animal's digestive tract, making the effects of intoxication less harmful. Currently, a series of adsorbents with different origins and levels of inclusion are available on the market (Sabater-Vilar, Malekinejad, Selman, Van Der Doelen, & Fink-Gremmels, 2007; Manafi, Narayanaswamy, & Pirany, 2009; Jacela et al., 2010; Carão, Burbarelli, Polycarpo, Albuquerque, & Oliveira, 2014; De Mil, Devreese, & De Baere, 2015).

Therefore, the objective of this study was to evaluate the effectiveness of different commercial adsorbents in diets made with corn naturally contaminated with mycotoxins on the productive performance, integrity of the gastrointestinal tract and serum parameters of broilers.

Material and methods

The experiment was conducted in an Experimental Aviary at the Federal University of Paraná and all procedures for raising animals and collecting biological material were approved by the Committee for Ethical Conduct in the Use of Animals (Protocol number: 01/2014).

A total of 1,296 1d-old male chicks from 40-week old Cobb broiler breeder were housed. The experimental design was completely randomized with 6 treatments and 6 repetitions, totaling 36 birds each experimental unit. The treatments consisted of: Treatment 1: Feed formulated with corn of better quality and considered adequate; Treatment 2: Feed formulated with corn naturally contaminated by mycotoxins (CCM); Treatment 3: CCM + adsorbent A; Treatment 4: CCM + adsorbent B; Treatment 5: CCM + adsorbent C; and Treatment 6: CCM + adsorbent D.

As adsorbent A, Toxfree® (1.0 kg t^{-1}, Nutrifarma Nutrição e Saúde Animal S.A) was used, composed of bentonite with indication for the control of aflatoxin, fumonisin. As adsorbent B, Calibrin Z® (1.0 kg t^{-1}, Elanco), composed of highly refined montmorillonite, with selectivity for zearalenone, was chosen. The adsorbent C was Toxinyll® (1.5 kg t^{-1}, Suifarma saúde animal) composed of oligosaccharides purified from yeast cell wall and botanical extracts of thistle, with indication of control of polar and non-polar mycotoxins and finally, as the adsorbent D Mycofix® (1.5 kg t^{-1}, Biomin) composed of bentonites, hydrated calcium and sodium aluminosilicates, zeolites and oligomannan, with selectivity for trichothecenes and zearalenone.

The Nutritional Program was divided into three phases: starter (1 - 18 days old), grower (19 - 35 days old) and finisher (36 - 42 days old). Table 1 shows the composition of the diets. The experimental feed, based on corn and soybean meal, were formulated in order to meet the nutritional requirements of the different phases according to the recommendations of local agro-industries. The corn considered to be of better quality was selected by using the gravimetric analysis and corn naturally contaminated by mycotoxins was obtained from a batch considered of lower quality due to the presence of a high concentration of damaged grains (savorless, borer, burned, sprouted, moldy, and cracked). Samples of corn considered adequate and inferior were sent for analysis of the occurrence of mycotoxins (SAMITC - Instituto de Soluções Analíticas Microbiológicas e Tecnológicas - Institute of Microbiological and Technological Analytical Solutions, Santa Maria, Rio Grande do Sul, Brazil).

Water and feed were provided ad libitum and the lighting program used was constant (24 hours of light) throughout the breeding period.

Every week, all birds and leftover feed were weighed to evaluate average weight, weight gain, feed intake and feed conversion. Mortality was recorded daily, and the feed conversion rate was calculated considering the weight of dead birds. Temperature and humidity were recorded daily and kept within the comfort range recommended for each phase of life of broilers.

Table 1. Composition and calculated nutritional levels of the experimental diets.

Ingredients (%)	Starter	Grower	Finisher
Corn (CP 7.5%)	58.50	60.76	62.41
Soybean Meal (CP 46%)	34.30	31.80	29.80
Soybean Oil	3.10	4.30	4.60
Limestone	1.14	1.00	0.95
Dicalcium Phosphate	1.20	0.95	0.80
Salt	0.32	0.39	0.37
Sodium Bicarbonate	0.20	-	-
Methionine (DL, 98%)	0.32	0.31	0.30
Lysine-HCl (L, 70%)	0.24	0.21	0.21
Choline Chloride	0.06	0.06	0.06
Threonine (L, 98%)	0.08	0.06	0.05
Inert*	1.50	1.50	1.50
Vitamin and mineral Premix[1,2]	0.40	0.40	0.30
Nutrient levels			
ME (kcal kg^{-1})	3,100	3,199	3,250
CP (%)	21.81	19.61	18.84
Calcium (%)	0.938	0.848	0.793
Available Phosphorus (%)	0.469	0.427	0.395
Lysine dig. (%)	1.240	1.101	1.051
Methionine + Cystine dig (%)	0.930	0.847	0.819
Threonine dig (%)	0.800	0.715	0.683
Tryptophan dig (%)	0.236	0.209	0.199
Arginine dig (%)	1.327	1.199	1.144
Choline (%)	1.802	1.602	1.507

*Replaced by the inclusion of adsorbents. [1]Vitamin premix (Content kg^{-1} of Premix): Vit. A 7,000,000 UI; Vit. D3 2,200,000 UI; Vit.E 11,000 mg; Vit. K3 1,600 mg; Vit. B1 2,000 mg; Vit. B2 5,000 mg, Vit. B12 12,000 mg; Niacina 35,000 mg; Ácido Pantotênico 13,000 mg; Ácido Fólico 800 mg; Antioxidant 100,000 mg. [2]Mineral Premix (Content kg^{-1} of Premix): Iron 10,000 mg; Copper 16,000 mg; Iodine 2,400 mg; Zinc 100,000 mg; Manganese 140,000 mg; Selenium 400 mg.

At 21 days of age, 6 birds from each repetition were randomly selected, weighed and euthanized by cervical dislocation for necropsy. The organs (gizzard, proventriculus, intestine, pancreas, liver and cloacal bursa) were weighed and the relative weight in relation to the live weight of the birds was calculated. Liver samples were collected, packed in 10% buffered formaldehyde solution and then embedded in paraffin. Each fragment was subjected to 5-µm-thick semi-serial sections and stained by hematoxylin-eosin for microscopic analysis.

The birds' blood was collected to study their serum biochemical profile. Specific DIALAB® brand kits were used to determine serum concentrations of liver function tests: ALT (Alanine aminotransferase), AST (Aspartate transaminase), GGT (Gamma-glutamyltransferase or GAMA-GT), AP (Alkaline phosphatase), and cholesterol. Biochemical analyzes were performed on the BS-200 Mindray® chemical analyzer (Mindray Medical International Limited).

At 42 days of age, 2 birds per experimental unit (12 birds per treatment), with live weight ± 2% of the average box weight, were slaughtered to calculate carcass yield. To calculate it, the weight of the hot eviscerated carcass, without feet, head and abdominal fat, in relation to live weight [% carcass yield = (carcass weight * 100/live weight)] was considered. For the performance of the cuts, the performance of the whole breast with skin and bones and of the legs (thigh) was also considered with bones and skin, wings and back calculated in relation to the weight of the eviscerated carcass. The abdominal fat present around the cloaca, the cloacal bursa, gizzard, proventriculus, and adjacent abdominal muscles were removed, weighed and also calculated in relation to the weight of the eviscerated carcass.

The data referring to the occurrence of fatty and hydropic degeneration, were submitted to Logistic Regression Analysis assuming binomial distribution with LOGIT link function through the generalized models procedure implemented in the SAS statistical software.

The other data were submitted to analysis of variance, considering the level of significance of 5%, and when there was an effect, the means were tested by the Tukey test. Statistical analyzes were performed with the aid of the SAS software.

Results and discussion

The results of the analysis of samples of soybean meal, control corn and corn naturally contaminated by mycotoxins (CCM) are shown in Table 2. No positive samples were found for aflatoxin, unlike fumonisin, present in all samples except for soybean meal used in grower and finisher feed. Corn naturally contaminated by mycotoxins samples showed extremely high levels for zearalenone used in the starter feed and fumonisin B1 and B2 in the samples of the grower and finisher feed.

Fumonisin B1 and B2 were found in the control diet, which demonstrates the wide contamination of food with mycotoxins. The FUM, NIV, DAS, ZEN levels were considered above the acceptable, as they were not in compliance with the regulations of the SAMITEC Institute (FUM = 100 μg/kg NIV = 20 μg/kg ZEN = 10 μg/kg DAS = 0 μg/kg = ppb) - With a Quality Management System certified by NBR ISSO 9001: 2008.

Worldwide, food and feed are seriously contaminated with mycotoxins, among which aflatoxin (AFL), zearalenone (ZEN), fumonisins (FUM), deoxynivalenol (DON), and ochratoxin A are the most commonly found (Streit et al., 2013). The presence of different mycotoxins in the feed can cause synergistic toxic effects, that is, the combination of these can increase their toxicity to animals (Pereira, Fernandes & Cunha 2013).

Table 2. Result of the analysis of mycotoxins from samples of soybean meal and control corn and corn naturally contaminated by mycotoxins (CCM).

	AFL B1	AFL B2	AFL G1	AFL G2	F B1	F B2	NIV	DAS	ZEN
Diets					Starter diet				
Soybean meal	DL	DL	DL	DL	409	133	DL	DL	DL
Control corn	DL	DL	DL	DL	260	DL	DL	DL	24
CCM	DL	DL	DL	DL	620	341	269	137	1429
					Grower and finisher diets				
Soybean meal	DL	DL	DL	DL	DL	DL	DL	DL	DL
Control corn	DL	DL	DL	DL	451	219	DL	DL	24
CCM	DL	DL	DL	DL	11700	5630	DL	DL	464

Express results in μg kg^{-1} (ppb) AFL: Aflatoxin; F: Fumonisins; NIV: Nivalenol; DAS: diacetoxiscirpenol; ZEN: Zearalenone; DL: < Detection limit; CCM: corn contaminated by mycotoxins.

At 7 days of age, there was a significant effect ($p < 0.05$) for body weight and weight gain. The control diet, consisting of corn considered adequate, resulted in greater live weight and weight gain. However, there was no significant difference ($p > 0.05$) for feed consumption and feed conversion (Table 3). Similar results were found by Swamy, Smith, Karrow, and Boermans (2004) who observed a linear decrease in the body weight gain of birds fed with contaminated grains during the grower phase. Yegani, Smith, Leeson, and Boermans (2006), observed inferior performance in the first week of life of chicks from broilers fed diets containing corn contaminated by trichothecenes. The first week of chicks' life is a complex phase, as it has a great maternal influence. Thus, pre-starter or starter feed naturally contaminated by mycotoxins can have a cumulative effect, given the influence of the maternal diet, as pointed out by these authors.

Table 3. Productive performance of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents at 7 days of age.

Diets	Live weight (g)	Body weight gain (g)	Feed intake (g)	Feed conversion ratio
Control	183.72^{a}	133.26^{a}	166.92	1.254
CCM	169.42^{b}	119.10^{b}	158.76	1.333
CCM + Ad A	173.32^{b}	123.43^{b}	155.04	1.255
CCM + Ad B	168.49^{b}	116.74^{b}	155.91	1.338
CCM + Ad C	169.97^{b}	120.38^{b}	165.10	1.371
CCM + Ad D	173.56^{b}	123.41^{b}	161.11	1.304
CV %	3.08	4.08	3.44	7.89
Value of P	0.0029	0.0001	0.5416	0.2242

CCM: corn contaminated by mycotoxins; AD: adsorbents.

Currently, one of the most practical and effective methods to reduce the intoxication of animals by mycotoxins is the use of adsorbents, which reduce the absorption of mycotoxins. However, at 7 days of age, the control treatment showed a significant difference in relation to the other treatments, presenting weight gain and live weight superior to the others. These results may be related to the fact that certain adsorbents may impair the use of some nutrients, since they may have chemical affinity with them, as well as high inclusion levels, being ineffective for improving the animals' performance (Sabater-Vilar et al., 2007; De Mil et al., 2015).

Furthermore, the results found in this study can be attributed to the fact that the diets composed by CCM in addition to the presence of mycotoxins, have a lower nutritional quality in relation to the corn of the control treatment considered adequate. Fungi, in addition to producing toxic metabolites, promote protein, carbohydrate degradation and germ discoloration (Santin, Maiorka, Zanella, & Magon, 2000), which reduces the oil content, and decreases the metabolizable energy value of the food (Silva et al., 2008). Thus, these factors contribute to the lower energy density of these grains, which for this reason, may not fully meet the energy requirement of birds (Pereira, Menten, Santa Rosa, Silva, & Tradi, 2011).

Another important fact that must be considered is the concentration of mycotoxins in the ingredients. Low concentrations are often considered to be of little importance but can have a greater effect than in high concentrations. The work of Alassane-Kpembi et al. (2013) showed that the effects on intestinal cell viability, per unit of mycotoxins, were greater at lower concentrations than at higher ones, and that the combination of different mycotoxins may result in additive and even synergistic effects.

For the productive performance from 1 to 21 days (Table 4), a significant effect was observed for all variables. The consumption of the control diet, with better quality corn, resulted in greater live weight and weight gain than the other treatments. Feed intake was higher for the control diet, a diet containing CCM + Ad A and CCM + Ad B. As for feed conversion, diets based on CCM, CCM + Ad A showed better results compared to the control treatment. It is important to note that the adsorbent's ability to bind to mycotoxins depends on intrinsic factors such as pH, molecular arrangement and the geographic region of origin. In addition to these factors, it must also be considered that the capacity to bind to each toxin has decreased as the number of toxins increases and this percentage reduction in binding may be due to the interaction between mycotoxins (Manafi et al., 2009).

Table 4. Productive performance of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents from 1 to 21 days of age.

Diets	Live weight (g)	Body weight gain (g)	Feed intake (g)	Feed conversion ratio
Control	976.57[a]	927.84[a]	1283.66[a]	1.370[a]
CCM	919.58[b]	870.80[b]	1128.23[b]	1.296[b]
CCM + Ad A	924.72 [b]	876.71[b]	1176.93[ab]	1.289[b]
CCM + Ad B	901.94 [b]	853.76[b]	1155.71[ab]	1.321[ab]
CCM + Ad C	919.47 [b]	871.36[b]	1151.89[b]	1.322[ab]
CCM + Ad D	911.13 [b]	862.12[b]	1152.30[b]	1.336[ab]
CV %	3.32	3.45	6.29	2.92
Value of P	0.0041	0.0037	0.0153	0.0292

CCM: corn contaminated by mycotoxins; AD: adsorbents.

These results are contrary to those found by Godoi et al. (2008) who observed a significant effect on the use of adsorbents on feed consumption. Rossi et al. (2013) found higher weight gain for animals fed with corn not contaminated with aflatoxin when compared to animals fed with corn contaminated with aflatoxin, which corroborates the values found in this research for weight gain.

The evaluation of the productive performance considering the whole period evaluated, from 1 to 42 days of age (Table 5), showed higher ($p < 0.05$) feed intake for the control diet compared to the diet containing CCM. This result can be explained due to some mechanisms used by mycotoxins in the organism of animals that lead to reduced intake or refusal of food, alteration in absorption, in the nutrient content of food, and alteration in the metabolism of contaminated nutrients (Freire et al., 2007), which may have influenced the lower feed intake of birds treated with corn contaminated with mycotoxins.

Table 5. Productive performance of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents from 1 to 42 days of age.

Diets	Live weight (g)	Body weight gain (g)	Feed intake (g)	Feed conversion ratio
Control	2966.92	2918.19	4825.48[a]	1.654
CCM	2834.37	2785.58	4515.23[b]	1.651
CCM + Ad A	2786.02	2738.01	4630.27[ab]	1.662
CCM + Ad B	2766.17	2718.00	4626.43[ab]	1.665
CCM + Ad C	2852.08	2803.98	4613.12[ab]	1.647
CCM + Ad D	2760.15	2711.14	4628.89[ab]	1.683
CV %	4.60	4.66	3.27	3.16
Value of P	0.0881	0.0873	0.0416	0.8903

CCM: corn contaminated by mycotoxins; AD: adsorbents.

The control diet resulted in greater carcass weight at 42 days when compared to the CCM + Ads D treatment (Table 6). This result can be explained due to the low and restricted selectivity of the adsorbent used, which has an action on trichothecenes and zearalenone, which may not have included other mycotoxins that were in this treatment. In addition, according to Santin et al. (2000), the use of aluminosilicate adsorbents, in which zeolites are included, can generate undesirable results, due to the possibility of adsorption of useful dietary components, such as minerals, vitamins, growth promoters and coccidiostats.

The other diets showed results similar to those observed with the use of the control diet, which demonstrates the positive effect of the other adsorbents and can be an interesting alternative when the raw materials are of inferior quality.

Accordingly, Rossi et al. (2013), when testing basal diets contaminated with aflatoxin demonstrated that supplementation with esterified glutamate or esterified glutamate + organic selenium adsorbents in the diets of the birds, showed higher carcass yield than the birds of the treatments that did not have adsorbent added to the diet. For the other variables analyzed, there was no significant difference ($p > 0.05$).

There was no significant difference for serum analysis at 21 days of age (Table 7). The effects on the animals' organisms vary according to the diversity of the chemical structures of mycotoxins, individual biological, nutritional and environmental factors related to the animal (Kiessling, 1986). Associated with these factors, the breeding environment has a great impact on the animals' organic responses to challenges. In experimental conditions, the poultry breeding environment offers adequate well-being, where in addition to thermal comfort temperatures, other well-being variables were provided to birds, such as lower housing density, and above all, free from health challenges, which may have spared the animal's organism from the deleterious effect of mycotoxins and therefore the absence of significant interference with metabolism or debilitating injuries, as observed in commercial breeding situations. In commercial farms, diets naturally contaminated with mycotoxins negatively affect performance, carcass characteristics, and some serum biochemical parameters of birds (Shang, Yang, Yang, Zhang, & Jiang, 2015).

Table 6. Yield of carcass, legs, breast and deposition of abdominal fat (absolute and relative weight) of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents.

Diets	Absolute weight (g)			
	Carcass	Legs	Breast	Fat
Control	2381.00[a]	609.33	797.33	65.71
CCM	2243.00[ab]	568.00	760.67	59.26
CCM + Ad A	2270.33[ab]	572.33	767.33	48.78
CCM + Ad B	2246.33[ab]	594.67	768.67	47.53
CCM + Ad C	2219.67[ab]	575.33	733.33	46.68
CCM + Ad D	2161.33[b]	553.67	713.67	53.25
CV, %	4.98	6.74	8.28	25.30
Value of P	0.0524	0.2011	0.2825	0.1362
Diets	Relative weight (%)			
Control	77.03	25.60	33.49	2.75
CCM	76.97	25.30	33.88	2.66
CCM + Ad A	76.65	25.21	33.78	2.14
CCM + Ad B	77.55	26.51	34.17	2.13
CCM + Ad C	76.09	25.94	32.98	2.10
CCM + Ad D	76.38	25.60	32.99	2.47
CV %	1.67	4.95	4.47	25.39
Value of P	0.4439	0.5329	0.6736	0.2697

CCM: corn contaminated by mycotoxins; AD: adsorbents.

Table 7. Serum biochemical profile of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents at 21 days of age.

Diets	ALT ($U L^{-1}$)	AST ($U L^{-1}$)	GGT ($U L^{-1}$)	FA ($U L^{-1}$)	COLEST ($mg dL^{-1}$)
Control	92.18	462.83	50.03	154.17	116.00
CCM	108.13	522.33	48.37	140.17	142.67
CCM + Ad A	104.00	456.33	48.63	195.33	139.50
CCM + Ad B	115.92	528.50	54.45	315.50	137.83
CCM + Ad C	95.13	489.17	36.38	259.83	145.50
CCM + Ad D	103.92	512.50	47.73	120.17	126.00
CV %	60.17	60.45	59.88	54.72	58.70
Value of P	0.9256	0.9545	0.8915	0.0799	0.7134

CCM: corn contaminated by mycotoxins; AD: adsorbents. ALT (Alanine aminotransferase), AST (Aspartate transaminase), GGT (Gamma-glutamyltransferase or GAMA-GT), AP (Alkaline phosphatase), and COLEST (Cholesterol).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, when found in high values, are considered indicators of high sensitivity of dysfunction/injury, liver inflammation and bile duct injury or obstruction (Kubena, 1998) thus worsening the digestion and utilization of food by animals. The results found in this study are contradictory to those found by Batina, Lopes, Santurio, Souza, and Martins (2005), who observed a decrease in serum cholesterol and triglyceride levels, an increase in ALT levels in diets testing adsorbents based on sodium montmorillonite on the biochemical profile of broilers intoxicated with aflatoxin.

There was a significant difference ($p < 0.05$) for the weight of intestine, proventriculus, gizzard, liver, and cloacal bursa (Table 8), assessed at 21 days of age. The highest relative intestinal weight observed was for birds that received an CCM-based diet and without the addition of adsorbent compared to the control diet. One of the main injuries observed in necropsies performed during the monitoring carried out on birds of commercial breeding and capable of reaching the intestinal mucosa is duodenitis with hyperemia, triggered by mycotoxins (Santin et al., 2000), which would justify the greater weight of the intestine of birds fed with poor quality corn contaminated with mycotoxins compared to the control diet and diets made with CCM and supplemented with adsorbents.

Table 8. Relative organ weights of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents at 21 days of age.

Diets	Intestine	Pancreas	Proventriculus	Gizzard	Liver	Cloacal bursa
			%			
Control	7.22^{b}	0.32	0.50^{b}	3.39^{b}	2.51^{b}	0.20^{b}
CCM	8.21^{a}	0.32	0.55^{ab}	3.72^{ab}	3.11^{a}	0.28^{a}
CCM + Ad A	7.95^{ab}	0.30	0.53^{ab}	3.98^{a}	2.66^{ab}	0.22^{ab}
CCM + Ad B	7.73^{ab}	0.33	0.66^{a}	4.03^{a}	2.95^{ab}	0.27^{a}
CCM + Ad C	7.57^{ab}	0.30	0.61^{a}	4.04^{a}	2.94^{ab}	0.28^{a}
CCM + Ad D	7.47^{ab}	0.28	0.57^{ab}	3.71^{ab}	2.54^{b}	0.25^{ab}
CV %	9.46	14.67	12.69	10.15	11.51	18.82
Value of P	0.0248	0.4738	0.0880	0.0441	0.0123	0.0307

CCM: corn contaminated by mycotoxins; AD: adsorbents.

Taranu et al. (2015) studied the effects of low concentrations of zearalenone on the expression of genes in the intestinal cells of pigs and observed that 1,954 genes had an altered profile and expression compared to the control. These authors also reported an increase in the expression of cytokines involved in inflammation and recruitment of immune cells. In this sense, it is important to consider that inflammation has an energy and nutrient cost and can compromise the integrity of the intestine and other organs by altering its metabolism.

The relative weight of the proventriculus was lower in birds that received a control diet compared to those that received a diet made with CCM + ads B and CCM + ads C. Similar results were found for the relative weight of gizzard, which was lower in birds fed with control diet compared to birds that received a diet made with CCM + ads A, CCM + ads. B and CCM + ads C. These results may be related to better corn quality and, consequently, the digestive process is faster and more effective and to the individual effect of adsorbents B and C, which may have been more effective in binding with mycotoxins that could affect the digestive process (Kolosova & Stroka, 2011). However, this issue needs to be studied and elucidated.

The relative weight of the liver was lower for the birds in the group receiving the control diet compared to the groups that received a diet based on CCM and CCM + ads D. This adsorbent consists of bentonites, hydrated calcium and sodium aluminosilicates, zeolites and oligomannan, that is, it has organic and inorganic components, different from the others that have inorganic components (ads. A and B) or only organic ones (ads. C).

Aflatoxins have a greater predilection for this organ, whose functioning is impaired by injuries resulting from hemorrhagic necrosis, proliferation of bile duct cells and fatty infiltration of hepatocytes (Kiessling, 1986; Freire et al., 2007). However, aflatoxins were not detected in the samples of CCM, control corn or soybean meal.

The same behavior was observed for the relative weight of the cloacal bursa (Table 8). Birds fed the control diet had a lower relative weight compared to diets containing CCM and added with adsorbents B and C. The cloacal bursa is the organ responsible for the maturation and differentiation of cells in the antibody-forming system (Tizard, 1998). Microbial challenges or immunosuppressive agents such as mycotoxins can induce lymphoid tissue hyperplasia in an attempt to increase the production of antibodies and immune defense signals.

The consumption of mycotoxins, at levels that do not cause clinical mycotoxicosis, suppresses immunity functions and can decrease resistance to infectious diseases. The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the vulnerability of continually proliferating and differentiating cells that participate in immune-mediated activities and regulate the complex communication network between cellular and humoral components (Elnabarawya et al., 2016). In commercial breeding situations, due to the negative effects of mycotoxins on the immune response, an increased susceptibility of birds to infections and decreased responses to vaccines are observed (Santin et al., 2002; Elnabarawya et al., 2016).

There was no significant effect ($p > 0.05$) of the diets on the occurrence of fatty and hydropic degeneration in the liver of broilers (Table 9). Although not significant, it is possible to observe that for the diet elaborated with CCM, the highest score values were observed for both fatty and hydropic degeneration, as well as the highest percentage of liver weight in relation to live weight (Table 8).

When a large amount of mycotoxins is ingested, the liver presents fatty degeneration, lobular necrosis with an increase in basophilic cells on the periphery of the lobe, bile duct hyperplasia and cirrhosis (Maciel et al., 2007; Bordini, Rossi, Hirooka, & Ono, 2013).

Table 9. Score of occurrence of lesions (%) of fatty and hydropic degeneration in the liver of broilers fed diets made with corn contaminated by mycotoxins plus commercial adsorbents at 21 days of age.

Diets	Fatty degeneration (%)	Hydropic degeneration (%)
Control	0.33	0.00
CCM	0.66	8.77
CCM + Ad A	0.00	2.25
CCM + Ad B	0.00	0.56
CCM + Ad C	0.15	1.79
CCM + Ad D	0.20	1.41
CV %	22.51	51.48
Value of P	0.822	0.541

CCM: corn contaminated by mycotoxins; AD: adsorbents.

Conclusion

The use of adsorbents in the broiler diet can mitigate the harmful effects of mycotoxins when the ingredients are of low quality. The choice of adsorbent must take into account the composition and history of occurrence of mycotoxins, since these products have specific binding capacity to the type of mycotoxin present in the feed offered to animals.

References

Alassane-Kpembi, I., Kolf-Clauw, M., Gauthier, T., Abrami, R., Abiola, F. A., & Oswald, I. P. (2013). New insights into mycotoxin mixtures: the toxicity of low doses of Type B trichothecenes on intestinal epithelial cells is synergistic. *Toxicology and Applied Pharmacology*, *272*(1), 191-198. DOI: https://doi.org/10.1016/j.taap.2013.05.023

Almeida, A. P., Sabino, M., Fonseca, H., & Corrêa, B. (2005). Milho recém-colhido no Brasil: interação da microbiota fúngica, fatores abióticos e ocorrência de fumonisinas. *Revista do Instituto Adolfo Lutz*, *64*(1), 1-9.

Batina, P. N., Lopes, S. T. A., Santurio, J. M., Souza, C., & Martins, D. B. (2005). Efeitos da adição de montmorilonita sódica na dieta sobre o perfil bioquímico de frangos de corte intoxicados com aflatoxina. *Ciência Rural*, *35*(4), 826-831 2. DOI: https://doi.org/10.1590/S0103-84782005000400012

Bordini, J. G., Rossi, C. N., Hirooka, E. Y., & Ono, E. Y. S. (2013). Impact of fumonisins, aflatoxins and ochratoxin A on poultry industry. *BBR - Biochemistry and Biotechnology Reports*, *2*(1), 68-88. DOI: https://doi.org/10.1590/0103-8478cr20180888

Carão, A. C. P., Burbarelli, M. F. C., Polycarpo, G. V., Albuquerque, A. R. S., & Oliveira, C. A. F. (2014). Métodos físicos e químicos de detoxificação de aflatoxinas e redução da contaminação fúngica na cadeia produtiva avícola. *Ciência Rural*, *44*(4), 699-705. DOI: https://doi.org/10.1590/S0103-84782014000400021

Costa, J. A., & Zanella, G. N. (2012). Identificação de fungos filamentosos em derivados de milho comercializados em Primavera do Leste – MT. *Revista Brasileira Farmácia*, *93*(1), 109-113. DOI: https://doi.org/10.1590/S0100-84042003000200010

D'Mello, J. P. F., & Macdonald, A. M. C. (1997) Mycotoxins. *Animal Feed Science and Technology*, *69*, 155-166. DOI: http://dx.doi.org/10.1016/S0377-8401(97)81630-6

De Mil, T., Devreese, M., & De Baere, S. (2015). Characterization of 27 mycotoxin binders and the relation with *in vitro* zearalenone adsorption at a single concentration. *Toxins*, *7*(1), 21-33. DOI: https://doi.org/10.3390/toxins7010021

Elnabarawya, A. M., Madian, K., Hassan Aly, E. A., & Madbouly, Y. M. (2016). The effect of mycotoxins on immune response of broilers to the live ND vaccines applied by different routes. *Middle East Journal of Applied Sciences*, *6*(1), 51-58.

Freire, F. C. O., Vieira, I. G. P., Guedes, M. I. F., & Mendes, F. N. P. (2007). *Micotoxinas: importância na alimentação e na saúde humana e animal*. Fortaleza, CE: Embrapa Agroindústria Tropical.

Gimeno, A., & Martins, M. L. (2011) *Mycotoxinas y micotoxicosis en animales y humanos*. (3rd. ed.). Miame, US: Special Nutrients.

Godoi, M. J. S., Albino, L. F. T., Rostagno, H. S. R., Gomes, P. C., Barreto, S. L. T., & Vargas J. J. G. (2008). Utilização de aditivos em rações formuladas com milho normal e de baixa qualidade para frangos de corte. *Revista Brasileira de Zootecnia*, *37*(6), 1005-1011. DOI: https://doi.org/10.1590/S1516-35982008000600008

Guerre, P. (2016). Worldwide mycotoxins exposure in pig and poultry feed formulations. *Toxins*, *8*, 1-25. DOI: https://doi.org/10.3390/toxins8120350

Jacela, J. Y., DeRouchey, J. M., Tokach, M. D., Goodband, R. D., Nelssen, J. L., Renter, D. G., & Dritz, S. S. (2010). Feed additives for swine: Fact sheets – flavors and mold inhibitors, mycotoxin binders, and antioxidants. *Journal of Swine Health and Production*, *18*(1), 27-32.

Kiessling, K. (1986). Biochemical Mechanism of action of mycotoxins. *Pure and Applied Chemistry*, *58*(2), 327-328. DOI: https://doi.org/10.1351/pac198658020327

Kolosova, A., & Stroka, J. (2011). Substances for reduction of the contamination of feed by mycotoxins: A review. *World Mycotoxin Jounal*, *4*(3), 225-256. DOI: https://doi.org/10.3920/WMJ2011.1288

Kubena, L. F. (1998). Effects of hydrated sodium calcium aluminosilicate (T-BindTM) on mycotoxicosis in young broiler chickens. *Poultry Science*, *77*(10), 1502-1509. DOI: https://doi.org/10.1093/ps/77.10.1502

Maciel, R. M., Lopes, S. T. A., Santurio, J. M., Martins, D. B., Rosa, A. P., & Emanuelli, M. P. (2007). Função hepática e renal de frangos de corte alimentados com dietas com aflatoxina e clinoptilolita natural. *Pesquisa Agropecuária* Brasileira, *42*(9), 1221-1225.

Manafi, M., Narayanaswamy, H. D., & Pirany, N. (2009). In vitro binding ability of mycotoxin binder in commercial broiler feed. *African Journal of Agricultural Research*, *4*(2), 141-143.

Maziero, M. T., & Bersot, L. S. (2010). Micotoxinas em alimentos produzidos no Brasil. *Revista Brasileira de Produtos Agroindustriais*, *12*(1), 89-99. DOI: http://dx.doi.org/10.15871/1517-8595/rbpa.v12n1p89-99.

Pereira, P. W. Z., Menten, J. F. M., Santarosa, J., Silva, C. S., & Traldi, A. B. R. (2011). Avaliação de milhos de diferentes densidades para frangos de corte. *Revista Brasileira de Zootecnia*, *40*(7), 1554-1561. DOI: https://doi.org/10.1590/S1516-35982011000700022

Pereira, V. L., Fernandes, J. O., & Cunha, S. C. (2013). Micotoxinas em Portugal: ocorrência e toxicidade. *Acta Farmacêutica Portuguesa*, *2*(1), 61-73. DOI: https://doi.org/10.17268/sci.agropecu.2019.04.13

Resanović, R. M., Nesic, K. N., Nesic, V. D., Palić, & Jacevic, V. (2009). Mycotoxins in poultry production. *Zbornik Matice Srpske Za Prirodne Nauke*, *116*, 7-14. DOI: https://doi.org/10.2298/ZMSPN0916007R

Rossi, P., Nunes, J. K., Rutz, F., Reis, J. S., Boursheudt, D., Rocha, A. A., ... Anciuti, M. A. (2013). Glucomanano esterificado e selênio orgânico em frangos alimentados com dietas com aflatoxinas. *Archivos de Zootecnia*, *62*(237), 33-43. DOI: https://doi.org/10.4321/S0004-05922013000100004

Sabater-Vilar, M., Malekinejad, H., Selman, M. H. J., Van Der Doelen, M. A. M., & Fink-Gremmels, J. (2007). *In vitro* assessment of adsorbents aiming to prevent deoxynivalenol and zearalenone mycotoxicoses. *Mycopathologia*, *163*(2), 81-90. DOI: https://doi.org/10.1007/s11046-007-0093-6

Santin, E., Maiorka, A., Zanella, I., & Magon, L. (2000). Micotoxinas do *Fusarium* spp. na avicultura comercial. *Revista Ciência Rural*, *31*(1), 185-190. DOI: https://doi.org/10.1590/S0103-84782001000100030

Santin, E., Paulillo, A. C, Maiorka, P. C., Alessi, A. C., Krabbe, E. L., & Maiorka, A. (2002). The effects of ochratoxin/aluminosilicate interaction on the tissues and humoral immune response of broilers. *Avian Pathology, 31*(1), 73-79. DOI: https://doi.org/10.1093/japr/11.1.22

Shang, Q. H., Yang, Z. B., Yang, W. R., LI, Z., Zhang, G. G., & Jiang, S. Z. (2015). Toxicity of mycotoxins from contaminated corn with or without yeast cell wall adsorbent on broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *29*(5), 674-680. DOI: https://doi.org/10.5713/ajas.15.0165

Silva, C. C., Couto, H. P., Ferreira, R. A, Fonseca, J. B., Gomes, A. V. C., & Soares, R. T. N. R. (2008). Valores nutricionais de milhos de diferentes qualidades para frangos de corte. *Revista Brasileira de Zootecnia, 37*(5), 883-889. DOI: https://doi.org/10.1590/S1516-35982008000500016

Streit, E. Naehrer, K., & Rodrigues, I. (2013). Mycotoxin occurrence in feed and feed raw materials worldwide: long-term analysis with special focus on Europe and Asia. *Journal of the Science of Food and Agriculture*, *93*(12), 2892-2899. DOI: https://doi.org/10.1002/jsfa.6225

Swamy, H. V. L., Smith, N. T. K., Karrow N. A., & Boermans, H. J. (2004). Effects of feeding blends of grains naturally contaminated with fusarium mycotoxins on growth and immunological parameters of broiler chickens. *Poultry Science Association*, *83*(4), 533-543. DOI: https://doi.org/10.1093/ps/83.4.533

Taranu, I., Braicu, C., Marin, D. E., Pistol, G. C, Motiu, M., Balacescu, L., Burlacu, R. (2015). Exposure to zearalenone mycotoxin alters in vitro porcine intestinal epithelial cells by differential gene expression. *Toxicology Letters*, *232*(1), 310-25. DOI: https://doi.org/10.3390/toxins7061979

Tizard, I. R. (1998). *Sistema complemento. Imunologia veterinária*. (5. ed.). São Paulo, SP: Editora Roca Ltda.

Yegani, M., Smith, T. K., Leeson, S., & Boermans, H. J. (2006). Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders. *Poultry Science*, *85*(9), 1541-1549. DOI: https://doi.org/10.1093/ps/85.9.1541