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Use of different adsorbents in broiler diets naturally contaminated by mycotoxins

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Abstract. This study investigated the effects of adding different adsorbent substances to broilers feed naturally contaminated by mycotoxins. Two hundred and eighty male 1-day-old chicks, Cobb Slow® lineage, were distributed in a randomized block design with 4 treatments, 5 repetitions with 14 birds each. The treatments consisted of: T1- basal feed naturally contaminated with mycotoxins. T2- basal feed + Bentonite, Thistle Extract, Yeast Extract, Vitamin E and Choline. T3- basal feed + Bentonite, Thistle Extract, yeast cell wall and Silymarin. T4- basal feed + Bentonite and Algae extract. Performance (weight gain, feed intake, feed conversion) at 7, 14, 21, 28 days were evaluated. At 28 days, a portion of the jejunum was collected in two birds by replicate to study the intestinal morphology. The relative weight of the gizzard, proventricle and total intestine was evaluated. The data obtained were analyzed using the statistical program SAS (9.3). With the use of any adsorbents studied, the performance and liver weight were improved in all evaluated periods. Thus, the inclusion of adsorbents improves the performance of the broiler chickens when the feed is contaminated by mycotoxins.

Keywords: intestinal morphometry; poultry farming; adsorbents sources; zootechnical parameters.

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Introduction

Animal production, mainly chicken meat, is characterized by being a very intensified sector, with large numbers of animals produced and cutting-edge technologies. Even so, there are a lot of concerns regarding animal health, that is, the productive losses due to the diseases affecting these birds.

One of these concerns in poultry is the presence of contaminants of fungal origin in feed. These fungi are present mainly in cereals and grains, such as corn, wheat, and soy, among other important ingredients that compose a large part of the daily diet of chickens. Thus, these fungi produce toxic metabolites, called mycotoxins (Andretta, Kipper, Lehnen, Vale, & Lovatto, 2011), and when ingested by animals, they cause large productive losses because they are detrimental substances.

According to Godoi, Albino, and Rostagno (2008), the use of low quality corn, contaminated by mycotoxins, in the formulation of broiler rations, generates productivity losses in addition to worsening the health and quality of the carcasses of birds. Consequently, it is possible to perceive the seriousness of this problem in animal farming, as it is believed that of all cultures produced worldwide, 30% are affected by toxigenic mycotoxins (Pinotti, Ottoboni, Giromini, Dell'orto, & Chell, 2016).

The main fungi responsible for the production of mycotoxins are of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps* and *Alternaria* (Maziero & Bersot, 2010). These fungi will produce the toxins found in feed, the most common and important are Aflatoxins (B1, B2, G1, G2 and M1), fumonisins (B1 and B2), fusaric acid, ochratoxins (A, B and C), zearalenone, patulin, citrinin, and trichothecenes (Murphy, Hendrich, Landgren, & Bryant, 2006; Maziero & Bersot, 2010; Mostrom, 2016).

The predisposing factors for fungi to colonize feed or food, especially corn, usually occur due to storage error of this raw material. According to Chu (1991), storage places with little ventilation and high humidity support both fungal contamination and the development of possible fungi present. The major problem with

mycotoxins ingested by birds is that they will cause carcinogenic problems such as changes in metabolic functions (Miazzo et al., 2005). Thus, impairing the health and productive response of animals, and reduction in weight gain is a common characteristic of mycotoxicosis (Swamy, 2002; Pappas et al., 2016). In addition, Abreu et al. (2008), report that when they have a diet with mycotoxins, animals start to develop mycotoxicosis, that is characterized by a diffuse syndrome, which can cause damage to several organs, such as the liver, kidneys or even the central nervous system.

The inclusion of adsorbents in the feed has been used as a way to minimize the damage caused by mycotoxins. These substances work by reducing the deleterious effects on the gastrointestinal tract, caused by mycotoxins, as well as reducing the absorption of toxic metabolites by the animal. (Swamy et al., 2002; Liu, Wangb, Denga, Gua, & Wanga, 2018). The main adsorption mechanism of these materials is related to the exchange of charges between the adsorbent and mycotoxin and an adsorbent must be of inert material and able to fix the mycotoxin to its surface, and leave the organism together with the feces, preventing the mycotoxin from being absorbed by the animal (Pappas et al., 2016).

Therefore, the objective of this research was to test the effectiveness of three products used as adsorbents in broiler diets on their performance and intestinal health.

Method and materials

The Ethics Committee on Animal Use (CEUA) UTFPR, Dois Vizinhos, approved this experiment under the protocol: 2016-033. The experiment was conducted at UNEPE Small Animal of Federal University of Technology of Paraná (UTFPR), Dois Vizinhos, PR, Brazil.

Experimental design

A total of 280 male one-day-old Cobb Slow® broiler chickens with an average weight of 45g were selected, vaccinated in the hatchery against Marek, Avipoxvirus, infectious bronchitis and Gumboro diseases. These animals were distributed in a randomized block design (DBC) with 4 treatments and 5 repetitions per treatment. Thus, there were 14 birds per repetition, totaling 20 per experimental unit.

Housing and management

These animals were housed in the experimental aviary for 28 days. The aviary is 12m long x 8m wide and the boxes are 1.2 m^2 each. The aviary has anti-bird screens and side curtains, a dirt floor that has been covered with wood shavings, a pressure cup type drinker and a tubular feeder. Feed and water were provided *ad libitum*. Heating was performed, when necessary, with a wood oven with a control panel. The light program was made according to the age of the birds, following recommendations according to the lineage manual.

Treatments

The treatments were conducted as follows: T1: basal feed naturally contaminated with mycotoxins and without the inclusion of adsorbent (control group); T2: basal feed + adsorbent 1 (Bentonite, Thistle Extract, Yeast Extract, Vitamin E and Choline); T3: basal feed + adsorbent 2 (Bentonite, Thistle Extract, yeast cell wall and Silymarin); T4: basal feed + adsorbent 3 (Bentonite e Algae Extract). The adsorbents are products of different commercial brands formulated based on bentonite and the dose used was 2 kg ton^{-1} as recommended in the products.

The experimental diets were based on corn and soybean meal and were formulated and adjusted according to Rostagno et al. (2011) in order to meet the minimum nutritional requirements for each phase (Table 1). In addition, the analysis of mycotoxins in the diet indicated the presence of fumonisin (B1 + B2) in total levels of 1321 ppb and zearalenone of 36 ppb.

Evaluated parameters

The birds were evaluated in relation to weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) and body weight (BW). For this, weighings were performed on the 1st day of age, on the 7^{th}, 14^{th}, 21^{st} and 28^{th} days. Feed intake was determined by weighing the feed offered and the feed residue at the beginning and end of the experimental period, respectively. Mortality was recorded daily for the correction of the performance rates mentioned above, as well as the weighing of the receptive feeder.

Table 1. Composition and nutritional levels of experimental diet for broilers 1-28 days of age.

Ingredients	1-28 days of age (kg)
Corn grain (7.5%)	62.24
Soybean meal (46%)	30.90
Soybean oil	4.00
Salt	0.20
Dicalcium phosphate 18%	0.80
Limestone	0.70
Vitamin and mineral supplement*	0.40
DL- Methionine	0.30
L- Lysine 78	0.15
L- Threonine 98	0.11
Inert[1]	0.20
Adsorbents [2]	0.20
Total	100.00
Calculated composition	
AME	3003.19 kcal Kg^{-1}
Crude protein	21.62%
Calcium	1.12%
phosphorus	0.69%
Available phosphorus	0.45%
Sodium	0.21%
Lysine	1.29%
Available Lysine	1.17%
Methionine	0.65%
Available Methionine	0.64%
Available Threonine	0.83%
Available Tryptophan	0.25%
Available Methionine + cysteine	0.91%

[1]- Included in the control diet (treatment 1). [2]- Included in the diet of Treatments 2, 3 and 4 to replace the inert. * Provided per kilogram of product: Folic acid (min) 7.5 mg, Pantothenic acid (min) 100 mg, Zinc Bacitracin 1.375 mg, Biotin (min) 0.5 mg, Calcium (min) 200 g, Calcium (max) 300 g kg^{-1}; Copper (min) 165 mg, Choline chloride (min) 3.750 mg, Iron (min) 1.375 mg, phosphorus (min) 58 g, Fluorine (max) 580 mg kg^{-1}, Iodine (min) 33 mg, manganese (min) 1.650 mg, Methionine (min) 18.2 g, Niancin (min) 300 mg, selenium (min) 5 mg, Sodium (min) 37.1 g, Vitamin A (min) 97.500 UI, Vitamin B1 (min) 10 mg, Vitamin B12 (min) 125 mcg, Vitamin B2 (min) 50 mg, Vitamin B6 (min) 15 mg, Vitamin D3 (min) 30.000 UI, Vitamin E (min) 162.5 UI, Vitamin K3 (min) 25 mg, Zinc (min) 1.650 mg.

For the analysis of intestinal morphometry, two birds per experimental unit, at 28 days, were euthanized by the cervical dislocation method (according to the Conselho Nacional de Controle de Experimentação Animal [CONCEA] guidelines, 2013) for later collection of the small intestine in the jejunum portion. The fragments of the intestinal segments were washed with 0.9% saline solution and immersed in Alfac fixative mixture (85 mL 80% ethanol, 15 mL formaldehyde (37-40%) and 5 mL glacial acetic acid) for 16 hours. Subsequently, they were dehydrated in an increasing series of alcohols (30 minutes in 80% alcohol, 30 minutes in 95% alcohol, 60 minutes in 100% alcohol), treated in toluene and included in paraffin as described by Ribeiro, Grötzner, and Filho (2012). The blocks were cut into 5-µm-thick sections using a microtome. The histological sections were stained with hematoxylin and eosin (HE) (Beçak & Paulete, 1976). With the aid of a light photomicroscope coupled to a computer with an image analysis system (UTHSCSA ImageTool. Version 3.0), 10 different villi were measured: villus height and width, crypt region and diameter, and muscle thickness of the mucous. In addition, the gizzard, proventricle and intestine (small and large) were collected and weighed to obtain the relative weight of the organs in relation to the body weight of the fasting birds.

Statistical analysis

The data were tabulated to perform the statistical analysis, being of qualitative origin. The performance data were subjected to analysis of variance and the means compared by the Tukey test at 5% probability, using the statistical program SAS version 9.3 (Statistical Analysis System [SAS], 2008).

Results and discussion

Table 2 shows the results of weight gain (WG), feed intake (FI), feed conversion ratio (FCR) and body weight (BW) for each of the treatments tested. These variables were analyzed in four periods: 7, 14, 21 and 28 days of age.

Table 2. Weight gain, feed intake, feed conversion ratio and body weight of chickens subjected to feed intake containing mycotoxins and addition of different adsorbents in the periods of 7, 14, 21 and 28 days of age.

Weight gain (g bird^{-1})				
Treatment	7-d-old	14-d-old	21-d-old	28-d-old
T1	61.38^{b}	106.22^{b}	211.29^{b}	330.78^{b}
T2	83.96^{a}	164.93^{a}	281.17^{a}	433.49^{a}
T3	79.22^{a}	151.40^{a}	271.10^{a}	413.77^{a}
T4	78.34^{a}	147.97^{a}	256.72^{ab}	382.63^{ab}
Coefficient of variation (%)	11.48	12.61	10.92	8.80
p value	0.004	0.005	0.013	0.011
Feed intake (g bird^{-1})				
Treatment	7-d-old	14-d-old	21-d-old	28-d-old
T1	124.77^{b}	478.79	353.87^{b}	603.27
T2	155.99^{a}	460.24	479.52^{a}	819.47
T3	150.80^{a}	450.14	445.87^{a}	670.69
T4	154.30^{a}	450.64	457.13^{a}	753.50
Coefficient of variation (%)	11.95	5.80	8.60	17.77
p value	0.040	0.322	0.004	0.148
Feed conversion ratio (g g^{-1})				
Treatment	7-d-old	14-d-old	21-d-old	28-d-old
T1	2.07	2.17	1.73	2.26
T2	1.86	2.04	1.71	1.98
T3	1.93	1.98	1.64	1.88
T4	1.95	2.79	1.79	2.09
p value	0.642	0.051	0.845	0.456
Body weigth (g)				
Treatment	7-d-old	14-d-old	21-d-old	28-d-old
T1	106.93^{b}	213.27^{b}	424.43^{b}	754.67^{b}
T2	129.82^{a}	294.74^{a}	575.91^{a}	1009.40^{a}
T3	124.30^{ab}	275.94^{a}	547.38^{a}	961.15^{a}
T4	124.75^{ab}	272.73^{a}	530.59^{a}	913.21^{a}
Coefficient of variation (%)	5.63	8.01	10.18	9.15
p value	0.010	0.012	0.017	0.018

T1: basal feed naturally contaminated with mycotoxins and without the inclusion of adsorbent (control group); T2: basal feed + adsorbent 1 (Bentonite, Thistle Extract, Yeast Extract, Vitamin E and Choline); T3: basal feed + adsorbent 2 (Bentonite, Thistle Extract, yeast cell wall and Silymarin); T4: basal feed + adsorbent 3 (Bentonite e Algae Extract). Means followed by equal letters in the same column do not differ by Tukey's test at 5% probability.

There was a difference ($p < 0.05$) in WG, in all evaluated periods. Since in the periods 7 d-old and 14 d-old, the WG in treatment 1 was significantly lower when compared with treatment 2, 3 and 4. In periods 21 and 28 days of age treatment 1 obtained the lowest average of WG.

In the study by Weibking, Ledoux, Bermudez, Turk, and Rottinghaus (1993), they fed broilers from 1 to 21 days of age with diets containing around 525,000 ppb kg^{-1} of fumonisin B1, and found that the birds that received had a significant decrease in WG, results that corroborate those found in this study. Similarly, in the work of Lopes, Rutz, Mallmann, and Toledo (2006), who evaluated the inclusion of adsorbent based on bentonite at different levels, observed a positive effect on the inclusion of adsorbents on the WG of animals.

Thus, in this study, in all evaluated periods, treatment without the inclusion of any adsorbent substance impaired the animals' performance. In all evaluated periods, any adsorbent substances that were used behaved similarly for the variable, with no statistical difference between them. Thus, the importance of adding this substance for greater WG of broilers when mycotoxins are present in the feed was demonstrated.

This occurs due to the ability of the adsorbents to adhere to the surface of mycotoxins, forming a mycotoxin and adsorbent complex and thus it is possible to eliminate them together with the feces, not being available for absorption by the animal organism during the passage through the gastrointestinal tract (Vila-Donat, Marín, Sanchis, & Ramos, 2018). In this way, it prevents the absorption of mycotoxin by the chicken and avoids the negative effects of this harmful agent for birds.

There was less feed consumption ($p < 0.05$) in treatment 1 in the periods 7 and 21 days of age in relation to the other treatments. It is possible to verify the harmful effect on FI in the diet without adsorbent. According to Pasha, Farooq, Khattak, Jabbar, & Khan (2007) there is a decrease in feed intake from 7 to 21 days of age in birds that ingested a diet containing mycotoxins when compared to animals feeding on the same diet but with the inclusion of adsorbents based on sodium bentonite.

In the studies by Liu et al. (2018), it was also observed that chickens 1 to 21 days of age fed with naturally contaminated diets (40 ppb of Aflatoxin) had worse feed conversion compared to birds fed with adsorbents (bentonite and lactic acid bacteria). Working with purified fumonisin at doses of 0, 20,000, 40,000 and 80,000 ppb kg^{-1} in the diet of chickens from 1 to 21 days of age, Henry and Wyatt (2000) observed that with these levels of toxin there were no negative effects on the FI of broilers and FCR. The authors inferred that fumonisins are more toxic to pigs and horses than to chickens.

According to resolution No. 7 of February 2011, of the National Health Surveillance Agency – ANVISA (Brasil, 2011), the maximum tolerated limits of fumonisin (B1 and B2) and zearalenone in corn samples are 5000 and 40 ppb, respectively. In this research, the feed samples are within the limits established by ANVISA (Brasil, 2011). However, the Laboratory of Mycotoxicological Analysis at the University of Santa Maria (Laboratório de Análises Toxicológicas da Universidade Federal de Santa Maria [LAMIC], 2011) presents the maximum limits of these mycotoxins (fumonisin and zearalenone) specific for farm animals, in which for broilers the tolerable limits in the starter phase are 100 ppb and 10ppb; grower phase 500 and 20ppb and in the final phase 500 and 20ppb, respectively. In this case, the levels of mycotoxins in this study would be above that recommended by LAMIC (2011) in broiler chickens. The difficulty in identifying the recommended level is noteworthy, as there are divergences in relation to these tolerable limits within the same animal category and between the breeding phases

There was a difference ($p < 0.05$) for BW in all evaluated periods. In the first period, only treatment 1 and 2 had a difference, with treatment 1 having the worst averages. In this sense, in the other periods evaluated, treatment 1 showed the worst averages of BW.

According to a meta-analytical study carried out by Andretta et al. (2011), they found a negative correlation of the final weight of the chickens when mycotoxins (aflatoxins, deoxynivalenol, ochratoxins, fumonisins, zearalenone) are present. This result corroborates our study which showed a difference in the final weight (at 28 days), and the control group that obtained the lowest weight averages in relation to treatments with the addition of adsorbents. Thus, for live weight the use of adsorbent substances reduces the deleterious effects on the performance of birds caused by the presence of mycotoxins in the feed.

As it can be seen in Table 3, there was no influence ($p > 0.05$) of the treatments, for the relative weight (%) of the gizzard, proventriculus and intestine of broilers at 28 days of age.

Table 3. Relative weight (%) of gizzards, proventriculus and intestines of broiler chickens at the 28th day of age submitted to the consumption of feed containing mycotoxins and addition of different adsorbents.

Treatments	Gizzard (%)	Proventriculus (%)	Intestine (%)
T1	4.0579	0.5622	7.4634
T2	4.1798	0.5632	7.4411
T3	3.8605	0.5665	7.7653
T4	4.3010	0.5783	7.4816
Coefficient of variation (%)	11.73	11.86	10.91
p value	0.090	0.350	0.180

T1: basal feed naturally contaminated with mycotoxins and without the inclusion of adsorbent (control group); T2: basal feed + adsorbent 1 (Bentonite, Thistle Extract, Yeast Extract, Vitamin E and Choline); T3: basal feed + adsorbent 2 (Bentonite, Thistle Extract, yeast cell wall and Silymarin); T4: basal feed + adsorbent 3 (Bentonite e Algae Extract).

In the studies carried out by Ledoux, Bermudez, Rottinghaus, and Broomhead (1995) and Santin, Maiorka, Zanella, and Magon (2001), the authors found a decrease in feed intake and an increase in gizzard, proventricle, heart and liver weights in chickens fed diets contaminated with fumonisin or zearalenone at levels above 100,000 ppb kg^{-1} and 800,000 ppb kg^{-1}, respectively. These results are different from the results we found. However, it must be considered that the levels of mycotoxins in the diets of our study are very distant from those tested by the authors, therefore, birds demonstrate greater resistance to clinical signs (mycotoxicological) to fumonisin and zearalenone.

In birds, the action of fumonisins is not fully understood, but it is considered that the target of the toxic action of this mycotoxin is in the biosynthesis of sphingolipids (Wang, Norred, Bacon, Riley, & Merill, 1991). Sphingolipids are important for the integrity and physiological activity of the cell membrane and as part of the membranes, they participate in the processes of cell growth and differentiation and changes in these functions favor oxidative stress (Ledoux et al., 1995; Jahanian, Mahdavi, & Asgary, 2016). Another toxic mechanism is due to the relationship with folate metabolism, since for the proper incorporation of folate in cells it is dependent on sphingomyelin (Santuario, 2000; Miazzo et al., 2005) resulting in less feed intake and weight gain and diarrhea. In some studies, it has been demonstrated that the levels of toxicity of fumonisin

are above 80,000 ppb kg^{-1} (Weibking et al., 1993;), which can show an increase in the relative weight of the liver, proventriculus and gizzard, marked mortality of the batch and ascites. In contrast, the toxic metabolite zearalenone produced by fungi of the same genus Fusarium can produce hyperestrogenic effects. However, birds show little pronounced effects based on experimental studies, if levels above 100,000 ppb kg^{-1} of food are needed to see the first signs of intoxication (Boudergue et al., 2009).

Table 4 shows the results of villus height, villus width, crypt depth and relationship villus:crypt of 28 days old for broiler jejunum for each treatments. There was no difference ($p > 0.05$) for the variables analyzed. Therefore, the amount of mycotoxins present in the diets were not sufficient to cause any change in the area of absorption of the jejunum despite the negative action on WG, FI and BW.

Table 4. Villus height (µm), villus width (µm), crypt depth (µm) and relationship villus:crypt of the jejunum of broilers with 28 d-age, submitted to the consumption of feed containing mycotoxins and addition of different adsorbents.

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Relationship villus:crypt
T1	479.015	63.135	105.584	4.886
T2	545.535	61.906	101.306	5.304
T3	398.685	69.69	84.44	5.305
T4	477.60	86.78	77.64	5.799
Coefficient of variation (%)	29.01	24.09	27.48	20.86
p value	0.082	0.140	0.290	0.102

T1: control group, without the inclusion of adsorbent in the basal feed; T2: Basal feed + Bentonite, Thistle Extract, Yeast Extract, Vitamin E and Choline; T3: Basal feed + Bentonite and Thistle Extract; T4: Basal feed + Bentonite and Algae extract.

In the study by Jahanian et al. (2016), exposure to a high level of aflatoxin (50 and 2000ppb) resulted in lower villus height, villus width, crypt depth and relationship villus:crypt in broilers at 28 and 42 days of age. The decrease in the absorption surface area contributed to a lesser absorption of nutrients and consequent growth depression. However, in the study of these authors, the addition of 2 g kg^{-1} of mannooligosaccharide (MOS) modulated the changes caused by mycotoxin, being positive in decreasing the pathogenic bacteria in the intestine and in the greater height and width of the villus and in the relationship villus: crypt.

Thus, it was expected that the different compositions of the adsorbents (yeast extracts, thistle extract and compounds with antioxidant actions) would also reflect in different responses due to the possible influence on the microbiota and cell integrity in the gastrointestinal tract of birds. The performance of the compounds in the adsorbent could contribute to a larger community of beneficial microorganisms, and in addition, maintain the structure and integrity of the cells of the tract, consequently providing better absorption of nutrients and performance of the birds (Macari & Maiorka, 2017). However, only the positive effect on productive performance characteristics was observed with the use of adsorbents in comparison to diets naturally contaminated with mycotoxins in this study.

Nevertheless, the importance of using adsorbents in the diet of broiler chickens is verified, with the objective of reducing the negative effects of toxic compounds on health and productive performance.

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Conclusion

In this study diets with adsorbents improved the performance of broilers from 1 to 28 days of age compared to diets naturally contaminated by fumonisins and zearalenone at the levels of 1321 and 36ppb, respectively.

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