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Performance and Nutrient Metabolizability in Broilers Fed Diets Containing Corn Contaminated with Fumonisin B1 and Esterified Glucomannan

■Author(s)

Oliveira EM[I]
Tanure CBGS
Castejon FV[I]
Castro RMAD[I]
Rocha FRT[II]
Carvalho FB[III]
Andrade MA[III]
Stringhini JH[III]

[I] Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Campus II Samambaia, Goiânia, 74690-900, Goiás, Brazil.
[II] Faculdade de Zootecnia, Universidade Estadual de Goiás, S. L. M. Belos, Brazil.
[III] Professor in Departamento de Produção Animal, UFG, Goiânia, Goiás, Brazil.

■Mail Address

Corresponding author e-mail address
Eduardo Miranda de Oliveira
Rua Claudio Manoel Costa N 50 Cond Morada Nova Bl X Ap 404 St. Cidade Jardim, CEP 74423-420, Goiânia, GO - Brazil.
Phone: 55 (062) 8596 56 74
E-mail: eduardozootec@gmail.com

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ABSTRACT

An experiment was conducted to evaluate the performance and nutrient metabolizability of broilers fed diets containing fumonisin B1 (FB1) and an esterified glucomannan (EGM). In total, 420 male broilers were distributed according to a 3 x 2 + 1 factorial arrangement, corresponding to three FB1 exposure times (seven, 21, or 35 days), two dietary glucomannan addition levels (0 or 0.1% EGM), and control diet, totaling seven treatments. The following diets were fed: 1) Control diet, 2) pre-starter diet containing FB1, 3) pre-starter diet containing FB1 and 0.1% EGM, 4) starter diet containing FB1, 5) starter diet containing FB1 and 0.1% EGM, 6) grower diet containing FB1, and 7) grower diet containing FB1 and 0.1% EGM. On d 7, broilers fed FB1 presented lower body weight gain and feed intake ($p<0.05$) compared with control treatment. On d 21, no significant performance differences were detected among treatment groups ($p>0.05$). At 35 days of exposure to FB1 body weight gain was reduced ($p<0.05$) compared with broilers fed fumonisin B1 for seven days. From 4 to 7 days and 18 to 21 days of age, FB1 reduced nutrient metabolizability ($p<0.05$). From 36 to 39 days of age, the EGM allowed maintaining apparent metabolizability for ether extract. It was concluded that the EGM did not reduce FB1 effects on performance or nutrient metabolizability in broilers, except for apparent metabolizability of ether extract.

INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi that may be present in animal and human diets by direct or indirect contamination of grains and cereals. The susceptibility to mycotoxins varies with animal species, age, sex, and the toxins involved. Depending on its physicochemical properties and on the animal species involved, each mycotoxin may affect a specific organ or system, leading to specific clinical manifestations of acute or chronic nature (Pier *et al.,* 1973).

Fumonisins are mainly produced by *Fusarium verticillioides* and *Fusarium proliferatum.* The most toxigenic is fumonisin B1 (FB1) and causes different pathologies, such as liver cancer in rodents (Gelderblom *et al.,* 1994), pulmonary edema in pigs (Diaz & Boermans, 1994), and leukoencephalomalacia in horses (Norred & Voss, 1994). Broilers contaminated with increasing dietary FB1 levels (up to 400 mg fumonisin/kg of feed) showed poor live performance, diarrhea, lack of appetite, increased liver size, high proventriculus, gizzard and kidney weight, and high mortality (Ledoux *et al*., 1992). In poultry, these serious symptoms were observed with doses greater than 150 mg fumonisin/kg of feed (Norred & Voss, 1994).

The mode of action of fumonisins is not fully understood yet. However, some investigators have raised the hypothesis that their mode of action may be related with the inhibition or interruption of the metabolism of sphingolipids. This inhibition, which occurs at the

level of the enzyme ceramide synthetase, results in an increase in the concentrations of sphingoid bases (sphinganine and sphingosine) in the serum of exposed animals (Wang *et al*., 1991).

The dietary inclusion of several organic and inorganic adsorbent materials has been employed to try to reduce the absorption and the adverse effects of mycotoxins. These supplements bind to mycotoxins, partially or totally transporting them out of digestive tract, thus preventing the occurrence of mycotoxin poisoning in test subjects (Gowda *et al*. 2008). Olver (1997) asserted that adsorbents have the ability to adhere the mycotoxin and prevent its absorption in the gastrointestinal tract, rendering the toxins inert and non-toxic to animals.

Among most commonly used adsorbents, esterified glucomannans (EGM) derived from the cell wall of the yeast *Saccharomyces cerevisiae* offer the advantages of not adsorbing vitamins and minerals, improving the performance and reducing the effects of mycotoxins on the digestion of poultry. Studies involving the dietary inclusion of EGM have demonstrated its efficacy in counteracting the negative effects of mycotoxins (Aravind *et al*., 2003; Chowdhury *et al.,* 2005; Girish & Smith, 2008).

The isolation of *Fusarium verticillioides* and *Fusarium proliferatum* and the co-occurrence of fumonisins in the same substrate, especially in corn, which is the main feedstuff of poultry feeds, have frequently been reported in literature (Espada *et al.,* 1994, Broomhead *et al*., 2002; Tessari *et al*., 2006; Tessari *et al.,* 2010). Therefore, the objective of the present study was to evaluate the performance and nutrient metabolizability in broilers fed diets containing corn contaminated with FB1 and with the addition of EGM.

MATERIALS AND METHODS

A total of 420 male broiler chicks, with 44.6 ± 0.5g average body weight at the beginning of the experiment, were reared in brooded batteries from one to 39 days of age in the Poultry Sector from of the School of Veterinary Medicine and Animal Science of Escola de Veterinária e Zootecnia da Universidade Federal de Goiás. Birds were distributed according to completely randomized experimental design in a 3 x 2 + 1 factorial arrangement, corresponding to three FB1 exposure times (seven, 21 or 35 days), two adsorbent dietary addition levels (0 or 0.1% EGM), and control diet, totaling seven treatments with five replicates of 12 birds each. The project was approved by the Ethics Committee on Animal Use (ECAU) of Universidade Federal de Goiás under protocol number 157/10.

The following treatments were applied: 1) pre-starter (1 to 7 days), starter (8 to 21 days), and grower (22 to 39 days) control diets, which was not contaminated with FB1 and did not contain EGM; 2) pre-starter diet containing FB1-contaminated corn; 3) pre-starter diet containing FB1-contaminated corn and 0.1% EGM; 4) starter diet containing FB1-contaminated corn; 5) starter diet containing FB1-contaminated corn and 0.1% EGM; 6) grower diet containing FB1-contaminated corn; and 7) grower diet containing FB1-contaminated corn and 0.1% EGM.

The diets were based on corn and soybean meal, and were formulated to meet the nutritional requirements of broilers as recommended by Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011) and to contain equal nitrogen and energy levels. The lighting program consisted of 24 hours of light. Birds were fed *ad libitum* and had free access to water throughout the experiment. Table 1 shows the ingredients and the nutrient composition of the basal diet.

Table 1 – Ingredients and nutrient composition of the experimental basal diet

	Diets		
Ingredients	Pre-Starter	Starter	Growth
Corn	56.82	59.32	63.29
Soybean meal (45% CP)	37.32	34.39	29.60
Soybean oil	1.50	2.37	3.00
Dicalcium phosphate	1.92	1.56	1.34
Limestone	0.81	0.85	0.82
NaCl	0.44	0.42	0.40
DL-methionine 99%	0.35	0.30	0.29
L-lysine HCL 79%	0.34	0.29	0.27
Mineral and Vitamin premix*	0.40	0.40	0.40
EGM or starch**	0.10	0.10	0.10
TOTAL	100.00	100.00	100.00
Metabolizable energy (kcal/kg)	2960	3050	3150
Crude protein (%)	22.40	21.20	19.80
Lysine (%)	1.32	1.21	1.13
Methionine + cysteine (%)	0.95	0.87	0.82
Calcium (%)	0.92	0.84	0.75
Available Phosphorus (%)	0.47	0.40	0.35
Sodium (%)	0.22	0.21	0.20

*Mineral and vitamin premix (composition/kg product): vitamin A (retinyl acetate) 3,125 IU; vitamin D (cholecalciferol) 550,000 IU; vitamin E (dl-α-tocopheryl acetate) 3,750 mg; vitamin K 625 mg; vitamin B1 250 mg; vitamin B2 1,125 mg; vitamin B6 250 mg; vitamin B12 3,750mg; niacin 9,500 mg; calcium 3,750 mg; folic acid 125 mg; DL-methionine 350,000 mg; choline chloride 150,000 mg 50%; growth promoter (avilamycin) 12,500 mg, coccidiostat (Nicarmix) 15,000 mg; selenium 50 mg; antioxidant 2,500 mg; vehicle q.s.p. 1,000 g; manganese 150,000 mg; zinc 100,000 mg; iron 100,000 mg; copper 16,000 mg; iodine 1,500mg.

**Esterified glucomannan was added at the expense of corn in the experimental diets containing this additive.

Birds and feeds were weighed when birds were seven, 21 and 35 days of age to calculate body weight gain, feed intake, feed conversion ratio, and mortality. Mortality and health status were visually observed

and recorded daily during the entire experimental period. Were used 63 broilers were housed in battery cages housed in batteries with (three 3 birds per cage), measuring (30 × 45 × 30 cm). Cages were equipped with trough feeders and nipple drinkers. An with feeder trough type, drinker pressure and aluminum tray was placed underneath each cage for excreta collection located in barn of brickwork controlled digital device and artificial light was provided for 24 hours a day. Three metabolic assays were performed through the excreta collection method.

Total excreta collection was performed daily for three consecutive 4-d periods: from 4 to 7 days (pre-starter phase), 18 to 21 days (starter phase), and 36 to 39 days (grower phase). The trays under the cages were detached, and the feathers were removed from the droppings before collection to prevent contamination. Excreta were weighed, placed in plastic bags, and frozen (- 20° C). Subsequently, excreta samples were dried in a forced-ventilation oven at 55°C for 72 hours). The dried samples were finely ground using mortar and pestle to 1-mm particle size and then stored in sealed containers for dry matter, ether extract, crude protein, and nitrogen content determination. The crude protein and nitrogen contents were determinated using the Kjeldahl method; extract ether by the Goldfish method and dry matter by means of drying the samples in an oven at 105°C. Based on these laboratory results, were calculated nitrogen balance (NB) and apparent metabolizability of dry matter (AMD), of apparent metabolizability nitrogen and apparent metabolizabilityof ether extract (AMEE) were calculated as described by Campos *et al.* (2004).

$$NB\ (\%) = \frac{\text{nitrogen intake ingested} - \text{excreted nitrogen excretion X 100}}{\text{nitrogen intake ingested (g)}}$$

$$AMDM\ (\%) = \frac{\text{dry matter intake ingested} - \text{excreted dry matter excretion X 100}}{\text{dry matter intake ingested (g)}}$$

$$AMN\ (\%) = \frac{\text{nitrogen intake ingested} - \text{excreted nitrogen excretion X 100}}{\text{nitrogen intake ingested (g)}}$$

$$AMEE\ (\%) = \frac{\text{ether extract intake ingested - excreted ether extract excretion X 100}}{\text{ether intake extract ingested (g)}}$$

Representative feed samples, approximately 500 g, were collected to determine dry matter, crude protein, and ether extract contents. The nutritional composition of contaminated corn and non-contaminated corn was determined.

Only dietary mycotoxin levels were analyzed. Samples were analyzed in the Veterinary Laboratory Mercolab, Cascavel unit, state of Paraná, Brazil, which is accredited by the Brazilian Ministry of Agriculture. Mycotoxin levels of 10 mg FB1 /kg were determined in corn samples, and were higher than those recommended by the European Commission (4 ppm) (Commission Regulation no. 1126/2007).

Analysis of variance was performed using Software T Team (2011) and differences among treatments were analyzed by the test of Tukey. Differences were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

No significant differences in mortality among the treatments was observed during the live performance trial. On day 7, no interaction ($p>0.05$) between FB1 contaminated corn and EGM addition was detected. However, the broilers fed the diets with FB1 contaminated corn, independently of EGM addition, presented lower body weight gain and feed intake compared with those offered the control diet (Table 2). Xu *et al.* (2011) reported that corn with low nutritional quality affected growth performance due to the presence of mycotoxins and FB1 significantly decreased body weight and feed intake and caused poor feed efficiency in broilers and turkeys. Tessari *et al.* (2006) observed that the dietary contamination with AFB1 and FB1, individually or in combination (at 50 mg/kg and 200 mg/kg, respectively), may adversely affect the body weight, liver structure, and immune responses of broilers.

Table 2 – Performance of 7-d-old broilers fed diets containing fumonisin B1 contaminated corn and esterified glucomannan.

Exposure time fumonisin B1 (FB1)	BWG[1] (g)	FI[1] (g)	FCR[1]
Control treatment (CT)	139.1	154.1	1.00
Seven days	123.6y	143.9y	1.05
Esterified glucomannan (EGM)			
0%	122.0y	143.2y	1.06
0.1%	125.2y	144.6y	1.05
Probability			
FB1	0.23	0.12	0.58
EGM	0.76	0.84	0.93
FB1*EGM	0.81	0.64	0.42
CT *Treatments	<0.01	<0.01	0.11
CV[2] (%)	6.22	5.66	5.12

BWG[1], body weight gain; FI, feed intake; FCR, feed conversion ratio; CV[2], coefficient of variation; y indicates significant difference from the control treatment by Tukey's test (5% probability level).

Broiler performance was not influenced by the treatments when evaluated at 21 days of age (Table 3). No interaction (p>0.05) between FB1 contaminated corn and EGM was detected. Henry *et al.* (2000) fed broilers with diets contaminated with 20, 40, or 80 mg FB1/kg up to 21 days and did not observe any significant differences in body weight gain in comparison with the control group. Li *et al.* (1999) did not find any significant in body weight gain differences in broilers offered feeds containing FB1 (at 50, 100, or 200 mg FB1/kg diet) either. However, although performance parameters may not be affected by the presence of low dietary concentrations of mycotoxins, the immune function of poultry may be impaired (Smith *et al.*, 1990).

Table 3 – Performance of 21-d-old broilers fed diets containing fumonisin B1 contaminated corn and esterified glucomannan.

Exposure time fumonisin B1 (FB1)	BWG[1] (g)	FI[1] (g)	FCR[1]
Control treatment (CT)	639.1	1023.9	1.42
Seven days	635.9	1025.6	1.45
21 days	633.9	1035.1	1.46
Esterified glucomannan (EGM)			
0%	626.9	1007.1	1.44
0.1%	642.9	1053.6	1.47
Probability			
FB1	0.98	0.36	0.16
EGM	0.22	0.46	0.92
FB1*EGM	0.86	0.42	0.27
CT *Treatments	0.76	0.83	0.75
CV[2] (%)	4.24	7.91	6.66

BWG[1], body weight gain; FI, feed intake; FCR, feed conversion ratio; CV[2], coefficient of variation.

When broilers were 35 days of age (Table 4), there was not interaction (p>0.05) between FB1 contaminated corn and EGM. The body weight gain, feed intake, and feed conversion ratio were not significantly affected (p>0.05) by the dietary treatments. This results may be attributed also to the non-selective mycotoxin binding properties of EGM. However, Aravind *et al*. (2003) concluded that the dietary addition of EGM (at 0.05%) was effective in counteracting the toxic effects of mycotoxins (168 ppb aflatoxin, 8.4 ppb ochratoxin, zearalenone at 54 ppb, and T-2 toxin 32 ppb) in broilers.

Chowdhury *et al.* (2005) showed that the dietary supplementation of 0.2% EGM prevented the adverse effect of *Fusarium* mycotoxins on basophil and monocyte counts in turkeys. Swamy *et al*. (2004) found that EGM (at 0.2% of the diet) did not prevent the negative effects of *Fusarium* mycotoxins on broiler growth performance, but inhibited mycotoxin-induced reduction of peripheral blood B-cell counts. Girish & Smith (2008) reported that EGM prevented the adverse effects caused by feeding *Fusarium* mycotoxins during early growth phase on small intestine morphology of turkeys.

Table 4 – Performance of 35-d-old broilers fed diets containing fumonisin B1 contaminated corn and esterified glucomannan.

Exposure time fumonisin B1 (FB1)	BWG[1] (g)	FI[1] (g)	FCR[1]
Control treatment (CT)	1558.4	2932.0	1.59
Seven days	1558.4a	2881.5	1.58
21 days	1602.1a	2906.0	1.58
35 days	1502.7b	2788.2	1.59
Esterified glucomannan (EGM)			
0%	1556.0	2849.8	1.59
0.1%	1552.7	2867.3	1.58
Probability			
FB1	<0.01	0.06	0.96
EGM	0.88	0.67	0.73
FB1*EGM	0.28	0.23	0.41
CT *Treatments	0.89	0.19	0.68
CV[2] (%)	4.03	3.92	4.19

BWG[1], body weight gain; FI, feed intake; FCR, feed conversion ratio; CV[2], coefficient of variation. Means followed by lowercase letters in the same column differ by Tukey's test (5% probability level).

Between 4 and 7 days (Table 5), there was not interaction between FB1 contaminated corn and EGM (p>0.05). Birds fed contaminated corn with or without EGM presented significantly reduced metabolic rates, including lower nitrogen balance and apparent metabolizability of dry matter, nitrogen and ether extract when compared with those fed the control diet. In addition, EGM dietary inclusion resulted in reduced nitrogen balance and apparent metabolizability of nitrogen when compared with the diet containing FB1 contaminated corn and no EGM inclusion. The results reported in literature are quite variable because they are influenced by the environment where poultry are reared and the possible health challenges. Are envolved enviromental question as the poultry are created and sanitary challenge that are submitted.

Table 5 – Nutrient metabolizability of diets containing fumonisin B1 contaminated corn and esterified glucomannan fed to broilers between 04 to 07 days of age.

Exposure time	AMDM[1]	NB[1]	AMN[1]	AMEE[1]
fumonisin B1 (FB1)	(%)	(g)	(%)	(%)
Control treatment (CT)	70.1	20.9	56.9	78.1
Seven days	68.1y	14.8y	47.0y	69.2y
Esterified glucomannan(EGM)				
0%	67.8y	17.4Ay	52.0Ay	69.7y
0.1%	68.3y	12.1By	41.9By	68.6y
Probability				
FB1	0.50	0.11	0.06	0.66
EGM	0.16	<0.01	<0.01	0.66
FB1*EGM	0.50	0.29	0.20	0.95
CT*Treatments	0.01	<0.01	<0.01	<0.01
CV[2] (%)	3.01	9.09	7.54	6.76

[1]AMDM, apparent metabolizability of dry matter; NB, nitrogen balance; AMN, apparent metabolizability of nitrogen; AMEE, apparent metabolizability of ether extract; CV[2], coefficient of variation. Means followed by the letter y indicate significant difference from the control treatment by Tukey's test (5% probability level). Means followed by capital letters in the same column are significantly different by Tukey's test (5% probability level).

Between 18 to 21 days (Table 6), there was not interaction to FB1 contaminated corn and EGM (p>0.05). However, apparent metabolizability values of nitrogen and of ether extract were reduced when FB1 was present in the feed. This indicates that the apparent metabolizability of nitrogen and of ether extract may have decreased with time because of FB1 accumulation.

Table 6 – Nutrient metabolizability of diets containing fumonisin B1 contaminated corn and esterified glucomannan fed to broilers between 18 and 21 days of age.

Exposure time	AMDM[1]	NB[1]	AMN[1]	AMEE[1]
fumonisin B1 (FB1)	(%)	(g)	(%)	(%)
Control treatment (CT)	74.8	41.8	53.7	90.8
Seven days	75.8	47.6	60.1a	90.7a
21 days	74.3	37.8	48.7b	88.7b
Esterified glucomannan(EGM)				
0%	73.5	37.5	51.9	89.4
0.1%	76.7	47.9	56.8	90.0
Probability				
FB1	0.26	0.11	<0.01	0.01
EGM	0.55	0.28	0.13	0.78
FB1*EGM	0.20	0.53	0.48	0.46
CT *Treatments	0.76	0.75	0.58	0.15
CV[2] (%)	6.27	3.78	11.20	3.64

AMDM[1], apparent metabolizability of dry matter; NB, nitrogen balance; AMN, apparent metabolizability of nitrogen; AMEE, apparent metabolizability of ether extract; CV[2], coefficient of variation; Means followed by different lowercase letters in the same column are different by Tukey's test (5% probability).

The fungi present in grains may change their nutritional profile and significantly reduce their nutrient values. Studies have also showed a high correlation between the presence of mycotoxins and reduced grain energy levels. For instance, Zaviezo and Contreras (2005) found that mycotoxins reduced metabolizable energy levels in 4% to 5% in poultry.

Between 36 and 39 days (Table 7), EGM did not minimize the negative effects of FB1 on nitrogen balance, but was able to maintain the apparent metabolizability ether extract, demonstrating that it may improve the metabolizability of fat, which is the main substrate for fungi growth.

Table 7 – Nutrient metabolizability of diets containing fumonisin B1 contaminated corn and esterified glucomannan fed to broilers between 36 and 39 days of age.

Exposure time	AMDM[1]	NB[1]	AMN	AMEE[1]
fumonisin B1 (FB1)	(%)	(g)	(%)	(%)
Control treatment (CT)	71.4	19.7A	43.8	91.2A
Esterified glucomannan(EGM)				
0%	71.3	17.2AB	45.8	87.8B
0.1%	70.4	15.0B	38.3	89.2AB
Probabilility	0.39	<0.01	0.25	<0.01
CV[2] (%)	1.78	9.16	15.79	1.47

[1]AMDM, apparent metabolizability of dry matter; NB, nitrogen balance; AMN, apparent metabolizability of nitrogen; AMEE, apparent metabolizability of ether extract; CV[2], coefficient of variation; Means followed by capital letters in the same column are significantly different by Tukey's test (5% probability level).

Many recent studies have focused on the reduction of the contamination of feeds by mycotoxins. The low concentration of FB1 and EGM used in this study may be the reason when no interactions or positive effects on performance and nutrient availability were observed. The Food and Drug Administration (FDA, 2001) establishes a maximum allowable level of total fumonisins in the diet of poultry for meat production of 100 mg/kg (no more than 50% of diet). This level was determined using data from 21-d studies, while in the present study, broiler were evaluated up to 39 days of age. Considering the volume of feed currently manufactured in the global poultry industry, it is unlikely that poultry will be fed diets containing high levels of FB1 for extended periods of time.

The results of the present study showed that broiler performance was negatively affected by to the presence of FB1 in the feed and that the dietary addition of EGM did not prevent the adverse effects of fungal contamination. Further studies on the effects of FB1 and using different mycotoxin adsorbent levels should be conducted with broilers.

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