The effects of necrotic enteritis, aflatoxin B_1, and virginiamycin on growth performance, necrotic enteritis lesion scores, and mortality in young broilers

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ABSTRACT The effects of increasing aflatoxin B_1 concentration (0, 0.75, 1.5 mg/kg) on broilers with or without necrotic enteritis or virginiamycin were determined. In the 23-d study, 22 male Cobb 500 chicks per pen were allotted to 12 treatments (3 × 2 × 2 factorial arrangement) with 8 replications. Intestines of 5 birds per pen were examined for lesions on d 21. Birds were allowed to consume feed and water ad libitum. Aflatoxin was included in the diets from d 0. All birds received a 10× dose of coccidiosis vaccine on d 10. Pens of birds where necrotic enteritis was being induced were on *Clostridium perfringens* pathogen (CPP) contaminated litter from d 0. Aflatoxin decreased gain and feed intake and resulted in poorer feed:gain, increased mortality, and higher lesion scores. Inducing necrotic enteritis increased lesion scores and decreased feed intake and gain. Adding virginiamycin to the diets improved gain, feed intake, feed conversion, and decreased mortality. There was a 3-way interaction (aflatoxin × virginiamycin × CPP) on gain; increasing aflatoxin decreased gain and the effects of CPP and virginiamycin were dependent on aflatoxin concentration. In the absence of aflatoxin virginiamycin increased gain but was unable to prevent the growth suppression caused by CPP. At 0.75 mg/kg of aflatoxin virginiamycin no longer increased growth in non-CPP challenged birds but was able to increase growth in CPP-challenged birds. At the 1.5 mg/kg of aflatoxin concentration, virginiamycin increased gain in non-CPP-challenged birds but challenging birds with CPP had no effect on gain. Virginiamycin improved overall feed conversion with the greatest improvement at 1.5 mg/kg (aflatoxin × virginiamycin, $P < 0.05$). Aflatoxin increased lesion scores in unchallenged birds but not in challenged birds (aflatoxin × CPP, $P < 0.001$). Aflatoxin and necrotic enteritis decrease broiler performance and interact to decrease weight gain, virginiamycin helps improve gain in challenged birds at 0.75 mg/kg of aflatoxin, but not at 1.5 mg/kg of aflatoxin.

Key words: necrotic enteritis, aflatoxin, coccidiosis, virginiamycin, *Clostridium perfringens*

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INTRODUCTION

Aflatoxin, a mycotoxin, is a secondary metabolite mainly produced by several species of *Aspergillus flavus* and *Aspergillus paracitus* but also by *Aspergillus nomius* and *Aspergillus pseudotamarii* (Council for Agricultural Science and Technology, 2003). The Food and Agriculture Organization (2012) estimates that over 25% of food crops worldwide are contaminated by mycotoxin-producing fungi and global losses due to mycotoxins are approximately 1,000 million metric tonnes annually, with a cost to the US and Canadian livestock and feed industries of approximately $5 billion (FAO/IAEA, 2001). The first mycotoxin elucidated, aflatoxin is also considered to be the most toxic of the commonly studied mycotoxins (Council for Agricultural Science and Technology, 2003; Yu et al., 2004; Devegowda and Murthy, 2005). In poultry, it causes reductions in feed intake and gain (Osweiler et al., 2010), morphological changes in the intestine (Applegate et al., 2009; Yunus et al., 2011) and liver (Miazzo et al., 2005; Yarru et al., 2009), and decreases immune function (Hegazy et al., 1991; Hegazy and Adachi, 2000; Verma et al., 2004).

Necrotic enteritis is a common infectious disease in broiler production. The actual cost of necrotic enteritis to the industry is unknown, but estimates are $2 to 3 billion in the United States due to the poor performance of infected birds and the cost of treatment (McDevitt et al., 2006; Lee et al., 2011). The disease develops when *Clostridium perfringens*, normally found in the gastrointestinal tract of poultry (Al-Sheikhly and Truscott, 1977) in small numbers, proliferates rapidly. This can be triggered by a variety of reasons including high dietary protein (Drew et al., 2004), coccidiosis (Ficken and Wages, 1997), or high viscosity di-

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ets (Jia et al., 2009). *Clostridium perfringens* produces toxins that cause the disease symptoms of necrotic enteritis: intestinal lesions, decreased water and feed intake, growth depression, and death. Antibiotics such as virginiamycin are used to prevent necrotic enteritis (George et al., 1982). McReynolds et al. (2004) showed that immunocompromised broilers had more severe responses to a necrotic enteritis challenge. The synergistic effect of aflatoxin and endotoxin lipopolysaccharide, a gram-negative bacterial cell wall, on growth performance reduction and an increase of mortality has been demonstrated in broilers and turkeys (Guaiume, 2005). An interaction of aflatoxin and infectious bursal disease in broilers has also been reported (Mahajan et al., 2002). However, the interaction of aflatoxicosis and necrotic enteritis has not been studied previously, even though the 2 diseases are commonly observed in the field. Therefore, the objective of the present study is to investigate the outcomes and relationships when aflatoxin and necrotic enteritis challenges are given simultaneously in broilers and their interactions with dietary virginiamycin as model development for future research.

MATERIALS AND METHODS

Birds and Treatments

A 23-d experiment was conducted with 2,112 male Cobb 500 chicks from a commercial hatchery. Methods used in this experiment concerning animal care were in accordance with the standards of Colorado Quality Research Institutional Animal Care and Use Committee, Wellington. Chicks at 1 d posthatch were allotted to 12 treatments with 8 replications and 22 birds per treatment replication in a 3 × 2 × 2 factorial arrangement. Factors were as follows: 3 levels of aflatoxin B_1, 2 levels of virginiamycin, and 2 levels of pathogen challenge. On d 7 the number of chicks per pen was equalized to 20. The facility was environmentally controlled with concrete floor pens measuring 1.219 m × 1.129 m. Incandescent lights were used in a commercial lighting program.

Diets and Aflatoxin B_1 Challenge

Cultured fungal aflatoxin was obtained from the Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia. It was prepared by inoculating whole grain rice and distilled water with a fungal culture of *Aspergillus paraciticus* (NRRL-2999), which was raised for 7 d; then the fungus was killed, dried, and ground. The fungal aflatoxin B_1 is hereafter known as aflatoxin.

Birds had ad libitum access to feed and water. Corn-soybean meal basal starter and grower diets (Table 1) were made and treatment diets were made by adding an aflatoxin-corn premix to reach 0, 0.75, or 1.50 mg/kg of aflatoxin B_1 in the complete feed with the addition of 0 or 22 mg/kg of virginiamycin (Phibro Animal Health, Teaneck, NJ). Ground corn replaced the aflatoxin-corn premix and sand replaced virginiamycin in treatments without those ingredients.

Table 1. Compositions of the basal diets for the starter (d 0 to 16) and grower (d 16 to 23) phases

Item	Starter	Grower
Ingredient, % (as-is basis)		
Corn	59.31	65.33
Soybean meal (48%)	31.42	25.20
Meat and bone meal	5.00	5.00
Animal fat	2.08	2.30
Deflourinated rock phosphate	0.71	0.60
Limestone	0.54	0.55
Salt	0.27	0.28
DL-Methionine	0.24	0.26
L-Lysine	0.06	0.12
Choline chloride (60%)	0.09	0.08
Vitamin premix[1]	0.18	0.18
Trace mineral premix[2]	0.10	0.10
Total	100.00	100.00
Nutrient composition		
ME, kcal/kg	3,018.00	3,083.00
CP, %	23.00	20.50
Ca, %	0.95	0.90
Available P, %	0.45	0.42
Lysine, %	1.28	1.16
Methionine, %	0.60	0.58
Methionine + cysteine, %	0.96	0.91
Na, %	0.21	0.21
Cl, %	0.25	0.26

[1]Supplied per kilogram of diet: 16,865 IU of vitamin A, 5,456 IU of vitamin D_3, 50 IU of vitamin E, 0.03 mg of vitamin B_{12}, 0.16 mg of biotin, 3.5 mg of vitamin K_3 (as menadione sodium sulfate complex), 3.5 mg of thiamine, 14 mg of riboflavin, 22 mg of calcium pantothenate, 5.6 mg of pyridoxine, 17 mg of niacin, and 1.8 mg of folic acid.

[2]Supplied per kilogram of diet: 120 mg of Mn (as manganese sulfate), 99 mg of Zn (as zinc sulfate), 40 mg of iron (as ferrous sulfate), 25 mg of Mg (as magnesium oxide), 10 mg of Cu (as copper chloride), 1.4 mg of I (as calcium iodate), 0.3 mg of Se (as sodium selenite).

Analysis of corn, soybean meal, and basal diets for the mycotoxins: aflatoxins, zearalenone, vomitoxin, ochratoxin-A, and fumonisin B_1 were performed using HPLC with detection limits of 10, 250, 500, 50, and 500 μg/kg, respectively. All mycotoxins were below detection limits in the basal diets. Analysis of diets for aflatoxin B_1 was as follows: starter 5 and 7) 850 μg/kg; starter 6 and 8) 910 μg/kg; starter 9 and 11) 1,720 μg/kg; starter 10 and 12) 1,765 μg/kg; grower 5 and 7) 795 μg/kg; grower 6 and 8) 785 μg/kg; grower 9 and 11) 1,620 μg/kg; and grower 11 and 12) 1,660 μg/kg with a detection limit of 10 μg/kg. No aflatoxin B_1 was detected in diets formulated to 0 μg/kg of aflatoxin B_1.

Necrotic Enteritis Challenge

Birds were vaccinated for Marek's disease at the hatchery. From d 0, *C. perfringens* pathogen (**CPP**) challenged birds were placed on recently used litter from chickens challenged via the feed with CPP cultures (CL-15, type A, α, and β2 toxins, Microbial Research Inc., Fort Collins, CO), whereas nonchallenged birds were placed on clean litter. All birds received a 10× dose of coccidia vaccine (Novus, St. Louis, MO)

administered in their feed on d 10. Birds and feeders were weighed at d 0 and 23 to determine daily feed intake, gain, and feed:gain.

Intestinal Lesion Scoring

Five birds per pen were randomly selected on d 21, weighed, euthanized by cervical dislocation, and intestines were removed for lesion scoring by a veterinarian. The entire length of the duodenum and jejunum and the first third of the ileum were examined for lesion scoring. Scores ranged from 0 to 5 and are as follows:

- 0 = normal: no necrotic enteritis lesions, small intestine has normal elasticity (rolls back to normal position after being opened);
- 1 = mild: small intestinal wall is thin and flaccid (remains flat when opened and does not roll back into normal position after being opened), thickened mucus covering mucus membrane;
- 2 = minor necrotic enteritis: 1 to 6 necrotic enteritis pocks (minor ulceration and necrosis of the intestinal wall);
- 3 = moderate necrotic enteritis pocks: more than 6 necrotic enteritis pocks or coalescing of pocks;
- 4 = severe: extensive area(s) of necrosis and ulceration of the small intestinal membrane, significant hemorrhage, layer of fibrin and necrotic debris on the mucus membrane (Turkish towel appearance);
- 5 = dead or moribund: a bird that would likely die within 24 h and has necrotic enteritis lesion score of 2 or more.

Statistical Analysis

Data were analyzed by a GLM for a factorial experimental design using Statistix (version 8.0, Analytical Software, Tallahassee, FL); pairwise comparisons for the means were performed by a least significant difference test ($P \leq 0.05$). Block was found to be nonsignificant and was removed from the model. To obtain better fit to a normal distribution, mortality and lesion score data were subjected to arc sine transformation before statistical analysis. Contrast statements were included to examine the main effects of aflatoxin, CPP challenge, and virginiamycin addition and the 2- and 3-way interactions of a 3 × 2 × 2 factorial arrangement of treatments. The pen of birds was the experimental unit for the analysis of all data.

RESULTS

Main Effects

Aflatoxin in the diet decreased gain and feed intake (Table 2), with significantly poorer results at each increasing aflatoxin concentration. Feed conversion was poorer when aflatoxin was added to the diet at the 1.5 mg/kg concentration. There was an increase in mortality when 1.5 mg/kg of aflatoxin was included in the diet, whereas lesion score was higher than the control when either concentration of aflatoxin was added but was not different between 0.75 and 1.5 mg/kg of aflatoxin. Birds that were challenged with CPP had decreased gain and feed intake and poorer feed conversion. Mortality was unaffected by CPP challenge. Lesion score was higher in birds challenged with CPP. Adding virginiamycin increased gain and feed intake and improved feed conversion. Mortality decreased with virginiamycin addition, but lesion score was increased when virginiamycin was added to the diet.

Interactions

Five birds per pen were euthanized on d 21 and intestines were removed for lesion scoring, with the scale ranging from 0 to 5. There was a significant 2-way interaction (Table 3) between aflatoxin level and CPP challenge on lesion score. Lesion scores were higher when birds were challenged with CPP or when aflatoxin was added to the diet. Lesion scores of non-CPP challenged birds not fed aflatoxin were low (lesion score = 0.19) and increased as dietary aflatoxin concentration increased, whereas lesion scores in CPP-challenged birds were higher than those in the unchallenged birds and were independent of aflatoxin concentration. There was also a 2-way interaction of CPP challenge and virginiamycin on lesion score. Birds fed virginiamycin had higher lesion scores, with similar scores for non-CPP birds without and with virginiamycin, but higher scores in CPP-challenged birds that were fed virginiamycin.

There was a 3-way interaction (Table 4) on gain as increasing aflatoxin concentration decreased gain and the effects of CPP and virginiamycin were dependent on aflatoxin concentration. In the absence of aflatoxin, adding virginiamycin increased gain but was unable to prevent the growth suppression caused by CPP. At 0.75 mg/kg of aflatoxin, virginiamycin no longer increased growth in the absence of the CPP challenge but was able to increase growth in CPP-challenged birds. At the 1.5 mg/kg of aflatoxin concentration, virginiamycin increased gain in non-CPP-challenged birds but challenging birds with CPP had no effect on gain. There was also a 3-way interaction on feed conversion as CPP and virginiamycin had no effect on feed conversion in the absence of aflatoxin, but at the 0.75 mg/kg of aflatoxin level challenging birds with CPP without virginiamycin decreased feed conversion relative to birds receiving only one of those challenges, and at 1.5 mg/kg of aflatoxin virginiamycin improved feed conversion regardless of the presence of CPP.

DISCUSSION

Adding increasing concentrations of aflatoxin decreased feed intake. This is a common effect of aflatoxin (Osweiler et al., 2010). The decrease in feed intake when aflatoxin is present in the diets may be an

Table 2. The main effects of aflatoxin, *Clostridium perfringens* pathogen challenge, and virginiamycin on broiler weight gain, feed conversion, mortality, and intestinal lesion score[1]

Item	Weight gain, g	Daily feed intake, g	Feed conversion ratio (feed:gain)	Mortality	Lesion score
Main effect					
AFL,[2] mg/kg					
0	923[a]	53.8[a]	1.363[b]	2.03[b]	0.82[b]
0.75	836[b]	48.6[b]	1.373[b]	2.19[b]	0.99[a]
1.50	649[c]	38.6[c]	1.412[a]	6.25[a]	1.12[a]
CPP[3]					
–	825[a]	47.9[a]	1.377[b]	3.85	0.60[b]
+	781[b]	46.1[b]	1.388[a]	3.13	1.35[a]
VM,[4] mg/kg					
0	792[b]	46.7[b]	1.396[ab]	3.96	0.91[b]
22	814[a]	47.4[a]	1.373[b]	3.02	1.04[a]
P-value					
AFL	0.001	0.001	0.001	0.001	0.001
CPP	0.001	0.001	0.022	0.435	0.001
VM	0.001	0.023	0.001	0.321	0.035

[a–c]Means within a main effect within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means were the average of 8 replicate pens with 22 birds initially (equalized to 20 on d 7); 5 birds/pen were euthanized on d 21 for lesion scoring.

[2]Aflatoxin (AFL), cultured material from Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia.

[3]A minus sign (−) indicates birds on this treatment were placed on clean litter, a plus sign (+) indicates treatments in which disease was induced by placing *Clostridum perfringens* pathogen (CPP) challenged birds on litter obtained from broilers that had undergone a recent *C. perfringens* challenge; all experimental birds were given a 10× dose of coccidiosis vaccine on d 10.

[4]Virginiamycin (VM, Phibro Animal Health, Teaneck, NJ) was added to diets at 0 or 22 mg/kg.

Table 3. The 2-way interactions of aflatoxin, *Clostridium perfringens* pathogen challenge, and virginiamycin on broiler weight gain, feed conversion, mortality, and intestinal lesion score[1]

Item		Weight gain, g	Daily feed intake, g	Feed conversion ratio (feed:gain)	Mortality	Lesion score
Interaction						
AFL,[2] mg/kg	VM,[3] mg/kg					
0	0	916[a]	53.5[a]	1.370[cd]	2.81[bc]	0.74[c]
0	22	931[a]	54.1[a]	1.356[d]	1.25[c]	0.90[bc]
0.75	0	826[b]	48.1[c]	1.382[bc]	3.44[bc]	0.84[c]
0.75	22	847[b]	49.2[b]	1.363[d]	0.94[c]	1.14[a]
1.50	0	634[d]	38.5[d]	1.436[a]	5.63[ab]	1.15[a]
1.50	22	664[c]	38.8[d]	1.389[b]	6.88[a]	1.09[ab]
AFL, mg/kg	CPP[4]					
0	–	942[a]	54.7[a]	1.359[c]	1.56[c]	0.19[c]
0	+	905[b]	52.9[b]	1.368[bc]	2.50[bc]	1.45[a]
0.75	–	865[c]	49.6[c]	1.362[c]	2.50[bc]	0.74[b]
0.75	+	807[d]	47.7[d]	1.384[b]	1.88[bc]	1.24[a]
1.50	–	667[e]	39.6[e]	1.411[a]	7.50[a]	0.86[b]
1.50	+	631[f]	37.6[f]	1.414[a]	5.00[ab]	1.38[a]
VM, mg/kg	CPP					
0	–	809[b]	47.6[a]	1.393[a]	4.17	0.60[c]
0	+	775[c]	45.8[b]	1.399[a]	3.75	1.22[b]
22	–	840[a]	48.3[a]	1.361[c]	3.54	0.59[c]
22	+	787[c]	46.4[b]	1.378[b]	2.50	1.49[a]
P-value						
AFL × VM		0.001	0.022	0.001	0.240	0.066
AFL × CCP		0.271	0.951	0.255	0.324	0.001
VM × CCP		0.127	0.821	0.263	0.736	0.025

[a–f]Means within an interaction effect within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means were the average of 8 replicate pens with 22 birds initially (equalized to 20 on d 7); 5 birds/pen were euthanized on d 21 for lesion scoring.

[2]Aflatoxin (AFL), cultured material from Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia.

[3]Virginiamycin (VM, Phibro Animal Health, Teaneck, NJ) was added to diets at 0 or 22 mg/kg.

[4]A minus sign (−) indicates birds on this treatment were placed on clean litter, and a plus sign (+) indicates treatments in which disease was induced by placing *Clostridum perfringens* pathogen (CPP) challenged birds on litter obtained from broilers that had undergone a recent *C. perfringens* challenge; all experimental birds were given a 10× dose of coccidiosis vaccine on d 10.

Table 4. The 3-way interactions of aflatoxin, *Clostridium perfringens* pathogen challenge, and virginiamycin on broiler weight gain, feed conversion, mortality, and intestinal lesion score[1]

Item			Weight gain, g	Daily feed intake, g	Feed conversion ratio (feed:gain)	Mortality	Lesion score
Interaction							
AFL,[2] mg/kg	VM,[3] mg/kg	CPP[4]					
0	0	–	925^{b}	54.4^{ab}	1.370^{def}	1.88^{cd}	0.15^{d}
0	0	+	906^{b}	52.6^{c}	1.371^{def}	3.75^{abcd}	1.33^{a}
0	22	–	958^{a}	55.0^{a}	1.347^{f}	1.25^{cd}	0.23^{d}
0	22	+	904^{b}	53.2^{bc}	1.365^{def}	1.25^{cd}	1.58^{a}
0.75	0	–	863^{c}	49.4^{d}	1.369^{def}	3.75^{abcd}	0.68^{c}
0.75	0	+	788^{e}	46.8^{e}	1.396^{bc}	3.13^{bcd}	1.00^{b}
0.75	22	–	867^{c}	49.8^{d}	1.354^{ef}	1.25^{cd}	0.80^{bc}
0.75	22	+	827^{d}	48.6^{d}	1.372^{cde}	0.63^{d}	1.48^{a}
1.50	0	–	638^{g}	39.0^{fg}	1.440^{a}	6.88^{ab}	0.98^{bc}
1.50	0	+	630^{g}	37.9^{gh}	1.431^{a}	4.38^{abcd}	1.33^{a}
1.50	22	–	696^{f}	40.2^{f}	1.381^{bcd}	8.13^{a}	0.75^{bc}
1.50	22	+	632^{g}	37.4^{h}	1.396^{bc}	5.63^{abc}	1.43^{a}
P-value							
AFL × VM × CCP			0.015	0.707	0.012	0.895	0.084

[a–h]Means within an interaction effect within a column with no common superscripts differ significantly ($P < 0.05$).

[1]Means were the average of 8 replicate pens with 22 birds initially (equalized to 20 on d 7); 5 birds/pen were euthanized on d 21 for lesion scoring.

[2]Aflatoxin (AFL), cultured material from Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia.

[3]Virginiamycin (VM, Phibro Animal Health, Teaneck, NJ) was added to diets at 0 or 22 mg/kg.

[4]A minus sign (−) indicates birds on this treatment were placed on clean litter, and a plus sign (+) indicates treatments in which disease was induced by placing *Clostridum perfringens* pathogen (CPP) challenged birds on litter obtained from broilers that had undergone a recent *C. perfringens* challenge; all experimental birds were given a 10× dose of coccidiosis vaccine on d 10.

attempt to decrease toxin load as is seen with pigs consuming vomitoxin (Council for Agricultural Science and Technology, 2003) or because of morphological changes in the intestine (Applegate et al., 2009; Yunus et al., 2011) and liver (Miazzo et al., 2005; Yarru et al., 2009) when aflatoxin is present in the diet. Decreased general health of the bird from an aflatoxin-induced decrease in immune function (Verma et al., 2004) may also reduce feed intake. Birds challenged to cause necrotic enteritis also had decreased feed intake, which is typical of clinical necrotic enteritis (Williams, 2005) and is often one of the main symptoms of a subclinical necrotic enteritis infection (McDevitt et al., 2006). A reduction in feed intake caused by this enteric disease should be expected as "the lesions of necrotic enteritis are among the most severe of any disease that occurs in the chicken intestine" (Long et al., 1974). Virginiamycin addition increased feed intake, although other researchers have seen little or no influence of virginiamycin on feed intake (O'Connor-Dennie and Southern, 2005).

In the current study, gain generally followed the patterns of feed intake, decreasing as dietary aflatoxin concentration increased or with CPP challenge and increasing when virginiamycin was added to the diet. Decreased gain is a symptom of necrotic enteritis in poultry (Lensing et al., 2010) or aflatoxicosis in most species (Rauber et al., 2007; Osweiler et al., 2010). Virginiamycin is a streptogramin antibiotic that that can inhibit bacterial growth, especially of gram-positive bacteria such as *C. perfringens* (Gaskins et al., 2002), whereas Zhou et al. (2007) showed increased amounts of *Enterococcus* in the ileum of broilers fed virginiamycin and theorized that antibiotics reduce harmful bacteria and increase bacteria that could be viewed as probiotics. Virginiamycin has been shown to increase gain in broilers and turkeys (Waibel et al., 1991; Cervantes et al., 2002; O'Connor-Dennie and Southern, 2005). Whereas the overall reduction in gain was 5.3% when the birds were challenged to induce necrotic enteritis, aflatoxin caused a considerably greater decrease. Aflatoxin contamination in the diets decreased overall gain 9.4% between 0 and 0.75 mg/kg and an even higher 20.3% decrease as aflatoxin increased from 0.75 to 1.5 mg/kg.

Aflatoxicosis decreases immune function in poultry (Thaxton et al., 1974; Hegazy et al., 1991; Hegazy and Adachi, 2000) and swine (Chaytor et al., 2011). McReynolds et al. (2004) showed that immunocompromised broilers had more severe responses to a necrotic enteritis challenge. Thus, aflatoxin contamination of the diet during a necrotic enteritis challenge will likely result in poorer performance of broilers than with either challenge alone. This was seen in the current study when the combination of dietary 0.75 mg/kg of aflatoxin and a CPP challenge decreased gain in a synergistic manner. A possible explanation for the nonchallenged and challenged birds having similar gain at 1.5 mg/kg of dietary aflatoxin is that at this concentration birds were so depressed in growth from aflatoxin that only minor decreases could result from the disease challenge, especially because necrotic enteritis and aflatoxicosis can affect the same organs (i.e., intestine and liver). Another hypothesis is that because necrotic enteritis is a disease that is induced and exacerbated by contamination from external sources, the higher concentration of aflatoxin reduced the typical behaviors of

the birds (i.e., preening and eating) to the point that the disease challenge was lower because of decreased ingestion of CPP when birds were fed 1.5 mg/kg of aflatoxin than that of the birds fed 0.75 mg/kg. A 28% decrease in feed intake was seen between the 0 and 1.5 mg/kg of aflatoxin concentrations, which supports this theory. At 1.5 mg/kg of aflatoxin, the unchallenged birds not receiving virginiamycin had poorer gain than those receiving virginiamycin. This disagrees with previous research where Abo-Norag et al. (1995) reported the poorest performance in broilers when virginiamycin was given to broilers in combination with aflatoxin.

Dietary aflatoxin at 1.5 mg/kg resulted in poorer feed efficiency, and both feed intake and feed-to-gain ratio were influenced by virginiamycin in the diet. The poorer feed conversion indicates that aflatoxin was decreasing nutrient absorption into the body (Applegate et al., 2009), perhaps as a result of changes in the intestine (Tejada-Castañeda et al., 2008, Yunus et al., 2011). Using a scanning electron microscope, Tejada-Castañeda et al. (2008) showed that aflatoxin altered microvilla and resulted in severe lesions affecting the tight junctions of the intestinal mucosa, which would cause decreased nutrient absorption. Applegate et al. (2009) reported decreased digestibility of AME_n and apparent N in hens fed 0.6 or 1.2 mg/kg of aflatoxin. Feeding broilers diets contaminated with aflatoxin resulted in decreased utilization of both protein and energy (Verma et al., 2002).

Increased nutrient absorption has been shown because of addition of virginiamycin to the diet (Pelura et al., 1980; O'Connor-Dennie and Southern, 2005). Feeding virginiamycin improved the feed-to-gain ratio throughout this experiment with the most effect in the birds fed 1.5 mg/kg of aflatoxin, possibly because of its effects on digestibility and disease. Birds challenged to induce necrotic enteritis also had a poorer feed-to-gain ratio. Necrotic enteritis causes intestinal changes ranging from an intestinal wall that is thin and remains flat when opened to the standard "Turkish towel" appearance, which shows an extensive area of necrosis and ulceration. The intestine may also fill with a brownish liquid and become friable. Thus, decreased feed efficiency is commonly reported (Kaldhusdal et al., 2001; Hofacre et al., 2003) with necrotic enteritis and should be expected.

Lesion scores increased in birds challenged with CPP in this experiment. In clinical cases of necrotic enteritis seen in the field, lesions might be higher than what we saw in this experiment, but it is generally accepted that subclinical cases of necrotic enteritis with ongoing reductions in feed intake, gain, and feed conversion are more costly to the poultry industry than the clinical outbreaks with high levels of mortality. This increase in lesion scores, together with the decreases in feed intake and gain and poorer feed efficiency in challenged birds, indicates that we succeeded in inducing necrotic enteritis. Aflatoxin concentration did not affect lesion scores in challenged birds, but adding aflatoxin to the diets of the unchallenged birds increased lesion scores. Lesion scores for the nonchallenged birds without aflatoxin were 0.19 on a scale of 0 to 5; the increase caused by aflatoxin indicates that it was causing changes in the gut of the birds in the absence of a CPP challenge. Previous reports have shown that dietary aflatoxin will cause adaptations and morphological changes in the intestine of broilers and hens (Applegate et al., 2009; Yunus et al., 2011). Changes such as this explain the increase in lesion scores caused by aflatoxin in unchallenged birds in this experiment. Minor lesions have been also been seen in unchallenged control birds in an experiment looking at necrotic enteritis reported by Olkowski et al. (2006).

Birds fed virginiamycin had higher lesion scores than those not fed virginiamycin, with no difference in score when nonchallenged birds were fed virginiamycin but higher scores when birds had the CPP challenge. This was an unexpected result as virginiamycin and other antibiotics have long been used to control necrotic enteritis and have been shown to decrease lesion scores (George et al., 1982; Hofacre et al., 1998; Brennan et al., 2003). However, virginiamycin is known to cause thinning of the intestinal wall (Henry et al., 1986) as well as decreased length and weight in the gastrointestinal tract of broilers (Miles et al., 2006) and such changes may account for the increase in lesion score. This did not seem to be detrimental to the birds as adding virginiamycin did improve feed intake, gain, and feed conversion, and decreased mortality in the current study.

Mortality is often the most visible indication that an avian flock has necrotic enteritis (McDevitt et al., 2006). In a review, Lee et al. (2011) stated that broiler mortality during a necrotic enteritis outbreak typically runs from 2 to 10% but up to 50% can be seen. In this study mortality that could be attributed to necrotic enteritis was negligible, but this was also seen by Olkowski et al. (2006) who reported no mortality in broilers from an oral challenge of high doses of *C. perfringens*. Mortality from all causes was higher when birds were fed 1.5 mg/kg of aflatoxin compared with those fed 0 or 0.75 mg/kg of aflatoxin. Aflatoxin is considered to be the most toxic (Yu et al., 2004; Devegowda and Murthy, 2005) of the commonly studied mycotoxins and has been shown to increase mortality in poultry, other livestock, and humans (FAO/IAEA, 2001; Council for Agricultural Science and Technology, 2003; Rauber et al., 2007). Aflatoxin B_1, is the most potent form, having a 50% lethal dose of 0.37 mg/kg in 1-d-old ducks, half that of aflatoxin G_1 (Council for Agricultural Science and Technology, 2003). Virginiamycin decreased mortality from all causes in the current experiment.

In conclusion, the study demonstrated that aflatoxin- and microbial toxin-challenged birds had significantly higher lesion scores and decreased gain; aflatoxin decreased feed intake, gain, and feed efficiency and virginiamycin improved feed:gain and feed intake. Dietary aflatoxin and necrotic enteritis interactions were noted, especially in gain, one of the most costly effects that

would occur during a subclinical necrotic enteritis outbreak in the field. The 0.75 mg/kg of aflatoxin concentration combined with necrotic enteritis decreased gain synergistically, but the 1.5 mg/kg concentration may be too high a dose to show further effects because of necrotic enteritis. Therefore, an aflatoxin concentration between 0.75 and 1.5 mg/kg should be used in subsequent experiments. This model can be used in future research that is needed to look at ways to alleviate the challenges of aflatoxin and necrotic enteritis, especially as the industry is trying to decrease the use of antibiotics. In conclusion, 0.75 mg/kg of dietary aflatoxin increased the effects of necrotic enteritis, whereas feeding virginiamycin decreased those effects.

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