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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Biological Effect of Naturally Occurring Mycotoxins Fed to Poult Reared to 21 Days of Age

Jesse L. Grimes<sup>1</sup>, Matthew D. Koci<sup>1</sup>, Charles R. Stark<sup>1</sup>, Doug P. Smith<sup>1</sup>, Prashant K. Nighot<sup>2</sup> and Teena Middleton<sup>3</sup>

<sup>1</sup>Department of Poultry Science, College of Agriculture and Life Sciences, NC State University, Raleigh, NC 27695, USA

<sup>2</sup>Department of Clinical Sciences, College of Veterinary Medicine, NC State University, Raleigh, NC 27695, USA

<sup>3</sup>Ag Provisions, LLC, Kenansville, NC, USA

**Abstract:** A trial was conducted to observe potential changes in turkey poult reared to 21 d by feeding diets with naturally occurring mycotoxins. Two sources of corn, one each with Aflatoxin (AFL) and Deoxynevalenol (DON), were obtained. Treatments (T) were: 1) clean corn (C), 2) AFL (A), 3) DON (D) and 4) ½A+½D (AD). A marker (celite, 1.5%) was added for 21 d AMEn determination. A basal ration with ingredients except corn was mixed. The basal and corn were mixed for T feeds. Feed was pelleted and crumbled. Male poult were placed into 24 pens in petersyme batteries (7 birds/pen; 6 pens/T). Feed consumption, by pen and BW were determined by wk. At 21 d, birds were euthanized. Heart (H), spleen (S), gizzard (G), liver (L) and bursa of fabricious (B) were weighed and Breast Muscle (BM) collected for color analysis. Light microscopic analysis of H&E stained L, B and S were performed to assess histopathological changes. One bird per pen was injected IV with 7% SRBC on d 7 and 14. Serum Albumin (SA) and Antibody Production (AB) were determined at 11, 14, 18 and 21 d (1 bird/pen). Data were analyzed using GLM ( $p < 0.05$ ). The D feed had 1.7 ppm DON, A had 97 ppb AFL and AD had an even mixture. Poult fed D and AD gained less to 21 d than C or A (456, 454 v 486, 502±12 g). D fed birds had the lowest feed consumption versus C, A, or AD (648 v 691, 701, 677±16 g). Poult fed AD had the highest feed:gain versus C, A and D (1.50 v 1.42, 1.45, 1.44±0.02). The AMEn (kcal/kg) at 21 d was increased for D (2,894) fed poult while for A (2,696) fed poult it was reduced versus C (2,767; AD = 2,758±29). Relative (R) S weight of D (0.11) fed birds was reduced versus A (0.14) but not C or AD (0.12, 0.13±0.006 g/g). The RL weight of birds fed A (2.26) and AD (2.52) were reduced versus C (2.68±0.07g/g). The A fed birds had reduced SA (by OD). Mortality, RH, RB and RG weights, G score and BM colors were not affected. There were no differences in AB. The L of the A fed birds had hepatic parenchyma with diffuse degenerative changes. The hepatocytic nuclei were swollen and had condensed nucleoli. Some hepatic cords had hepatocyte necrosis. There was some sinusoidal congestion with dilatation/congestion of few central veins. These lesions were suggestive of aflatoxin toxicity. The effects of AFL and DON in this study were reflective of what has been reported in the literature. Feeding naturally occurring mycotoxins to poult can be used as a model to study interventions.

**Key words:** Turkey, mycotoxin, aflatoxin, deoxynivalenol, body weight, feed conversion

### INTRODUCTION

"The impact of fungal toxins on animals extends far beyond the obvious effect of causing death. The economic impact of lowered productivity, decreased weight gain, decreased feed efficiency, decreased meat and egg production, increased disease incidence because of immune system suppression, subtle damage to vital organs and interferences with reproduction is many times greater than that of immediate morbidity and lethality" (CAST, 2003).

This same concern has been voiced by members of the swine and poultry industries in North Carolina with first-hand experience of mycotoxin presence in grain and feed. Due to concerns about the most effective strategy

to deal with production losses, there have been interests voiced to determine if there is any performance liability due to low levels of various mycotoxins naturally occurring and to determine the most effective way to alleviate any production losses. Therefore, the objective of this work was to determine the effect of naturally occurring mycotoxins on turkey poult performance and to ascertain a poult model for testing methods to alleviate the negative effects of dietary mycotoxins.

### MATERIALS AND METHODS

A trial was conducted to determine if negative production or immune events could be observed in turkey poult reared to 21 days by feeding diets with low levels of

naturally occurring mycotoxins. All birds were handled in accordance with the institutional animal care and use committee. Two sources of corn with naturally levels of mycotoxins, one with Aflatoxin (AFL) and one with DON were identified and transported to the university feed mill for processing. Samples from these two sources of corn as well as a source of "clean" corn were sent to a private lab for mycotoxin analysis. Four turkey starter diets were made using either AFL, DON, AFL+DON or "clean" corn. The "clean" corn was used as the control. This provided four feed treatments: 1) control, 2) AFL, 3) DON and 4) AFL+DON (AD). The AD treatment was formed using ½ the ration corn from AFL and ½ the ration corn from DON so that the strength of each mycotoxin in this treatment was ½ of the original. An indigestible marker (celite @ 1.5%) was added to all four rations for AMEn (apparent metabolizable energy) determination at 21 days. A basal ration including all ingredients except corn was mixed. Appropriate amounts of basal and corn of each type were mixed to provide the four treatments. The feed was pelleted and crumbled.

Male poults of one strain (Nicholas, Aviagen Turkeys, Lewisburg, WV) were placed into 24 pens in two petersyme batteries with 7 poults per pen. Each bird was wing banded. The four treatments were randomized across the 24 pens (6 replicate pens per treatment). Birds were weighed individually weekly. Feed consumption, by pen, was determined weekly. Mortality (with body weight) was noted daily. At 21 days of age, birds were euthanized with the following organs weighed: heart, spleen, gizzard, liver and bursa of fabricious. Breast muscle was collected for color analysis. Light microscopic analysis of H&E stained liver, bursa and spleen were performed to assess histopathological changes. Serum albumin was determined at 11, 14, 18 and 21 days of age on one bird per pen. Feed and fecal samples were collected for AMEn determination. One bird per pen was injected IV with 7% Sheep Red Blood Cells (SRBC) on day 7 to examine humoral immunity. Secondary injection was given on day 14. Serum was collected at 11, 14, 18 and 21 days of age to monitor for antibody production. The pen was the experimental unit. All data were analyzed using the GLM procedure of SAS (SAS Institute, 1998). Calculated means for each measurable parameter from each treatment group were compared by using LSD to determine significant differences due to feed treatment. Statements of significance are based on P<0.05.

## RESULTS AND DISCUSSION

The ration used for all four diets is shown in Table 1. The DON corn was analyzed to contain 5.3 ppm DON and 0.7 ppm Zearalenone (ZON). The aflatoxin corn was analyzed twice, once at 263 ppb and then again at 130-150 ppb. The treatment feeds contained 50% corn. The

Table 1: Turkey starter ration used to 21 days of age

Ingredient	%
Corn	50.00
SBM (48%)	34.00
Poultry meal	8.00
Fat	2.00
DiCal Phos	3.00
Limestone	0.90
Lysine	0.55
DL Meth	0.40
Salt	0.40
Choline CL	0.20
Threonine	0.10
Trace Min <sup>1</sup>	0.10
Vitamin premix <sup>2</sup>	0.10
Se premix <sup>3</sup>	0.10
Celite	0.15
Total	100.00
<b>Calculated nutrient content</b>	
Dry matter (%)	90.00
Crude protein (%)	26.00
Crude fat (%)	5.00
ME (kcal/lb)	1314.00
Lysine (%)	1.80
Meth (%)	0.80
M+C (%)	1.20
Calcium (%)	1.35
P avail.	0.83
Na (%)	0.22

### Nutrient and mycotoxin analysis

	Control	Aflatoxin diet	DON diet	AD diet
CP (%)	22.3	25.3	24.9	24.9
Aflatoxin B1 (ppb)	ND	97	ND	37.0
Aflatoxin B2 (ppb)	ND	8	ND	3.0
DON (ppm)	ND	ND	1.7	0.9

CP analysis by NCDA. Mycotoxin analysis by Romer Labs, Union, MO. ND = None Detected. Aflatoxins G1 and G2 were not detected in any sample. 15 Acetyl-DON and 3 Acetyl-DON were not detected in any sample.

<sup>1</sup>Minerals mix supplied the following per kilogram of diet: 120 mg Zn as ZnSO<sub>4</sub> H<sub>2</sub>O; 120 mg Mn as MnSO<sub>4</sub> H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub> H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Cu(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>2</sup>Vitamin mix supplied the following per kilogram of diet when added at 0.2%: vitamin A, 6,600 IU; vitamin D<sub>3</sub>, 2000 ICU; vitamin E, 33 IU; vitamin B<sub>12</sub>, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; d-pantothenate, 11 mg menadione, 2 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; d-biotin, 126 µg; ethoxyquin, 50 mg.

<sup>3</sup>Selenium premix supplied 0.21 mg Se, as Na<sub>2</sub>SeO<sub>3</sub>

DON treatment feed (1.7 ppm DON) contained close to the original DON rate. The aflatoxin treatment feed (97 ppb aflatoxin) contained AFL at a rate that was between the two original samples. The analysis of the AD feed treatment, designed to contain half of each corn source, reflected an even mixture. While these results mimic the variability of mycotoxins in grains, even those that test positive for a particular toxin, we were able to provide the birds with a level of mycotoxin challenge reflective of the original corn sources.

Table 2: Body weights and gains of poult fed the four treatment feeds fed to poult to 21 days of age

Diet	BW0	GN1	BW1	GN2	BW2	GN3	BW3	C GN
Control	53	62	114	177 <sup>a</sup>	292 <sup>a</sup>	247	539 <sup>a</sup>	486 <sup>a</sup>
AFL	52	65	117	184 <sup>a</sup>	301 <sup>a</sup>	253	555 <sup>a</sup>	502 <sup>a</sup>
DON	53	56	109	162 <sup>b</sup>	272 <sup>b</sup>	238	509 <sup>b</sup>	456 <sup>b</sup>
AD	52	58	110	161 <sup>b</sup>	271 <sup>b</sup>	236	507 <sup>b</sup>	454 <sup>b</sup>
SEM	0.6	2.4	2.6	4.6	5.6	7.4	11.8	11.7
P	NS	0.07	NS	0.005	0.002	NS	0.02	0.02

<sup>a,b</sup>Means within a column with different superscripts are significantly different ( $p \leq 0.05$ )

Table 3: Feed to gain ratios, feed per bird (gm) and AMEn (kcal/kg) for the four treatment feeds fed to poult to 21 days of age

Diet	FD:GN1	FD:GN2	FD:GN3	FD:GN1-2	FD:GN2-3	FD/BD1	FD/BD2	FD/BD3	CUM F/B	AMEn
Control	1.37 <sup>b</sup>	1.32 <sup>b</sup>	1.42 <sup>b</sup>	1.31 <sup>b</sup>	1.52	82 <sup>b</sup>	232	377	691 <sup>a</sup>	2767 <sup>b</sup>
AFL	1.42 <sup>b</sup>	1.34 <sup>b</sup>	1.45 <sup>b</sup>	1.31 <sup>b</sup>	1.58	88 <sup>b</sup>	233	380	701 <sup>a</sup>	2696 <sup>c</sup>
DON	1.56 <sup>a</sup>	1.41 <sup>a</sup>	1.44 <sup>b</sup>	1.36 <sup>b</sup>	1.47	87 <sup>b</sup>	216	345	648 <sup>b</sup>	2894 <sup>a</sup>
AD	1.69 <sup>a</sup>	1.50 <sup>a</sup>	1.50 <sup>a</sup>	1.43 <sup>a</sup>	1.50	97 <sup>a</sup>	227	353	677 <sup>ab</sup>	2758 <sup>bc</sup>
SEM	0.05	0.02	0.02	0.03	0.04	2.7	6.3	11.5	16.3	29
P	0.003	0.0001	0.04	0.02	NS	0.008	NS	0.1	0.15	0.001

<sup>a,b</sup>Means within a column with different superscripts are significantly different ( $p \leq 0.05$ )

Body weights and BW gains are presented in Table 2. The effects on poult of the grains with naturally occurring mycotoxins used in this study were consistent with what has been reported (CAST, 2003). Poults fed the DON and the AD treatment gained less to 21 days compared to poult fed the control and aflatoxin treatment feeds. Reduced BW gain is the usual response reported for poult consuming aflatoxin or DON. There usually is a dose response. In this case, the aflatoxin level may not have been high enough or may not have been fed long enough to illicit a negative growth response.

Feed conversion, feed consumption and AMEn values are presented in Table 3. The highest (worse) feed conversion was experienced by the poult fed the AD treatment feed. The feed conversions of birds fed the AFL or DON feeds were not different than those fed the control diet. However, feeding the treatment feeds longer may have generated a greater and therefore significant, difference in the responses. Feed consumption was lowest for the birds fed the DON feed. This type of response, reduced feed intake, is reported consistently for animals fed DON, especially for swine.

The AMEn at 21 days was increased for DON fed to poult while that of aflatoxin feed was reduced compared to the control feed. Feeding the AD feed resulted in a response intermediate between the control and aflatoxin treatments. Reduced AMEn due to aflatoxin content is another usual response. While the increase in AMEn for the DON feed may appear to be unusual, it is not without precedent. Goyarts *et al.* (2005) fed DON at 6 mg/kg to swine for 11 weeks. They reported increased AMEn and nitrogen retention for swine fed the DON feed.

Matthaus *et al.* (2004) reported increased crude protein content and increased activities of protease, amylase and several NSP-degrading enzyme activities in wheat grown after it was inoculated with *Fusarium culmorum*

compared to the non-inoculated control wheat. The inoculated wheat of the Matthaus study contained both DON (7.8mg/kg) and Zearalenone (100ug/kg) at harvest. Organ weights and relative organ weights of birds are presented in Table 4. As one might expect, organ weights were reduced in birds with reduced body weight. The spleen of birds fed DON was reduced on a relative weight basis. The liver of birds fed aflatoxin and DON were reduced on a relative body weight basis while the liver of those fed AD were intermediate. Relative heart, bursa and gizzard weights were not affected by treatment feeds. Gizzard score was also not affected by treatment feeds. There were no differences in red color, yellow color or lightness of breast meat among the treatments (data not shown).

Microscopic analysis of the histological changes to the bursa and spleen demonstrated few significant changes across any of the treatment groups; however, the livers of the AFL treated animals demonstrated hepatic parenchyma with diffuse degenerative changes, leaving only few patches of normal architecture between portal triads and centri-lobular areas. The hepatocytic nuclei were swollen and had condensed nucleoli. Occasionally, hepatic cords revealed necrosis of individual or small group of hepatocytes. There was some degree of sinusoidal congestion with dilatation/congestion of few central veins. Overall, the lesions were suggestive of AFL toxicity.

Interestingly, analysis of serum albumin, as an indirect assay of liver function, demonstrated that the AFL treated animals had significantly reduced serum albumin levels relative to control or DON or AD treated animals. These results seem to support the histological analysis above and suggest AFL treatment alters liver function.

To assess the affect of mycotoxin treatment on immune function poult were vaccinated with sheep red blood cells (7% SRBC) at 7 days of age. Secondary injection was given at 14 days of age and serum was collected at

Table 4: Organ weights and relative organ weights of poult fed the four treatment feeds to 21 days of age

Diet	Heart	RHW*	Spleen	RSPW*	Bursa	RBRW*	Liver	RLWT*	Gizzard	RGWT*	G Score**
Control	3.66 <sup>a</sup>	0.68	0.64 <sup>ab</sup>	0.12 <sup>ab</sup>	0.95	0.18	14.47 <sup>a</sup>	2.68 <sup>a</sup>	12.12 <sup>ab</sup>	2.25	0.22
AFL	3.77 <sup>a</sup>	0.68	0.75 <sup>a</sup>	0.14 <sup>a</sup>	0.99	0.18	12.50 <sup>b</sup>	2.26 <sup>b</sup>	12.72 <sup>a</sup>	2.32	0.44
DON	3.31 <sup>ab</sup>	0.65	0.56 <sup>b</sup>	0.11 <sup>b</sup>	0.89	0.17	12.84 <sup>b</sup>	2.52 <sup>ab</sup>	11.37 <sup>b</sup>	2.37	0.67
AD	3.03 <sup>b</sup>	0.62	0.65 <sup>ab</sup>	0.13 <sup>ab</sup>	0.98	0.20	11.74 <sup>b</sup>	2.41 <sup>b</sup>	11.51 <sup>ab</sup>	2.38	0.56
SEM	0.14	0.02	0.04	0.006	0.05	0.01	0.44	0.07	0.36	0.07	0.20
P	0.001	NS	0.004	0.007	NS	NS	0.0005	0.0009	0.04	NS	NS

Weight = grams; \*Relative = gm per 100 gm; \*\*Gizzard lining score: 0 = none, 3 = erosion through lining.

<sup>a,b</sup>Means within a column with different superscripts are significantly different (p<0.05)

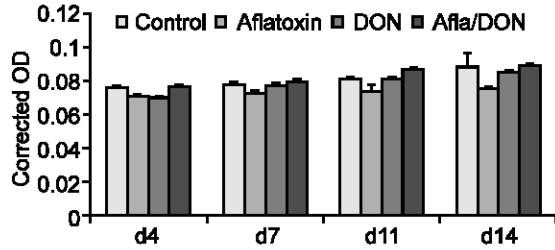


Fig. 1: Serum albumin (as indicated by OD) in birds at 11, 14, 18 and 21 days fed four treatment feeds

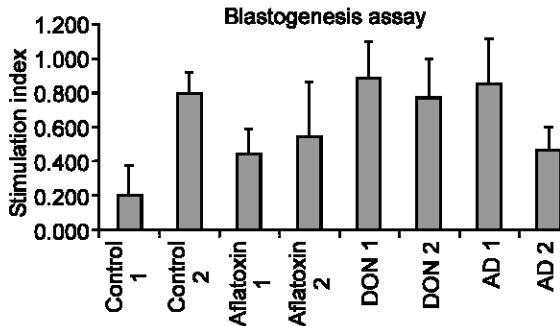


Fig. 2: Mitogenic response of PBMCs isolated at 21 days of age

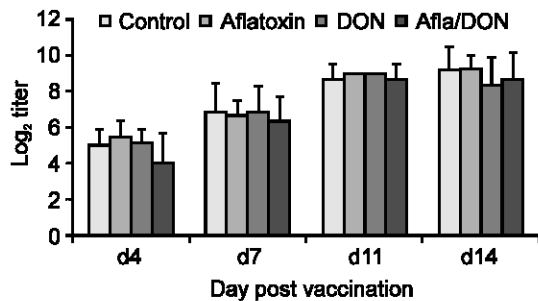


Fig. 3: Immune response of poult to sheep red blood cell administration by treatment

4 and 7 days post both vaccinations (d 11 and d 14 in Fig. 1). The anti-SRBC antibody titer was determined using serially diluted whole serum. No statistical difference was observed in the antibody response across the treatment groups. Additionally, white blood

cells were isolated from the circulation and assayed for differences in their proliferation response to mitogen (200nM PMA/ 200,000cells/well). Based on these data, we conclude there are no differences in response to SRBC across the treatment groups.

The effects of AFL and DON in this study were reflective of what has been reported in the literature based on the levels fed. Basically the model worked as expected. However, the model may be improved by feeding the diets longer to 4 weeks of age in Alternative Design cages or possibly even to 5 or 6 weeks of age in litter floor pens at the NCSU turkey unit. Also, grain with higher levels of mycotoxin might be secured. This may be more important for the AFL corn rather than the DON corn. Alternatively, the corn on hand can be enhanced with cultivated mycotoxins, especially aflatoxin for the AFL corn. However, this would be in contrast with the goal of using naturally occurring levels of mycotoxin. In addition, more applied or practical immune challenges might be employed to ascertain the immune response such as using a current vaccine rather than SRBC. Once the options are discussed and agreed upon, we are ready to begin the next phase.

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