Effects of mycotoxin-contaminated diets and deactivating compound in laying hens: 2. Effects on white shell egg quality and characteristics

J. T. Lee,*[1] K. A. Jessen,* R. Beltran,† V. Starkl,† G. Schatzmayr,† R. Borutova,† and D. J. Caldwell

*Poultry Science Department, Texas AgriLife Research, Texas A&M System, College Station, TX 77843; and †Biomin GmbH, 3130 Herzogenburg, Austria

ABSTRACT An experiment was conducted to determine the effect of dietary inclusion of Mycofix Select (Biomin GmbH, Herzogenburg, Austria) on discrete egg parameters and quality characteristics of hens fed mycotoxin-contaminated diets (aflatoxin; AFLA) and deoxynivalenol (DON)) during a 10-wk trial. A 4 × 2 factorial design was used with 4 contamination levels: control, low (0.5 mg/kg of AFLA + 1.0 mg/kg of DON), medium (1.5 mg/kg of AFLA + 1.5 mg/kg of DON), and high (2.0 mg/kg of AFLA + 2.0 mg/kg of DON) with or without the inclusion of mycotoxin deactivating compound. Three hundred and eighty-four 25-wk-old laying hens were housed 3 per cage. Birds were fed contaminated diets for a 6-wk phase of toxin administration followed by a 4-wk recovery phase, when all birds were fed mycotoxin-free diets. Parameters evaluated included egg weight, Haugh unit value, specific gravity, eggshell thickness, egg shape index, and relative albumen and yolk weights. Albumen height and Haugh unit value were depressed ($P < 0.05$) at the high mycotoxin level 2 wk postinclusion. Egg weight was significantly reduced ($P < 0.05$) with the high toxins level by the third week of toxin administration and remained throughout the study during toxin administration. Egg shape index indicated a variation ($P < 0.05$) in shape with all toxin levels compared with the control. Relative yolk weight was decreased ($P < 0.05$) by the high toxin level. An interaction existed between the deactivating compound inclusion and toxins level with regard to specific gravity. Following the toxin phase, the deactivating compound inclusion increased ($P < 0.05$) egg specific gravity in the control and low toxin groups whereas a decrease ($P < 0.05$) was observed at the high toxin level. These data indicate that mycotoxins present in feed can reduce egg quality, size, yolk weight, and alter egg shape and that inclusion of a mycotoxin deactivating compound can ameliorate some of the negative effects of mycotoxin consumption.

Key words: egg, mycotoxin, laying hen, deoxynivalenol, aflatoxin

2012 Poultry Science 91:2096–2104
http://dx.doi.org/10.3382/ps.2012-02137

INTRODUCTION

Mycotoxins are secondary metabolites produced by toxigenic strains of different species of fungi and are of toxicological concern because they can affect human health and livestock and poultry health or productivity. The toxigenic genera *Aspergillus*, *Penicillium*, and *Fusarium* have been shown to produce more than 400 different mycotoxins (Bünger et al., 2004). Aflatoxins B_1 (**AFB_1**), B_2, G_1, and G_2 are produced by various *Aspergillus* species, including *A. flavus*, *A. parasiticus*, and *A. nomius*, which are the main contaminants of plants and plant products (Smith, 2002). In laying hens, aflatoxin contamination of feed has been reported to negatively affect reproductive status, including egg production and egg weight (Danicke et al., 2002; Rizzi et al., 2003). Histological examination of the ovaries of laying hens fed a diet contaminated with 8.1 mg/kg AFB_1 and 1.6 mg/kg AFG_1 for 3 wk showed follicular atresia in ovaries (Hafez et al., 1982). Reduced egg production, enlarged liver, and increased liver fat are the most prominent manifestations of aflatoxicosis in layers (Leeson et al., 1995). Jacobson and Wiseman (1974) recorded that the carryover of AFB_1 from layer feed to eggs was demonstrated in hens where dietary levels of 100 to 400 ppb AFB_1 resulted in AFB_1 levels of 0.2 to 3.3 ppb in eggs.

Deoxynivalenol (**DON**) is trichothecene mycotoxin produced by fungi of *Fusarium* sp. (*F. graminearum*, *F. culmorum*) and is one of the most widely distributed mycotoxins in food and feed worldwide. Deoxynivalenol is a well-known inhibitor of protein synthesis. The toxin binds to peptidyl transferase (Feinberg and McLaughlin 1989), inhibits the synthesis of RNA and DNA via binding to the ribosome. It is assumed that cells and tis-

©2012 Poultry Science Association Inc.
Received January 2, 2012.
Accepted April 26, 2012.
[1]Corresponding author: jlee@poultry.tamu.edu

sues with high protein turnover rates, such as the small intestine, liver, and the immune system, are most severely affected by DON intoxication (Döll et al., 2003). Hamilton et al. (1981) fed laying pullets wheat-soybean diets containing 0.35 and 0.7 mg/kg of DON for 70 d observing no meaningful change in performance. However, egg and shell weight, shell thickness, and percent shell decreased linearly with increasing levels of dietary DON. Aluminosilicates can effectively adsorb aflatoxins but are not effective against trichothecene mycotoxins, such as DAS or T-2 toxin (Kubena et al., 1993, Phillips, 1999). A more specific dietary treatment has been recently developed using enzymes capable of inactivating the trichothecenes through modification of the basic trichothecene structure. The inhibitory activity of trichothecenes requires the presence of the C-12,13 epoxide, and opening of the 12,13-epoxide ring results in loss of any apparent toxicity (Binder et al., 2000). The aim of the present study was to investigate the adverse effects of dietary aflatoxin (**AFLA**) and DON in laying hens and possible effectiveness of commercially available mycotoxin deactivation product with demonstrated efficacy against both aflatoxins and trichothecenes.

MATERIALS AND METHODS

Birds

To evaluate influence of mycotoxin-contaminated diets and effectiveness of a potential deactivating agent (Mycofix Select, Biomin GmbH, Herzogenburg, Austria) on egg quality and characteristics in peak producing laying performance in 450 Lohmann LSL-LITE. Pullets (14 wk of age) were obtained from a local egg production facility and moved to the Texas A&M University Poultry Research Center. Pullets were allowed to acclimate to the research facility and begin egg production before the start of the trial. Hens were housed in commercial-type laying cages at densities that met acceptable placement densities outlined by the 2007–2008 United Egg Producers Animal Welfare Guidelines. All husbandry practices, including the space requirements for unrestricted feeder and water access followed these published guidelines and were conducted in accordance with an Animal Use Protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. Upon arrival at the research facility, pullets were fed a corn-soybean-based developer ration that met or exceeded nutrient recommendations specified in the Lohmann LSL-LITE management guide. Subsequent feed and lighting changes following the management guide were made allowing for the initiation for egg production. Birds were placed in commercial-type cages located in 2 windowless environmentally controlled laying facilities. The cages used during the trial had dimensions of 30.5 × 50.8 cm, which allowed for 1,548 cm^2 of rearing space. Three pullets were place per cage to achieve 516 cm^2 of rearing space per bird.

Experimental Design

The experimental design consisted of a 4 × 2 factorial design evaluating multiple toxin levels combined with and without mycotoxin deactivating agent inclusion, yielding a total of 8 treatment groups. Four toxin administration levels were evaluated and included a control (diet free of mycotoxin), low (0.5 mg/kg of AFLA + 1.0 mg/kg of DON), medium (1.5 mg/kg of AFLA + 1.5 mg/kg of DON), and high (2.0 mg/kg of AFLA + 2.0 mg/kg of DON) with and without the inclusion of deactivation compound (2.27 kg/ton). Each treatment consisted of 16 replicate cages with each replicate containing 3 birds. Treatments were assigned to individual replicates based on wk 24 BW and egg production levels to ensure that each treatment began the experiment on wk 25 with statistically equivalent BW and egg production levels.

Mycotoxin Contamination and Administration

Naturally contaminated corn with an assayed contamination level of 4.0 mg/kg of DON and purified aflatoxin in rice culture was used to reach experimental contamination levels. To increase contamination levels of the diets, contaminated corn and rice culture were used to replace noncontaminated corn. Basal diets of each contamination level were divided into 2 subsamples and the deactivating agent was added to one portion. Three samples of each treatment (500 g) were shipped to Romer Labs for AFLA and DON determination via HPLC analysis (Table 1). In short, purification was accomplished by using immunoaffinity clean up columns (Romer Labs, Austria). The HPLC analyses were performed with 1100 and 1200 series HPLC systems equipped with a Fluorescence detector (G1321A).

The experiment initiated on wk 25 of age with feeding of each dietary treatment to producing laying hens for 6 consecutive weeks. Following the 6 wk administration period, all laying hens were fed a diet devoid of all mycotoxin for a subsequent 4 wk (recovery period), at which time the experiment was concluded. Parameters evaluated throughout the experiment included egg weight, specific gravity, albumen height, yolk color, eggshell thickness, length, width, relative albumen, and yolk weight. All evaluated parameters were conducted on a biweekly basis using all eggs produced over a 24-h period with the exception of egg weight which was determined on a weekly basis. Specific gravity measurements were determined using the saline flotation method (Holder and Bradford, 1979). Egg length and width were determined using a veriner caliper and egg shape index was calculated as length/width (Lin et al., 2004). Albumen height and Haugh unit measurements were determined using an automated Egg Analyzer (Orka Food Technology, Ramat HaSharon, Israel). Large and small end shell thickness were measured with a barrel anvil micrometer to obtain a shell thickness average.

Table 1. Ingredient profile and nutrient concentration of the diet fed to 25- to 31-wk-old White Leghorn hens[1]

Ingredient	%
Corn	49.26
Soybean meal (48%)	31.37
Limestone	10.39
A/V fat blend	6.27
Mono-calcium PO_4	1.78
Sodium chloride	0.41
DL-Methionine (99%)	0.17
Vitamins[2]	0.25
Minerals[3]	0.05
Pigment[4]	0.05
Calculated nutrient concentration	
Protein (%)	19.5
ME (kcal/kg)	2,950
Methionine (%)	0.47
Total sulfur amino acids (%)	0.79
Lysine (%)	1.05
Threonine (%)	0.73
Tryptophan (%)	0.24
Calcium	4.33
Sodium	0.18
Available phosphorus	0.48

[1]Naturally contaminated deoxynivalenol (DON) corn and rice culture containing aflatoxin (AFLA) spared corn to produce the 3 evaluated mycotoxin levels. Assayed values of AFLA B1 as determined by HPLC for the low, medium, and high levels, respectively. Week 1–3: 0.66 mg/kg, 1.85 mg/kg, and 2.65 mg/kg; wk 4–6: 0.58 mg/kg, 1.65 mg/kg, and 2.40 mg/kg.

[2]Vitamin premix added at this rate yields 11,023 IU vitamin A; 3,858 IU vitamin D_3; 46 IU vitamin E; 0.0165 mg B_{12}; 5.845 mg riboflavin; 45.93 mg niacin; 20.21 mg D-pantothenic acid; 477.67 mg choline; 1.47 mg menadione; 1.75 mg folic acid; 7.17 mg pyroxidine; 2.94 mg thiamine; 0.55 mg biotin per kg of diet. The carrier is ground rice hulls.

[3]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[4]Yellow Pixafil 20—Xanthophyll extract from Aztec marigold (*Tagetes erecta*).

Statistical Analysis

Data were analyzed via a 4 (toxin) × 2 (deactivation compound) factorial ANOVA using the GLM procedure with main effect means deemed significantly determined at $P \leq 0.05$ (SPSS v. 18.0, Chicago, IL). Main effect means were separated using Duncan's multiple range test. In cases were a significant interaction was present between toxin level and deactivation compound inclusion, data were subjected to a one-way ANOVA with means deemed different at $P \leq 0.05$. In these cases, individual treatment means were separated using Duncan's multiple range test. Prior to analysis, relative yolk and albumen weight was subjected to an arcsin transformation.

RESULTS

Beginning with the third week of toxins administration, the high toxin level caused a decrease ($P < 0.05$) in egg weight compared with the control group and the low-toxin administered group (Table 2). Following the fourth week of toxin administration, an interaction was observed between toxin administration and deactivating compound inclusion. Egg weights from hens fed the high toxin diet alone had the lowest ($P < 0.05$) egg weights compared with all other groups, whereas inclusion of deactivating compound into the high toxin diet increased ($P < 0.05$) egg weight to a level comparable to the nonsupplemented control. Following the fifth and sixth weeks of toxins administration, egg weights in both the medium and high toxin levels were reduced ($P < 0.05$) compared with the control and low-toxin diets with high-toxin administration producing the lowest observed egg weight. No difference in egg weight was observed between the controls and low toxin level and the conclusion of the toxin phase. High-toxin administration continued to depress egg weight through 2 wk following toxins administration. Inclusion of the deactivating compound in the high-toxin diet increased egg weight during the first week following toxin removal to a level comparable to the nonsupplemented control diet. No difference in egg weights between any of the toxin levels and the control were observed at the conclusion of the 4-wk recovery period.

Differences in specific gravity were not observed until the final week of toxin administration when an interaction was observed between toxin level and Mycofix inclusion (Table 3). Increases ($P < 0.05$) in specific gravity were observed with deactivating compound inclusion in both the control and low-level toxin administration treatments; however, a reduction ($P < 0.05$) was observed in specific gravity with deactivating compound inclusion in the high-level diet. Increases ($P < 0.05$) in specific gravity in nonsupplemented diets were observed as the toxin level was increased with the high-toxin level, yielding the highest specific gravity value. The high-toxins diet continued to yield increased specific gravity values compared with the rest of the toxin treatments through the second week of the recovery period. This may be as a result of the reduced egg weights from this treatment. At the conclusion of the recovery period, all treatments expressed similar specific gravity values.

Egg shape index suggests a variation in egg shape ($P < 0.05$) following the fourth and sixth weeks of toxin administration at all toxin levels as compared with the control diet (Table 4). Following 4 wk of toxin administration, decreases ($P < 0.05$) in egg length were observed in hens fed the medium and high toxin levels as compared with the control and low toxin levels. This observation continued through the remainder of toxin administration. Similar observations were observed 2 wk following toxin removal with eggs produced by high-toxin administered hens expressing decreased length compared with eggs produced by non-toxin-administered hens. Similar results were observed in egg width. Eggs produced by high-toxin fed hens were observed to have decreased ($P < 0.05$) egg width as compared with eggs produced by non-toxin-administered hens following 4 wk of toxin administration and continued through the remainder of toxin administration. No differences

Table 2. Egg weight of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Egg weight (g)									
Diet	DC[2]	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10
Treatment mean											
Control	No	58.4	59.4	59.3	59.5^{abc}	60.5	60.0	62.2^{b}	62.5	62.4	63.2
Control	Yes	57.2	58.7	58.6	59.4^{bc}	60.6	61.5	61.1^{bc}	61.3	62.2	61.8
Low[3]	No	59.9	59.8	59.9	61.1^{a}	61.7	62.1	64.3^{a}	63.7	62.8	64.5
Low	Yes	58.3	58.5	59.1	60.3^{ab}	60.6	60.6	61.2^{bc}	62.0	61.7	64.3
Medium[4]	No	59.4	59.0	58.9	59.2^{bc}	59.5	58.7	61.3^{b}	62.3	62.3	64.0
Medium	Yes	59.4	58.6	57.9	57.8^{cd}	58.5	59.2	61.1^{bc}	61.9	61.9	63.7
High[5]	No	57.1	57.5	56.9	56.5^{d}	57.1	57.2	59.1^{c}	60.7	61.1	63.0
High	Yes	58.2	58.9	58.3	58.4^{c}	57.6	58.1	60.2^{bc}	60.6	62.0	63.2
Main effect											
Control		57.8^{bc}	59.1	59.0^{ab}	59.5	60.5^{a}	61.3^{a}	61.7	61.9^{ab}	62.3	62.5
Low		59.1^{ab}	59.2	59.5^{a}	60.7	61.2^{a}	61.4^{a}	62.6	62.8^{a}	62.3	64.3
Medium		59.4^{a}	58.8	58.4^{bc}	58.5	59.5^{b}	58.9^{b}	61.2	62.3^{a}	62.1	63.9
High		57.7^{c}	58.3	57.6^{c}	57.4	57.4^{c}	57.6^{c}	59.7	60.6^{b}	61.5	63.1
	No	58.7	59.0	58.8	59.1	59.8	59.2	61.7	62.3	62.2	63.8
	Yes	58.3	58.7	58.4	59.0	59.4	59.9	60.9	61.5	61.9	63.4
P-value											
Toxin		0.021	0.198	0.002	>0.001	>0.001	>0.001	>0.001	0.007	0.694	0.073
DC		0.397	0.298	0.447	0.796	0.379	0.921	0.072	0.070	0.653	0.505
Toxin × DC		0.190	0.087	0.069	0.020	0.355	0.150	0.013	0.547	0.547	0.797
PSEM		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3

[a–d]Main effect and treatment means with different superscripts differ at $P \leq 0.05$. Groupings of individual treatment means indicate the presence of a significant interaction between toxin administration and DC inclusion.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

Table 3. Egg-specific gravity of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Specific gravity				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	1.090	1.089	1.084^{d}	1.087	1.088
Control	Yes	1.090	1.090	1.086^{bc}	1.087	1.087
Low[3]	No	1.089	1.088	1.086^{cd}	1.088	1.087
Low	Yes	1.091	1.090	1.088^{ab}	1.089	1.087
Medium[4]	No	1.090	1.090	1.087^{bc}	1.089	1.086
Medium	Yes	1.090	1.090	1.088^{ab}	1.088	1.087
High[5]	No	1.090	1.091	1.089^{a}	1.091	1.088
High	Yes	1.091	1.090	1.087^{bc}	1.090	1.089
Main effect						
Control		1.090	1.089	1.085	1.087^{b}	1.087
Low		1.090	1.089	1.087	1.088^{b}	1.087
Medium		1.090	1.090	1.087	1.088^{b}	1.087
High		1.091	1.090	1.088	1.090^{a}	1.088
	No	1.090	1.089	1.087	1.089	1.087
	Yes	1.090	1.090	1.087	1.088	1.087
P-value						
Toxin		0.363	0.190	>0.001	>0.001	0.336
DC		0.234	0.345	0.102	0.931	0.542
Toxin × DC		0.400	0.123	0.002	0.325	0.802
PSEM		0.0002	0.0003	0.0003	0.0003	0.0003

[a–d]Main effect and treatment means with different superscripts differ at $P \leq 0.05$. Groupings of individual treatment means indicate the presence of a significant interaction between toxin administration and DC inclusion.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

Table 4. Egg shape index (length:width ration) of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Shape index (length:width ratio)				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	1.33	1.33	1.34	1.35	1.34
Control	Yes	1.30	1.33	1.33	1.34	1.35
Low[3]	No	1.31	1.32	1.32	1.32	1.34
Low	Yes	1.31	1.31	1.32	1.32	1.33
Medium[4]	No	1.31	1.31	1.32	1.33	1.35
Medium	Yes	1.31	1.30	1.31	1.33	1.33
High[5]	No	1.31	1.31	1.31	1.33	1.33
High	Yes	1.31	1.30	1.32	1.32	1.34
Main effect						
Control		1.32	1.33[a]	1.33[a]	1.35[a]	1.34
Low		1.31	1.31[b]	1.32[b]	1.32[b]	1.33
Medium		1.31	1.31[b]	1.31[b]	1.33[ab]	1.34
High		1.31	1.31[b]	1.31[b]	1.32[b]	1.33
	No	1.32	1.32	1.32	1.33	1.34
	Yes	1.31	1.31	1.32	1.33	1.34
P-value						
Toxin		0.669	0.002	0.001	0.016	0.245
DC		0.101	0.76	0.611	0.351	0.521
Toxin × DC		0.284	0.600	0.222	0.890	0.148
PSEM		0.002	0.002	0.002	0.003	0.003

[a,b]Main effect and treatment means with different superscripts differ at $P \leq 0.05$.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

were observed in egg shape index due to the inclusion of the deactivating compound. Following the recovery phase, no differences were observed in egg shape index due to toxin administration, indicating that egg shape will return to normal when mycotoxins are removed from the diet.

An immediate effect of toxin was observed with all toxin levels yielding decreased ($P < 0.05$) shell thickness compared with the control diet following 2 wk of administration (Table 5) although no differences were observed during the fourth week of toxin administration. An interaction between toxin and deactivating compound inclusion was observed on shell thickness following the toxin period. In non-deactivating compound diets, shell thickness in this region was increased ($P < 0.05$) in the high-level treatment compared with all other treatments. Deactivating compound inclusion improved shell thickness in the control, low, and medium toxin levels, however not to a level that reached significance ($P > 0.05$). No differences were observed during any point of the recovery phase with regard to shell thickness.

Following the second week of toxins administration, albumen height was reduced ($P < 0.05$) due to high toxin level inclusion (Table 6). This effect was also observed following the second week of the recovery phase. No differences in albumen height were observed at the conclusion of the recovery period. The reduction in albumen height resulted in an observed reduction in Haugh unit value ($P < 0.05$) during the second week of toxins administration and the second week of the recovery phase for eggs from hens fed the high toxins diet as compared with the control group (Table 7).

An interaction with regard to relative yolk weight was observed on wk 4 of the toxin administration period. As the level of toxins increased, relative yolk weight decreased ($P < 0.05$) resulting in a significant reduction at the high level of inclusion compared with the controls (Table 8). Inclusion of the deactivating compound increased relative yolk weight in the low-toxin level diet. Following the toxin phase, the high level of inclusion decreased ($P < 0.05$) relative yolk weight compared with all other treatment groups. No differences were observed due to toxin level during any point of the recovery phase with regard to relative yolk weight. During the fourth week of toxins administration, relative albumen weight increased ($P < 0.05$) in the medium and high toxin level treatments (Table 9) as compared with the control diet. After the fourth week of recovery, the inclusion of the deactivating compound decreased ($P < 0.05$) relative albumen weight and increased relative yolk weight. This observation had not been seen through the previous 9 wk of the experiment.

DISCUSSION

In the current study, multiple egg quality parameters were negatively affected with the presence of dietary

Table 5. Eggshell thickness (mm) of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Small end (mm)				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	0.385	0.372	0.373[bc]	0.321	0.367
Control	Yes	0.377	0.371	0.376[b]	0.328	0.369
Low[3]	No	0.376	0.375	0.370[bc]	0.331	0.366
Low	Yes	0.370	0.371	0.374[bc]	0.329	0.366
Medium[4]	No	0.377	0.380	0.366[c]	0.327	0.369
Medium	Yes	0.372	0.377	0.371[bc]	0.330	0.373
High[5]	No	0.375	0.384	0.384[a]	0.334	0.373
High	Yes	0.372	0.379	0.376[b]	0.337	0.370
Main effect						
Control		0.381[a]	0.376	0.374	0.325	0.368
Low		0.373[b]	0.376	0.372	0.330	0.366
Medium		0.374[b]	0.380	0.368	0.328	0.371
High		0.374[b]	0.385	0.379	0.336	0.371
	No	0.378[a]	0.380	0.373	0.328	0.368
	Yes	0.373[b]	0.378	0.374	0.331	0.369
P-value						
Toxin		0.026	0.109	0.001	0.254	0.329
DC		0.014	0.254	0.695	0.529	0.681
Toxin × DC		0.829	0.976	0.040	0.873	0.687
PSEM		0.001	0.002	0.001	0.002	0.001

[a–c]Main effect and treatment means with different superscripts differ at $P \leq 0.05$. Groupings of individual treatment means indicate the presence of a significant interaction between toxin administration and DC inclusion.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

Table 6. Albumen height (mm) of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Albumen height (mm)				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	6.39	6.36	6.23	6.80	6.57
Control	Yes	6.36	6.16	6.21	6.43	6.24
Low[3]	No	6.29	6.42	6.16	6.90	6.37
Low	Yes	6.19	6.35	5.96	6.63	6.25
Medium[4]	No	6.14	6.38	6.06	6.74	6.27
Medium	Yes	6.19	6.51	6.31	6.65	6.44
High[5]	No	6.02	6.20	6.00	6.33	6.08
High	Yes	6.05	6.29	6.14	6.34	6.32
Main effect						
Control		6.37[a]	6.26	6.22	6.61[a]	6.40
Low		6.24[a]	6.38	6.07	6.75[a]	6.31
Medium		6.19[ab]	6.44	6.18	6.69[a]	6.35
High		6.04[b]	6.24	6.18	6.33[b]	6.20
	No	6.21	6.34	6.11	6.69[a]	6.33
	Yes	6.21	6.33	6.14	6.51[b]	6.31
P-value						
Toxin		0.045	0.107	0.304	0.007	0.330
DC		0.942	0.722	0.565	0.043	0.977
Toxin × DC		0.821	0.211	0.161	0.475	0.080
PSEM		0.04	0.04	0.04	0.05	0.05

[a,b]Main effect and treatment means with different superscripts differ at $P \leq 0.05$.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

Table 7. Haugh unit value of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Haugh unit value				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	79.63	79.39	77.80	81.35	79.53
Control	Yes	79.66	77.99	77.61	79.23	77.48
Low[3]	No	78.75	79.16	77.05	81.50	77.64
Low	Yes	78.49	78.94	76.47	80.28	77.02
Medium[4]	No	77.94	79.59	77.50	80.84	77.08
Medium	Yes	78.98	80.94	79.05	80.52	78.37
High[5]	No	77.65	79.30	78.43	78.51	76.04
High	Yes	77.31	79.29	78.26	78.71	77.55
Main effect						
Control		79.64[a]	78.71	77.71	80.30[a]	78.51
Low		78.62[ab]	79.05	76.78	80.84[a]	77.33
Medium		78.45[ab]	80.25	78.23	80.69[a]	77.70
High		77.47[b]	79.29	78.35	78.60[b]	76.78
	No	78.50	79.36	77.70	80.53	77.59
	Yes	78.60	79.29	77.85	79.70	77.60
P-value						
Toxin		0.041	0.070	0.101	0.030	0.187
DC		0.700	0.580	0.620	0.113	0.898
Toxin × DC		0.779	0.120	0.333	0.579	
PSEM		0.39	0.28	0.26	0.31	0.40

[a,b]Main effect and treatment means with different superscripts differ at $P \leq 0.05$.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

Table 8. Relative yolk weight of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Relative yolk weight (%)				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	0.262	0.268[ab]	0.278	0.280	0.285
Control	Yes	0.263	0.268[ab]	0.279	0.279	0.285
Low[3]	No	0.263	0.263[bc]	0.277	0.268	0.278
Low	Yes	0.271	0.272[a]	0.284	0.279	0.287
Medium[4]	No	0.268	0.264[bc]	0.275	0.277	0.282
Medium	Yes	0.262	0.262[bcd]	0.276	0.285	0.292
High[5]	No	0.261	0.259[cd]	0.269	0.278	0.284
High	Yes	0.262	0.253[d]	0.270	0.281	0.283
Main effect						
Control		0.263	0.268	0.279[a]	0.279	0.285
Low		0.267	0.268	0.281[a]	0.274	0.283
Medium		0.265	0.263	0.275[a]	0.281	0.287
High		0.261	0.256	0.269[b]	0.279	0.283
	No	0.264	0.264	0.275	0.276	0.282[b]
	Yes	0.264	0.264	0.277	0.281	0.287[a]
P-value						
Toxin		0.165	>0.001	0.001	0.264	0.525
DC		0.711	0.919	0.250	0.062	0.047
Toxin × DC		0.105	0.020	0.768	0.405	0.181
PSEM		0.001	0.001	0.001	0.001	0.001

[a–d]Main effect and treatment means with different superscripts differ at $P \leq 0.05$. Groupings of individual treatment means indicate the presence of a significant interaction between toxin administration and DC inclusion.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 2.0 mg/kg of deoxynivalenol.

Table 9. Relative albumen weight of Lohmann LSL White Leghorn hens fed diets contaminated with aflatoxin and deoxynivalenol with and without the addition of deactivation compound (DC)[1]

		Percent albumen				
Diet	DC[2]	2nd wk Toxin	4th wk Toxin	6th wk Toxin	2nd wk Recovery	4th wk Recovery
Treatment mean						
Control	No	0.590	0.589	0.585	0.596	0.581
Control	Yes	0.593	0.586	0.582	0.587	0.566
Low[3]	No	0.595	0.590	0.586	0.606	0.580
Low	Yes	0.581	0.585	0.578	0.593	0.570
Medium[4]	No	0.589	0.595	0.583	0.595	0.571
Medium	Yes	0.588	0.601	0.583	0.586	0.561
High[5]	No	0.604	0.595	0.582	0.580	0.568
High	Yes	0.592	0.601	0.583	0.584	0.572
Main effect						
Control		0.591	0.588[b]	0.583	0.592	0.574
Low		0.588	0.587[b]	0.582	0.599	0.575
Medium		0.589	0.598[a]	0.583	0.591	0.565
High		0.598	0.598[a]	0.583	0.582	0.570
	No	0.595	0.592	0.584	0.594	0.575[a]
	Yes	0.589	0.593	0.582	0.587	0.567[b]
P-value						
Toxin		0.271	0.007	0.992	0.070	0.196
DC		0.132	0.730	0.388	0.141	0.021
Toxin × DC		0.356	0.418	0.641	0.631	0.235
PSEM		0.002	0.002	0.001	0.002	0.002

[a,b]Main effect and treatment means with different superscripts differ at $P \leq 0.05$.

[1]Contaminated diets were fed for 6 consecutive weeks followed by a 4-wk recovery period in which the diet was free of mycotoxins.

[2]Mycofix Select (Biomin GmbH, Herzogenburg, Austria) inclusion of 2.27 kg/ton.

[3]Target level of 0.5 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

[4]Target level of 1.5 mg/kg of aflatoxin B1 and 1.5 mg/kg of deoxynivalenol.

[5]Target level of 2.0 mg/kg of aflatoxin B1 and 1.0 mg/kg of deoxynivalenol.

mycotoxin contamination. The combination of AFLA and DON resulted in reduced egg weight, reduced Haugh units, reduced yolk weight, and reduced albumen height. However, following the 4-wk recovery period in which all layers were fed a mycotoxin-free diet, all egg quality measurements returned to levels equal to that of eggs produced by control laying hens. Reduction of egg weight due to the presence of dietary myctoxin has been previously reported. Rizzi et al. (2003) reported the observation of reduced egg weights from laying hens fed diets contaminated with 2.5 mg/kg of AFLA B_1 which is a similar level to the high inclusion rate in the current study which also resulted in a reduction of egg weight within 3 wk after mycotoxin administration. Danicke et al. (2002) observed reduced egg weight with *Fusarium* toxin-contaminated corn containing DON and zearalenone. In the current study, reduced egg weight was also observed at the medium inclusion level (1.5 mg/kg of AFLA and DON) at the conclusion of the mycotoxin administration period. Following the removal of toxins from the diet, egg weights from hens fed the medium toxin level immediately increased within 7 d to a level equivalent to eggs produced by toxin-free laying hens. Egg weights of laying hens fed the high toxin contamination level required greater than 2 wk of feeding a mycotoxin-free diet before egg weights increased to a level similar to control laying hen egg weights. This indicates that removal of mycotoxin-contaminated diets will result in a quick recovery and increased performance, however, time is dependent upon mycotoxin contamination level.

In addition to reduced egg weight, additional reductions in yolk weight, shell weight, and interior quality have been reported. Washburn et al. (1984) observed reduced egg and yolk weights produced by laying hens fed diets contaminated with 5 μg of AFLA B_1 per gram, which is twice that used in the current study. Zaghini et al. (2005) reported reduced eggshell weight associated with the feeding of 2.5 mg/kg of AFLA. Reduced yolk weight and Haugh unit value were reported by Rizzi et al. (2003) when feeding a diet contaminated with 2.5 mg/kg of AFLA B1. Reduced yolk weight and Haugh unit value were also observed in the current study, confirming that feeding mycotoxin-contaminated diets will negatively affect interior egg quality.

In the current study, high levels of mycotoxin contamination in feed fed to laying hens resulted in eggs with increased specific gravity measurements and eggshell thickness as compared with eggs produced by hens fed noncontaminated diets. Washburn et al. (1984) reported an increase in shell strength in hens fed AFLA B_1-contaminated diets which was attributed to the fact that shell weight was not decreased in proportion to the decrease in total egg weight, resulting in a higher shell percentage for smaller eggs. This observation can explain the observed differences in specific gravity and shell thickness in the current study between high toxin level and the control diet as the differences were

observed during time periods of observed reduced egg weight in the high-toxins diet fed to layers. Additional effects of mycotoxin contamination in the current study included egg length, egg width, and egg shape index as mycotoxin-contaminated feed being fed at all investigated levels altered the egg shape index as compared with eggs produced by noncontaminated feed fed to laying hens.

Inclusion of the mycotoxin deactivating compound in the current study resulted in multiple positive observations with regard to egg quality measurements. The inclusion of the deactivating compound delayed the onset of reduced egg weight in the high toxins treatment group. Additionally, the inclusion of deactivating compound increased specific gravity measurements in control, low, and medium toxins fed to laying hens. The lack of improved shell thickness and specific gravity in the high-toxins administration group is likely due to the reduction in egg size observed in this particular treatment. Danicke et al. (2002) reported the ability of this deactivating compound to increase egg weight in laying hens fed *Fusarium* toxin-contaminated corn containing DON and zearalenone. This group (Danicke et al., 2002) also reported improvements in nutrient digestibility and metabolizability of gross energy with the inclusion of this deactivating compound. Taken together, this study illustrates the significant negative effect of mycotoxin contamination on egg quality characteristics and demonstrates the ability of a deactivating compound to reduce or eliminate such observed effects.

ACKNOWLEDGMENTS

The authors thank Todd Applegate of Purdue University (West Lafayette, IN) for his technical assistance during the project.

REFERENCES

Binder, E. M., D. Heidler, G. Schatzmayr, N. Thimm, E. Fuchs, R. Krska, and J. Binder. 2000. Microbial detoxification of mycotoxins in animal feed. Pages 21–25 in Proceedings of the 10th Int. IUPAC Symp. Mycotoxins and Phycotoxins. Sao Paulo, Brazil.

Bünger, J., G. Westphal, A. Mönnich, B. Hinnendahl, E. Hallier, and M. Müller. 2004. Cytotoxicity of occupationally and environmentally relevant mycotoxins. Toxicology 202:199–211.

Danicke, S. K., H. Ueberschar, I. Halle, S. Matthes, H. Valenta, and G. Flachowsky. 2002. Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or *Fusarium* toxin-contaminated maize on performance of hens and on carryover of zearalenone. Poult. Sci. 81:1671–1680.

Döll, S., S. Dänicke, K. H. Ueberschär, H. Valenta, U. Schnurrbusch, M. Ganter, F. Klobasa, and G. Flachowsky. 2003. Effects of graded levels of *Fusarium* toxin-contaminated maize in diets for female weaned piglets. Arch. Tierernahr. 57:311–334.

Feinberg, B., and C. S. McLaughlin. 1989. Biochemical mechanism of the action of trichothecene mycotoxins. Pages 27–35 in Trichothecene Mycotoxins Pathophysiologic Effects. V. R. Beasley, ed. CRC Press, Boca Raton, FL.

Hafez, A. H., S. E. Megalla, H. M. Abdel-Fattah, and Y. Y. Kamel. 1982. Aflatoxin and aflatoxicosis. II. Effects of aflatoxin on ovaries and testicles in mature domestic fowls. Mycopathologia 77:137–139.

Hamilton, R. M. G., B. K. Thomson, and H. L. Trenholm. 1981. Feed intake, egg production, and shell quality of hens given diets that contained vomitoxin contaminated wheat. Poult. Sci. 60:1666. (Abstr.)

Holder, D. D., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs observed and percent shell. Poult. Sci. 58:250–251.

Jacobson, W. C., and H. G. Wiseman. 1974. The transmission of aflatoxin B_1 into eggs. Poult. Sci. 53:1743–1745.

Kubena, L. F., R. B. Harvey, W. E. Huff, M. H. Elissalde, A. G. Yersin, T. D. Phillips, and G. E. Rottinghaus. 1993. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxiscirpenol. Poult. Sci. 72:51–59.

Leeson, S., G. J. Diaz, and J. D. Summers. 1995. Pages 249–298 in Poultry metabolic disorders and mycotoxins. University Books, Guelph, Ontario, Canada.

Lin, H., K. Mertens, B. Kemps, T. Govaerts, B. De Ketelaere, J. De-Baerdemaeder, E. Decuypere, and J. Buyse. 2004. New approach of testing the effect of heat stress on eggshell quality: Mechanical and material properties of eggshell and membrane. Br. Poult. Sci. 45:476–482.

Phillips, T. D. 1999. Dietary clay in the chemoprevention of aflatoxin-induced disease. Toxicol. Sci. 52:118–126.

Rizzi, L., M. Simioli, P. Roncada, and A. Zaghini. 2003. Aflatoxin B1 and Clinoptilolite in feed for laying hens: Effects on egg quality, mycotoxin residues in livers, and hepatic mixed-function oxygenase activities. J. Food Prot. 66:860–865.

Smith, B. P. 2002. Pages 1627–1637 in Large Animal Internal Medicine. 3rd ed. Mosby Inc., St. Louis, MO.

Washburn, K. W., R. D. Wyatt, P. L. Potts, and G. M. Lanza. 1984. Effects and mechansims of aflatoxin on variation in shell strength. Poult. Sci. 64:1302–1305.

Zaghini, A., G. Martelli, P. Roncada, M. Simioli, and L. Rizzi. 2005. Mannanoligosaccharides and Aflatoxin B1 in feed for laying hens: Effects on egg quality, aflatoxins B1 and M1 residues in eggs, and aflatoxin B1 levels in liver. Poult. Sci. 84:825–832.