Efficacy of a New Ochratoxin-Binding Agent (OcraTox) to Counteract the Deleterious Effects of Ochratoxin A in Laying Hens

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ABSTRACT An experiment was conducted to evaluate the efficacy of a new ochratoxin-binding agent (OcraTox, 5 g/kg of feed) in offsetting the toxic effects of ochratoxin A (OTA, 2 mg/kg of feed) in laying hen diets. Performance, serum biochemistry, OTA residue in the liver and eggs, and egg quality parameters were evaluated. Twenty-eight Hisex Brown laying hens, 47 wk of age, were allocated to 1 of 4 experimental treatments for 3 wk: control, OTA (containing 2 mg of OTA/kg of feed), OcraTox (containing 5 g of OcraTox/kg of feed), and OTA + OcraTox (containing 2 mg of OTA and 5 g of OcraTox/kg of feed). Laying hens fed OcraTox showed results similar to the control hens ($P > 0.05$). The OTA diet significantly ($P < 0.05$) reduced daily feed consumption, egg mass production, and serum triglyceride concentrations, and increased the relative liver weight, the serum activity of alkaline phosphatase, and the serum concentration of uric acid as compared with the control diet. Addition of OcraTox to the contaminated diet alleviated ($P < 0.05$) the negative effects resulting from OTA, reaching values not significantly different from the control diet for most of the parameters except the relative weight of the liver. Birds fed the OTA treatment showed a greater content of OTA in the liver (15.1 µg/kg) than those fed the control diet (<0.05 µg/kg). Supplementing the contaminated diet with OcraTox (OTA + OcraTox) reduced the values to 12.0 µg/kg. Residues of OTA were not detected above our detection limit (0.05 µg/kg) in any of the analyzed eggs. In conclusion, our results indicated that addition of OcraTox can counteract the deleterious effects caused by OTA in laying hens.

Key words: ochratoxin A, laying hen, performance, egg quality, binding agent

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INTRODUCTION

Ochratoxin A (**OTA**) is a secondary metabolite produced by some strains of *Aspergillus ochraceus* and *Penicillium verrucosum* that can be found in various feed ingredients. Ochratoxin A is considered a potent nephrotoxic, hepatotoxic, and immunosuppressive (Stormer and Lea, 1995) compound in all mammalian species, and the International Agency for Research on Cancer (1993) has classified it as a possible carcinogen (group 2B) to humans. The mechanism of action of OTA is unclear. However, some authors have suggested that OTA may interfere with the synthesis of enzymes and other proteins by competitively inhibiting phenylalanine-tRNA (Ueno, 1991; Council for Agricultural Science and Technology, 2003). In commercial birds, consumption of OTA-contaminated diets has been related to clinical signs of toxicosis and reduced feed intake, egg production, and egg quality (Prior and Sisodia, 1978; Haazele et al., 1993; Verma et al., 2003). Consumption of OTA by birds may also be associated with the presence of the toxin and its derivatives in eggs (Piskorska-Pliszczyńka and Juszkiewicz, 1990).

Batches of cereal grains coming into the feed mill are routinely analyzed for mycotoxin content. However, some mycotoxins that are located in isolated areas of the batch are often not detected, and feed contamination may be identified when mycotoxicosis is detected in animals. A variety of dietary treatments have been used for eliminating or reducing the toxic effects of OTA in animals, including the use of specific adsorbents to block mycotoxin in the digestive content and the use of antioxidant compounds (Bauer, 1994; Hoehler and Marquardt, 1996). Hydrated sodium calcium aluminosilicate (Huff et al., 1992; Santin et al., 2002), activated charcoal, bentonite and cholestyramine (Bauer, 1994), and esterified glucomannan (Raju and Devegowda, 2000) have been used in animal feeds to diminish the adverse effects of OTA. However, many of these agents have failed to prevent ochratoxicosis in animals. Animal production takes place within a framework that

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establishes the fulfillment of legal requirements in food safety subjects. These requirements imply Hazard Analysis and Critical Control Points (HACCP) implementation and avoiding the presence of aflatoxins, deoxinivalenol, zearalenone, ochratoxins, T-2 and HT-2, and fumonisins in products for animal nutrition. In this way, mycotoxin-binding agents are designed to be used as therapeutics, that is, at the moment at which the problem is confirmed.

OcraTox (Adiveter SL Pol. Ind., Agro-Reus, Reus, Tarragona, Spain) is a new additive resulting from the modification and activation of diatomaceous earth, which is a natural material extracted from a quarry with a maximum of 70% silicon dioxide. OcraTox is physicochemically inert and presents a highly porous surface with high cationic interchange capacity. OcraTox was designed to comply with the demands listed above. It has a high efficacy in binding ochratoxin at a low dosage in feed, which allows its use as a preventive agent. The main objective of this study was to determine the protective effects of using OcraTox in OTA-contaminated diets on egg production, egg quality characteristics, serum biochemistry, and the presence of OTA residues in laying hens.

MATERIALS AND METHODS

The experiment was performed at the Animal Facility Research Center of the Universitat Autònoma of Barcelona and was conducted according to the guidelines for animal experimentation of the same university.

Birds and Diets

A total of 40 Hisex Brown laying hens were obtained from a commercial facility at 45 wk of age and placed in a light-controlled (16L:8D) and temperature-controlled (22°C) room. Hens were individually allocated to wire cages (41 × 41 cm), each equipped with a feeder and a nipple drinker. For 2 wk, hens were allowed to adapt to the basal diet, which was based on barley, wheat, and soybean meal (Table 1) and formulated to meet NRC (1994) requirements. Twenty-eight hens (1.793 ± 18 g of BW and 99% egg production) were selected and randomly divided into 4 experimental groups (7 birds per group). The 4 experimental treatments resulted from a 2 × 2 factorial arrangement, in which the 2 variation factors were the level of OTA (0 and 2 mg of OTA/kg of feed) and the level of the adsorbent OcraTox (0 and 5 g of OcraTox/kg of feed) in the basal diet. Experimental diets were the control, OcraTox, OTA, and OTA + OcraTox. Pure crystalline OTA (Sigma-Aldrich, Madrid, Spain) was dissolved in absolute ethanol (1 mg/mL), and the solution was sprayed onto 500 g of ground control diet. The treated feed was left overnight at room temperature for the solvent to evaporate. After this time, it was mixed into the basal diet to obtain the desired level of 2 mg of OTA/kg of diet. Ochratoxin A concentration in the diets was analyzed by HPLC (Monaci et al., 2005) to confirm the OTA concentrations in the experimentally contaminated diets. Feed and water were provided ad libitum for 3 wk.

Table 1. Ingredient contents and calculated composition of the basal diet

Item	Amount
Ingredient (g/kg)	
Barley	359.7
Wheat	300.0
Full-fat soybeans	109.2
Soybean meal (44% CP)	95.0
Sunflower oil	30.0
Calcium carbonate	86.3
Dicalcium phosphate	12.8
Iodized salt	1.8
DL-Methionine (98%)	1.5
Premix[1]	2.5
Choline	0.1
Glucanase and xylanase premix[2]	0.5
Natural pigments[3]	0.6
Total	1,000.0
Calculated nutrient and energy (as-is basis)	
CP (g/kg)	157.7
ME (kcal/kg)	2,750
Ca (g/kg)	38.7
Available P (g/kg)	3.9

[1]Provided (per kg of diet): vitamin A, 8,000 IU; vitamin D_3, 1,200 IU; vitamin E, 10 IU; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; pyroxidine, 0.2 mg; vitamin B_{12}, 0.03 mg; pantothenic acid, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 0.5 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

[2]Capsozyme C/2 [Industrial Técnica Pecuaria SA, Barcelona Spain; ß-glucanase (no. CE: 19, no. IUB: EC3.2.1.6): 48,000 glucanase units/kg; and xylanase (no. CE: 21, no. IUB: EC3.2.1.8): 112,000 xylanase units/kg].

[3]Capsantal TR (Industrial Técnica Pecuaria SA, Barcelona Spain, 30 g of total carotenoids/kg).

Performance and Egg Quality

Eggs were collected daily to measure egg weight and calculate egg mass production (egg production × egg weight). Body weights were recorded at the beginning and end of the experiment. Feed consumption was also registered, and the feed conversion rate was calculated as the feed consumed per gram of egg [(g of feed/hen per day)/(g of egg/hen per day)].

During wk 3, the last 3 eggs collected from each bird were used to determine the egg quality and OTA residues. The egg shape index (ES) was calculated by using the formula ES = L/B, where L is the length and B the breadth of the egg. These eggs were kept at 4°C and analyzed within 2 d after collection to determine the shell thickness, albumen height, Haugh unit score, and yolk color. Eggs were broken onto a flat surface and the albumen height (H) and eggshell thickness were measured by a micrometer (Ames S-6428, the nearest precision 0.1 mm; B. C. Ames Co., Waltham, MA). Haugh units were calculated by using the following formula:

$$\text{Haugh units} = 100 \log(H - 1.7G^{0.37} + 7.6),$$

where *H* is the height of the thick albumen in millimeters and *G* is the mass of the whole egg in grams. Yolk color was measured by using a Minolta Chroma Meter CR-200/08 (Minolta Corporation, Ramsey, NJ). The Chroma Meter measures Hunter L, a, and b values, where L is a measure of dark to light, with a greater value indicating a lighter color; a is a measure of green to red, with a greater value indicating a redder color; and b is a measure of blue to yellow, with a greater value indicating a more yellow color. The yolk from each egg was placed in a Petri dish, and the contents were spread evenly into a circle with a diameter similar to that of the light-projection tube on the color meter.

Serum Biochemistry and Organ Weight

At the end of the experiment, blood samples (2 mL) were collected from all birds for serum biochemical determination, and the hens were killed by neck dislocation and bleeding. The liver and spleen were removed, weighed, and frozen until analyzed for OTA concentration. Data were expressed as relative organ weights (grams of organ per 100 g of BW). Within 1 h, the serum was obtained by centrifuging the blood at 2,500 × *g* at 4°C for 15 min and stored at −80°C until further analysis. Serum biochemical parameters were measured to evaluate the hepatobiliary and kidney toxicity and the mineral and protein metabolism. Different serum proteins and metabolites and the activity of certain enzymes were analyzed as sensitive indicators of ochratoxicosis (Marquardt and Frohlich, 1992). Concentrations of total protein, uric acid, cholesterol, calcium, phosphorus, triglycerides, and activities of alkaline phosphatase (**ALP**), aspartate aminotransferase, γ-glutamyltransferase (**GGT**), and alanine aminotransferase in serum were measured by using an Olympus automatic analyzer (AU 400, Olympus, Mishima, Japan). In addition, the liver and spleen were removed, weighed (data expressed as relative organ weights − grams of organ per 100 g of BW), and frozen until analyzed for OTA concentration.

Analysis of OTA Residue in the Liver and Eggs

Six egg samples per treatment consisting of the pool of the last 3 eggs produced by the hen at the end of the study were analyzed for OTA residues. The procedure followed was a modification of the method developed by Monaci et al. (2005). Standards were prepared by adding an appropriate volume of methanol solution of the toxin to the homogenized egg and remaining liver samples for a least 1 h at room temperature to allow equilibration. Ochratoxin A was determined by HPLC with fluorometric detection (Waters Spherisorb ODS-2 chromatographic column, 150 × 4.6 mm × 5 µm, Waters Corporation, Milford, MA). Liquid chromatographic conditions were mobile phase, acetonitrile:water:acetic acid (102:96:2); injection volume, 100 µL; flow rate 1.0 mL/min, run time for a cycle, 10 min; fluorescence detection, excitation at 333 nm and emission at 460 nm. The quantification of OTA was calculated automatically according to the peak area of one set of standards of OTA (0.70 to 60.0 ng/mL). The recovery of the overall procedure was satisfactorily high (92 ± 9%). A calibration curve was obtained by spiking homogenized blank samples with OTA covering the range from 0.15 to 10.00 ng/g. The calibration curve was described by the following equation: peak area = 1.038 C − 0.3718, R = 0.992, where peak area was in arbitrary units and C was expressed as nanograms per gram of homogenate. The limit of detection and the limit of quantification, calculated as a 3- and 10-fold signal-to-noise ratio, were 0.05 and 0.15 ng/g, respectively.

Statistics

Statistical analyses were performed with SAS for Windows, version 8 (SAS Institute Inc., Cary, NC). Results of the parameters were analyzed by ANOVA with the GLM procedure of SAS by using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, α_i is the effect of OTA (i = 1, 2); β_j is the effect of OcraTox (j = 1, 2); $(\alpha\beta)_{ij}$ is the interaction between OTA and OcraTox; and $\varepsilon \sim N(0,\sigma^2_\varepsilon)$ represents the unexplained random error. The α level used for determination of significance for all the analyses was 0.05. Differences between means were tested by Tukey's least significant difference when an interaction between OTA and OcraTox was significant. Data are presented as means and SEM.

RESULTS

Results of feed consumption, BW changes, egg production, and feed conversion rate are shown in Table 2. Ochratoxin A decreased daily feed consumption compared with the control group, whereas no significant differences were observed in hens fed on the OcraTox diets. The lower feed intake with OTA decreased egg mass production and the average egg weight. However, there were no differences between treatments regarding BW changes and feed-to-gain ratio. Incorporating OcraTox into the OTA-containing diets partially ameliorated the adverse effects of OTA on daily feed consumption and egg production (%/hen). The OTA treatment showed the least egg production.

Egg quality parameters are shown in Table 3. There were no effects of treatments on shell thickness, shape index, Haugh units, and yolk brightness. OcraTox increased the albumen height (6.2 vs. 5.3 mm) and yolk

Table 2. Effects of dietary OcraTox and ochratoxin A (OTA) on daily feed intake, BW gain, egg production, egg mass, feed conversion rate (FCR), egg mass, and egg weight changes in laying hens[1]

	Treatment group[2]					Main effect, *P*-value		
Item	Control	OcraTox	OTA	OTA + OcraTox	SEM	OTA	OcraTox	OTA × OcraTox
Feed intake (g/hen per day)	147.3[a]	141.9[a]	126.7[b]	136.7[ab]	4.18	0.005	0.594	0.756
BW gain (g)	39.2	39.0	21.2	27.6	23.77	0.544	0.893	0.900
Egg production (%/hen)	99.2[a]	96.2[ab]	92.4[b]	99.2[a]	2.18	0.397	0.397	0.035
Egg mass (g/hen per day)	62.0[a]	62.9[a]	55.4[b]	59.4[ab]	1.90	0.014	0.220	0.434
FCR [(g of feed/hen per day)/(g of egg/hen per day)]	2.22	2.19	2.15	2.00	0.820	0.097	0.311	0.497
Egg weight (g)	67.3[a]	68.9[a]	63.5[b]	65.6[ab]	1.59	0.033	0.261	0.863
Egg weight changes[3] (g)	1.72	0.59	−1.47	−0.40	1.01	0.050	0.974	0.289

[a,b]Means with different superscripts differ significantly ($P < 0.05$)

[1]Each value represents the least squares mean from 7 laying hens per treatment.

[2]Treatments: control = 0 mg of OTA/kg of feed + 0 g of OcraTox/kg of feed; OcraTox = 0 mg of OTA/kg of feed + 5 g of OcraTox/kg of feed; OTA = 2 mg of ochratoxin A/kg of feed + 0 g of OcraTox/kg of feed; OTA + OcraTox = 2 mg of ochratoxin A/kg of feed + 5 g of OcraTox/kg of feed.

[3]Difference between the egg weight on d 1 and the egg weight at the end of the experiment.

redness color (21.4 vs. 19.5) compared with the unsupplemented treatments.

The effects of dietary treatments on serum biochemistry are summarized in Table 4. The serum activities of alanine aminotransferase and aspartate aminotransferase, and total protein concentration were not affected by treatments. Ochratoxin A in the diet significantly increased the serum activity of ALP (1,685 vs. 526 U/L) and the concentration of uric acid (5.7 vs. 3.3 mg/dL), whereas it decreased the serum concentration of phosphorus (4.4 vs. 5.8 mg/dL). In contrast, feeding OcraTox reduced the serum activity of GGT (16.9 vs. 22.5 U/L) and increased the serum calcium concentration (26.3 vs. 23.7 mg/dL). A significant interaction between OTA and OcraTox was observed in the serum concentrations of uric acid, cholesterol, and triglycerides as a consequence of the change observed on the OTA treatment.

The relative weights of the liver and spleen, and the content of OTA residues in the liver are shown in Table 5. The contaminated diets increased the relative weight of the liver (2.90 vs. 2.42%) but did not affect the relative weight of the spleen. Significant amounts of OTA were detected in the liver of birds fed the OTA treatment, but not in those of birds fed the control or OcraTox diet (<0.05 µg/kg). Supplementing the contaminated diet with OcraTox (OTA + OcraTox) significantly reduced the values from 15.1 to 12.0 µg/kg in the OTA and OTA + OcraTox diet, respectively. We did not detect residues of OTA above our detection limit (0.05 µg/kg) in the eggs.

DISCUSSION

Effects of OTA on Laying Hens

Birds from the OTA group presented clinical signs of toxicosis. Differences were observed between toxicosis hens (OTA) and the control group in daily feed consumption, egg production and egg quality, relative liver weights, hepatic function, serum triglycerides, and serum uric acid levels. The production results are in agreement with those reported by Haazele et al. (1993), who observed decreased feed consumption in

Table 3. Effects of dietary OcraTox and ochratoxin A (OTA) on shell thickness, albumen height, shape index, Haugh units, and yolk color in the eggs[1]

	Treatment group[2]					Main effect, *P*-value		
Item	Control	OcraTox	OTA	OTA + OcraTox	SEM	OTA	OcraTox	OTA × OcraTox
Shell thickness (mm)	0.352	0.352	0.360	0.361	0.009	0.402	0.983	0.960
Albumen height (mm)	5.6[ab]	6.4[a]	4.9[b]	5.9[ab]	0.32	0.060	0.017	0.798
Shape index (length/height, %)	76.7	77.4	76.8	77.1	0.86	0.543	0.602	0.704
Haugh units	76.2	79.7	72.0	75.3	2.65	0.432	0.756	0.776
Yolk color[3]								
L	65.7	65.7	65.6	65.4	0.57	0.760	0.816	0.893
a	20.1[ab]	21.7[a]	18.8[b]	21.0[ab]	0.69	0.167	0.010	0.668
b	46.8	47.2	44.5	46.3	0.80	0.087	0.171	0.418

[a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Each value represents the least square mean from 7 laying hens per each treatment.

[2]Treatments: control = 0 mg of OTA/kg of feed + 0 g of OcraTox/kg of feed; OcraTox = 0 mg of OTA/kg of feed + 5 g of OcraTox/kg of feed; OTA = 2 mg of ochratoxin A/kg of feed + 0 g of OcraTox/kg of feed; OTA + OcraTox = 2 mg of ochratoxin A/kg of feed + 5 g of OcraTox/kg of feed.

[3]L = brightness; a = redness; b = yellowness.

Table 4. Effects of dietary OcraTox and ochratoxin A (OTA) on the serum biochemistry[1] of laying hens

	Treatment group[2]					Main effect, *P*-value		
Item	Control	OcraTox	OTA	OTA + OcraTox	SEM	OTA	OcraTox	OTA × OcraTox
γ-Glutamyltransferase (U/L)	19.0[ab]	18.0[ab]	26.0[a]	15.9[b]	2.60	0.370	0.041	0.098
Aspartate aminotransferase (U/L)	170.6	182.3	213.3	180.0	11.96	0.104	0.376	0.077
Alanine aminotransferase (U/L)	1.1	1.3	1.0	2.3	0.39	0.284	0.080	0.157
Alkaline phosphatase (U/L)	474[b]	578[b]	1,940[a]	1,430[a]	294.62	0.0005	0.479	0.292
Uric acid (mg/dL)	2.6[c]	3.9[bc]	6.2[a]	5.1[ab]	0.59	0.0003	0.849	0.041
Total protein (g/dL)	5.2	5.1	4.7	5.0	0.23	0.200	0.797	0.365
Cholesterol (mg/dL)	103.4[a]	96.4[ab]	87.3[b]	114.5[a]	7.94	0.895	0.211	0.039
Triglycerides (mg/dL)	1,071[a]	975[ab]	785[b]	1,110[a]	78.69	0.332	0.146	0.011
Calcium (mg/dL)	24.6[ab]	26.4[a]	22.9[b]	26.3[a]	1.04	0.355	0.014	0.404
Phosphorus (mg/dL)	5.7[a]	5.9[a]	4.2[b]	4.7[ab]	0.34	0.0005	0.262	0.632

[a–c]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Treatments: control = 0 mg of OTA/kg of feed + 0 g of OcraTox/kg of feed; OcraTox = 0 mg of OTA/kg of feed + 5 g of OcraTox/kg of feed; OTA = 2 mg of ochratoxin A/kg of feed + 0 g of OcraTox/kg of feed; OTA + OcraTox = 2 mg of ochratoxin A/kg of feed + 5 g of OcraTox/kg of feed.

[2]Each value represents the least squares mean from 7 laying hens per treatment.

laying hens fed 1.7 mg of OTA/kg of feed for 2 wk, and by Verma et al. (2003), who reported reductions in the egg mass production of laying hens at levels of 1, 2, and 4 mg of OTA/kg of diet. The decrease observed in our experiment in egg mass production was associated with a decrease in daily feed consumption rather than with changes in the feed conversion rate. The lower energy and essential amino acid intake of the hens exhibiting ochratoxicosis could mainly explain the observed reductions in egg production. In contrast, OTA did not cause significant changes in BW, which is also in agreement with the findings of Verma et al. (2003).

Changes such as the serum concentrations of several proteins and metabolites and the activity of certain enzymes can be used as sensitive indicators of ochratoxicosis (Marquardt and Frohlich, 1992). Biochemical signs of ochratoxicosis reported in the literature in poultry include decreases in cholesterol, total protein, albumin, globulin, potassium, and triglyceride levels, and increases in uric acid and creatine levels and in the activities of serum ALP and GGT (Huff et al., 1988). In our study, significant increases were observed in serum ALP activity and uric acid concentration in birds exposed to OTA in the diet ($P < 0.05$). Similar observations attributable to ochratoxicosis have been reported by Kalorey et al. (2005) within these parameters. The increase observed in ALP activity is known to be indicative of hepatobiliary disease (Kaplan, 1987; Gentles et al., 1999). In fact, the reductions in serum cholesterol and triglyceride concentrations during ochratoxicosis may confirm impaired liver metabolism (Kalorey et al., 2005). Our data also showed an increase in the relative liver weights in groups treated with OTA. Similar increases in relative liver weights have also been observed in chickens exposed to OTA (Huff et al., 1992), and an increase in the concentration of serum uric acid has been observed in chickens fed OTA-contaminated diets (Hoehler and Marquardt, 1996; Huff et al., 1992). Feeding OTA-contaminated diets significantly decreased serum phosphorus levels. Similar results were reported previously by Bailey et al. (1989) and Gupta et al. (2005), and Huff et al. (1980) reported that feeding 2 mg/kg of OTA to broiler chicks decreased bone-breaking strength. Kidney dysfunction has been hypothesized as the cause of the decrease in phosphorus concentration.

However, the most critical aspect of mycotoxins in animal production is the likely presence of mycotoxins in animal products. Ochratoxin A has been described as showing potential teratogenic (Fukui et al., 1987) and genotoxic (Creppy et al., 1985) effects, and has been classified by the International Agency for Research on Cancer (1993) as a possible carcinogen agent (group 2B) to humans. There is a correlation between

Table 5. Effects of dietary OcraTox and ochratoxin A (OTA) on relative weights of the liver and spleen and concentrations of OTA in the liver of laying hens[1]

	Treatment group[2]					Main effect, *P*-value		
Item	Control	OcraTox	OTA	OTA + OcraTox	SEM	OTA	OcraTox	OTA × OcraTox
Liver weight (g/100 g of BW)	2.36[b]	2.48[b]	2.90[a]	2.90[a]	0.009	0.002	0.628	0.703
Spleen weight (g/100 g of BW)	0.12	0.14	0.15	0.12	0.14	0.564	0.817	0.303
Liver ochratoxin A (μg/kg)	ND[3]	ND	15.1	12.0	0.20	0.0001	0.0001	0.0001

[a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Each value represents the least squares mean from 7 laying hens per treatment.

[2]Treatments: control = 0 mg of OTA/kg of feed + 0 g of OcraTox/kg of feed; OcraTox = 0 mg of OTA/kg of feed + 5 g of OcraTox/kg of feed; OTA = 2 mg of ochratoxin A/kg of feed + 0 g of OcraTox/kg of feed; OTA + OcraTox = 2 mg of ochratoxin A/kg of feed + 5 g of OcraTox/kg of feed.

[3]ND = not detected.

OTA concentration in feed and its residues in animal tissues (Krogh, 1976). In pigs, ochratoxin is accumulated mostly in the kidneys, followed by the liver and muscle (Malagutti et al., 2005). In our study, we observed significant amounts of OTA in the liver of all birds fed the contaminated diets. We did not observe OTA residues in eggs when considering detection limits of 0.05 and 0.15 ng/g. Similarly, Krogh (1987) reported no detection of OTA in eggs of laying hens fed diets containing 0.3 and 1 mg of OTA/kg. In contrast, Piskorska-Pliszczyńka and Juszkiewicz (1990) reported that OTA was detected in the eggs of laying hens fed diets containing a greater level of OTA (10 mg/kg). The differences between studies may have been due to the concentrations of OTA in the diet.

Efficacy of OcraTox

The most promising and economical approach for reducing mycotoxicosis in animal feeding is the use of adsorbents, which bind mycotoxins efficiently in the gastrointestinal tract and prevent their adsorption (Dakovic et al., 2005). In vivo studies have demonstrated that aluminosilicates and many proposed adsorbents are capable of absorbing aflatoxins but do not prevent the toxicity of dietary OTA (Danicke, 2002) because they have different chemical structures (Phillips, 1999). OcraTox is a new additive resulting from the modification and activation of diatomaceous earth. OcraTox is physicochemically inert and presents a highly porous surface with a high cationic interchange capacity that yields a high binding capacity for OTA.

Our results showed that OcraTox did not cause any adverse effects in hens. However, OcraTox increased the egg albumen height, the redness color of the egg yolk, and the serum calcium concentration, which suggest likely effects of the product on the absorption of minerals and carotene. Moreover, when OcraTox was incorporated into the OTA-contaminated diets, the adsorbent increased egg production to values not significantly different from the control, and it ameliorated the negative effects on some of the serum variables altered by OTA, such as the serum concentrations of uric acid, cholesterol, and triglycerides. These results and the lower content of OTA in the liver of birds fed the OTA + OcraTox diet appear to support the suggestion that OcraTox may provide protection against the toxic effects of OTA.

In conclusion, results of the study confirmed the toxic effects in laying hens of a prolonged dietary intake of OTA. However, the inclusion of new adsorbent products in the diet, such as OcraTox, can significantly ameliorate many of its adverse effects.

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