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Effect of Zearalenone on Female White Leghorn Chickens†

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Acute toxic effects of purified zearalenone were studied in growing female White Leghorn chickens. In the first experiment, zearalenone in gelatin capsules was administered to 10 chickens (zearalenone-treated chickens [ZC]) in a single oral dose of 15.0 g/kg. Another 10 control chickens (CC) received empty gelatin capsules. All chickens survived the 10-day experiment and did not show any noticeable gross or histopathological lesions. There were no differences between CC and ZC in weight gain, oviduct, comb and liver weights, hematological parameters, and serum cholesterol. ZC had significantly less ($P < 0.05$) serum calcium but significantly greater ($P < 0.01$) serum phosphorus than CC. In the second experiment, zearalenone was administered orally or intramuscularly (pectoral muscle) at levels of 0, 50, 200, 400, and 800 mg/kg for 7 consecutive days. The oviduct weight increased with increasing toxin levels in both orally (OZC) and intramuscularly (IZC) administered groups: there were more pronounced effects in the IZC. The liver weight increased and comb weight decreased in IZC. The relative estrogenic biopotency of zearalenone in IZC, using estradiol dipropionate as a standard, was 1.37%. The results of this experiment demonstrate that chickens are highly tolerant to zearalenone and that the estrogenic effects of the toxin are greater when it is administered in multiple doses than in a single dose and in IZC than in OZC.

Zearalenone, a secondary metabolite produced by a number of species of *Fusarium* (4), has been demonstrated to possess estrogenic properties which cause uterine proliferation in rats (6) and hyperestrogenism in swine (12) and turkeys (14). Zearalenone is found most commonly in maize but also in other cereal grains and commercial feeds.

A quadratic effect on the growth rate of chickens was observed by increasing the level of zearalenone from 0 to 1,600 μg/g (19). Under conditions characterized by high moisture and alternate warm days followed by cold nights, it is possible for some *Fusarium* species to produce copious amounts of zearalenone while colonizing grain, which could cause intoxification in animals when such grains are fed. The objective of the present study was to evaluate the acute effects, if any, of zearalenone on growing White Leghorn female chickens in single or seven consecutive daily doses. The relative estrogenic biopotency of zearalenone was also estimated by oviduct growth response using estradiol 3,17-dipropionate (EDP) as a standard.

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MATERIALS AND METHODS

This study consisted of two experiments. One-day-old White Leghorn female chickens were obtained by hatching eggs purchased from a commercial source. Chickens were reared with a stock ration in electrically heated battery brooders with raised wire-mesh floors. Feed and water were provided ad libitum during the preexperimental and experimental period. The diet was a corn-soybean meal type.

Toxin source. Zearalenone was obtained from the Food and Drug Administration, Bureau of Veterinary Medicine, Beltsville, Md., and was purified by recrystallization from boiling ethyl ether after decolorization with charcoal. The purity (greater than 99%) and authenticity of the toxin were determined by analysis via combination gas chromatography and mass spectroscopy.

Experiment 1. At 2 weeks of age, 20 chickens were selected for uniformity in body weight and randomly housed in two pens so that each group included 10 chickens. One group received zearalenone at a level of 15.0 g/kg of body weight (1.8 to 2.0 g of zearalenone per chicken) in gelatin capsules. The other group (control) received the same number of empty gelatin capsules. Birds were closely observed during the 24-h period after dosing and thereafter two times daily. The experiment was terminated on day 10 after dosing.

Birds and feed were weighed at the beginning and end of the experiment. Blood samples were taken by heart puncture at the end of the experiment, and afterwards chickens were necropsied and examined. Tissue samples of liver, heart, lung, brain, oviduct,

kidney, bursa of Fabricius, spleen, and gastrointestinal tract were excised and kept in 10% neutral buffered formaldehyde for histopathological examination. The weights of the liver, oviduct, and comb were determined.

The serum concentrations of calcium (11), phosphorus (5), and cholesterol (22) were analyzed by standard methods. The packed cell volume, hemoglobin, erythrocyte counts, and leukocyte counts were determined on ethylenediaminetetraacetate-treated whole blood by standard methods described by Schalm (18). The differential leukocyte counts were performed based on 300 cell counts after staining with Wright-Giemsa stain. The tissues were fixed in 10% neutral buffered Formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

Experiment 2. Healthy chickens, uniform in body weight, were selected at 2 weeks of age and distributed randomly in the treatment. Each treatment included 10 chickens. Zearalenone was intubated into the crop of the first five groups of chickens by a plastic tube attached to a syringe: 0 (control), 50, 200, 400, and 800 mg/kg of body weight per day. Another five groups of chickens were injected with the toxin into the pectoral muscle at the same levels used in oral intubation. EDP was injected into the pectoral muscle in the third group of chickens: 0.5, 2.0, 4.0, and 8.0 mg/kg of body weight per day. Zearalenone was dissolved in 50 to 75% aqueous ethanol and administered at a volume of 0.5 ml per dose except for the treatment of 800 mg/kg per day (0.75 ml). Control groups for oral intubation and intramuscular injection received the same amount of aqueous ethanol as the zearalenone-treated groups. Zearalenone and EDP were administered for 7 consecutive days, and birds were sacrificed 24 h after the last dose. The liver, heart, spleen, oviduct, comb, and bursa of Fabricius were weighed and expressed per 100 g of body weight.

Statistical analysis. Data were analyzed using an analysis of variance technique, and comparisons among treatment means were made using Duncan's multiple-range test; two treatment means were compared by t test (21). The relative biopotency of zearalenone and its confidence intervals were calculated using published equations (1, 2).

RESULTS AND DISCUSSION

Tolerance of chickens to zearalenone. All chickens administered 15 g of zearalenone per kg of body weight at a single oral dose survived the treatment and did not show any noticeable gross or histopathological signs. On day 10 after dosing, there was no difference between control and zearalenone-treated chickens in body weight and weights of the oviduct, comb, and liver (Table 1). Birds treated with zearalenone had significantly ($P < 0.05$) lower serum calcium but significantly ($P < 0.01$) higher serum phosphorus concentrations than control chickens (Table 2). Hematological criteria (hematocrit, erythrocyte, leukocyte, and differential leukocyte counts) and serum cholesterol were similar between two groups. Chickens appeared to tolerate a large single oral dose of zearalenone; the 50% lethal dose of the toxin for growing chickens is greater than 15 g/kg of body weight.

Most investigators agree that blood calcium and phosphorus levels increase when birds begin laying and that these increases are due mainly to the effects of estrogen elaborated by the mature ovary (7, 10). In this study, blood was collected 10 days after zearalenone treatment. There were no appreciable estrogenic effects on blood components and tissues; this may be due to metabolism and excretion of the toxin during the 10-day period. Estrogen also causes increases in the blood lipids, including cholesterol contents (20). In this study, zearalenone-treated birds did not show such an effect at 10 days after administration.

Relative estrogenic biopotency of zearalenone. The weight gains of birds orally intubated with zearalenone were significantly less ($P < 0.01$) than those of chickens injected with zearalenone into the pectoral muscle at all levels (Table 3). When chickens were orally intubated with the toxin, the weight gains of birds receiving 800 mg/kg per day decreased significantly ($P < 0.01$) as compared with the control group. Chickens injected intramuscularly with varying amounts of zearalenone gained similarly to the control group injected with a plain vehicle solution. The weight gains of birds injected with EDP were similar to those of zearalenone-injected chickens. The smaller weight gains of chickens orally intubated with the toxin as compared to intramuscularly injected groups might be due to the effect of ethanol used as a vehicle solution, because the same pattern was observed with control chickens. One bird in each group

TABLE 1. *Effect of a single oral administration of zearalenone on weight gain and organ weight in chicks*[a]

Treatment[b]	Wt gain (g)	Oviduct wt (mg)	Comb wt (mg)	Liver wt (g)
Control	112.3 ± 7.5	38.23 ± 4.97	40.53 ± 21.13	2.68 ± 0.16
Zearalenone	100.0 ± 7.1	49.37 ± 11.95	42.96 ± 7.86	2.77 ± 0.26

[a] Mean value of 10 chicks ± standard deviation. Weight gain and organ weights were determined 10 days after treatment.

[b] Zearalenone was administered orally in capsules at 15 g/kg of body weight. The control group received empty capsules.

TABLE 2. *Effect of single oral administration of zearalenone on chick serum calcium phosphorus and cholesterol levels and peripheral blood*[a]

Determination (units)	Control chicks	Zearalenone-treated chicks[b]
Serum calcium (mg/dl)	8.18 ± 0.95[c]	6.36 ± 0.82[c, d]
Serum phosphorus (mg/dl)	5.73 ± 0.33[c]	7.98 ± 1.58[e]
Serum cholesterol (mg/dl)	145.80 ± 16.60	125.80 ± 31.00
Hematocrit (%)	27.60 ± 2.40[f]	27.20 ± 1.90
Hemoglobin (g/dl)	9.90 ± 0.50[f]	9.20 ± 0.60
Erythrocytes ($\times 10^6$/mm^3)	2.96 ± 0.28[f]	2.87 ± 0.27
Leukocytes ($\times 10^3$/mm^3)	33.10 ± 13.50[f]	37.80 ± 12.60
Differential leukocyte counts[g]		
Lymphocytes (%)	62.88 ± 8.24[f]	75.80 ± 9.33
Total neutrophils (%)	23.63 ± 5.01[f]	16.72 ± 7.04
Eosinophils (%)	0	0
Basophils (%)	9.76 ± 3.62[f]	7.20 ± 2.10
Monocytes (%)	3.73 ± 3.20[f]	0.30 ± 0.38

[a] Mean value of 10 chicks ± standard deviation. Blood samples were collected 10 days after zearalenone treatment.
[b] Zearalenone was orally administered in capsules at 15 g/kg of body weight. The control group received empty capsules.
[c] Mean value of nine chicks ± standard deviation.
[d] Significantly different from the control group ($P < 0.05$).
[e] Significantly different from the control group ($P < 0.01$).
[f] Mean value of eight chicks ± standard deviation.
[g] Based on 300 cell counts and divided by 3 to obtain percent.

(control and 50, 200, and 800 mg/kg per day, intubated orally) died; gross examination after necropsy revealed mechanical damage in crops, possibly by intubation procedure.

When the zearalenone dose was increased, there were increases in the oviduct weight in both orally and intramuscularly administered chickens (Tables 4 and 5). The comb weight decreased and the liver weight increased in chickens injected intramuscularly with the toxin (Table 5). The effect of zearalenone on the oviduct weight was greater in injected birds than in intubated ones. Chickens injected with EDP showed the same effect as in the chickens injected intramuscularly with zearalenone (Table 6). The weights of heart, spleen, and bursa of Fabricius were not significantly altered by administration of zearalenone or EDP.

The most pronounced effect of zearalenone is hyperestrogenism (16). The increase in the size of the oviduct in the hen with sexual maturity is due to the effect of estrogen. The sensitivity of the avian oviduct to estrogenic hormones has been used as an assay method for potency of estrogenic substances (9, 13). Estrogen has been reported to be antagonistic to comb growth in female chickens (3).

The increased size of the liver in chickens treated with zearalenone or EDP confirmed the results of previous studies with zearalenone that the toxin is estrogenic. Chickens treated with estrogen increased in liver fat, liver protein, and consequently liver weight (7, 8). Ranney and Chaikoff (17) demonstrated that the liver is the principal organ concerned in the increased synthesis of lipids induced by estrogen. Estradiol is

TABLE 3. *Body weight gain of chicks administered zearalenone and EDP*

Dose[a] (mg/kg per day)	Zearalenone[b]		EDP[b] (intramuscular injection) (g)
	Oral intubation (g)	Intramuscular injection (g)	
0 (control)	57.6 ± 17.4[c]	83.7 ± 11.0[d]	
0.5			82.7 ± 7.4
2.0			88.7 ± 7.4
4.0			93.8 ± 5.0
8.0			90.7 ± 6.0
50	56.0 ± 23.1[c]	87.2 ± 6.4[d]	
200	57.4 ± 20.9[c]	89.3 ± 6.3[d]	
400	53.7 ± 19.1	91.0 ± 8.8[d]	
800	28.6 ± 9.8[c, e]	84.4 ± 16.9[d]	

[a] Zearalenone and EDP were orally intubated or intramuscularly injected for 7 consecutive days. The control group received the same amount of the vehicle solution as the treated groups.
[b] Mean values of 10 chicks ± standard deviation.
[c] Mean values of nine chicks ± standard deviation.
[d] Significantly different from the oral intubation group ($P < 0.01$)
[e] Significantly different from the control group ($P < 0.01$).

TABLE 4. *Organ weights of chicks intubated orally with zearalenone*[a]

Dose[b] (mg/kg per day)	Oviduct (mg)	Comb (mg)	Liver (g)	Heart (g)	Spleen (g)	Bursa of Fabricius (g)
Control	52.1 ± 12.1	30.6 ± 7.6	2.8 ± 0.19	0.59 ± 0.05	0.19 ± 0.03	0.68 ± 0.13
50	54.2 ± 14.2	29.0 ± 6.0	2.7 ± 0.28	0.56 ± 0.04	0.20 ± 0.05	0.67 ± 0.13
200	76.4 ± 16.7	30.4 ± 6.6	2.9 ± 0.14	0.61 ± 0.06	0.19 ± 0.04	0.71 ± 0.13
400	107.4 ± 15.1[c]	27.9 ± 6.4	2.9 ± 0.18	0.56 ± 0.03	0.17 ± 0.03	0.73 ± 0.12
800	171.0 ± 16.7[c]	27.4 ± 8.1	3.1 ± 0.27	0.56 ± 0.04	0.16 ± 0.03	0.57 ± 0.08

[a] Organ weight per 100 g of body weight. Mean values of nine chicks ± standard deviation. The 400-mg/kg per day group had 10 chicks.
[b] Zearalenone was orally intubated for 7 consecutive days, and the organ weight was determined 24 h after the last dose. The control group received the same amount of the vehicle solution as the treated groups.
[c] Significantly different from the control group ($P < 0.01$).

TABLE 5. *Organ weight of chicks injected intramuscularly with zearalenone*[a]

Dose[b] (mg/kg per day)	Oviduct (mg)	Comb (mg)	Liver (g)	Heart (g)	Spleen (g)	Bursa of Fabricius (g)
Control	50.5 ± 10.1	32.6 ± 7.8	2.71 ± 0.11	0.57 ± 0.09	0.22 ± 0.04	0.72 ± 0.13
50	123.8 ± 13.5[c]	28.8 ± 7.4	2.89 ± 0.20	0.56 ± 0.04	0.21 ± 0.04	0.76 ± 0.13
200	559.2 ± 63.2[c]	17.0 ± 2.0[c]	3.17 ± 0.21[c]	0.58 ± 0.04	0.18 ± 0.04	0.73 ± 0.05
400	955.8 ± 61.4[c]	17.0 ± 3.7[c]	3.31 ± 0.13[c]	0.56 ± 0.06	0.16 ± 0.03	0.64 ± 0.13
800	1,198 ± 76.7[c]	14.7 ± 4.2[c]	3.71 ± 0.30[c]	0.60 ± 0.05	0.17 ± 0.05	0.67 ± 0.13

[a] Organ weight per 100 g of body weight. Mean values of 10 chicks ± standard deviation.
[b] Zearalenone was intramuscularly injected for 7 consecutive days, and organ weights were determined 24 h after the last dose. The control group received the same amount of the vehicle solution as the treated groups.
[c] Significantly different from the control group ($P < 0.01$).

TABLE 6. *Organ weight of chicks injected intramuscularly with EDP*[a]

Dose[b] (mg/kg per day)	Oviduct (mg)	Comb (mg)	Liver (g)	Heart (g)	Spleen (g)	Bursa of Fabricius (g)
Control	50.5 ± 10.1	32.6 ± 7.8	2.71 ± 0.11	0.57 ± 0.09	0.22 ± 0.04	0.72 ± 0.13
0.5	95.8 ± 13.5[c]	28.0 ± 5.1	2.85 ± 0.18	0.59 ± 0.06	0.16 ± 0.02	0.84 ± 0.11
2.0	434.1 ± 39.0[c]	19.5 ± 4.5[d]	3.18 ± 0.25[d]	0.58 ± 0.04	0.17 ± 0.03	0.82 ± 0.10
4.0	710.6 ± 67.9[c]	17.2 ± 3.3[c]	4.24 ± 0.33[c]	0.60 ± 0.05	0.16 ± 0.02	0.86 ± 0.15
8.0	1,100.3 ± 107.4[c]	13.9 ± 3.0[c]	4.76 ± 0.42[c]	0.61 ± 0.04	0.15 ± 0.02	0.75 ± 0.10

[a] Organ weight per 100 g of body weight. Mean values of 10 chicks ± standard deviation.
[b] EDP was intramuscularly injected for 7 consecutive days, and organ weight was determined 24 h after the last dose. The control group received the same amount of the vehicle solution as the treated groups.
[c] Significantly different from the control group ($P < 0.01$).
[d] Significantly different from the control group ($P < 0.05$).

effective on involution of the bursa of Fabricius in chickens (23). Birds injected with high levels of zearalenone or EDP showed similar effects.

Chickens injected intramuscularly with zearalenone at all levels showed an effect on the growth of the oviduct (Table 7). However, in orally intubated groups, only 45% of chickens receiving 200 mg/kg per day, and all chickens receiving 400 and 800 mg/kg per day, were affected. The 50% effective dose of zearalenone for the oviduct growth in intramuscularly injected chickens was less than 50 mg/kg per day (350 mg/kg during the 7 consecutive days), and for orally intubated chickens it was near 200 mg/kg per day (1,400 mg/kg during 7 consecutive days), according to the method of Miller and Tainter (15). The estrogenic effect of zearalenone was much greater when the toxin was administered in seven doses, as in experiment 2, than in a single large dose, as in experiment 1.

The relative estrogenic biopotency of zearalenone based on oviduct weight using EDP as a standard was estimated to be 1.37% by the slope-ratio assay technique described by Bliss (1) and Bliss and White (2). The confidence intervals of the value of 5% probability were estimated as 1.275 to 1.465%. The dose responses of the oviduct growth to zearalenone were more linear when the toxin was injected into the pectoral muscle than when it was orally intubated. The oral potency might be influenced by differences of the individual bird in absorption and metabolism of zearalenone in the gut, variation in feed consumption, and systemic destruction and excretion; therefore, the intramuscular route was used for biopotency determination.

TABLE 7. *Effect of zearalenone in chicks based on the weight of the oviduct*

Dose[a] (mg/kg)	No. of chicks affected/no. tested[b]	
	Oral intubation[c]	Intramuscular injection[d]
350	0/9	10/10
1,400	4/9	10/10
2,800	10/10	10/10
5,600	9/9	10/10

[a] The total amount of zearalenone administered over 7 consecutive days.
[b] Number of chicks affected by zearalenone in the oviduct growth out of the total number of chicks. The weight of oviduct in the control group + 2 standard deviations was used as a criterion.
[c] The oviduct weight of the control group + 2 standard deviations was 76.5 mg per 100 g of body weight.
[d] The oviduct weight of the control group + 2 standard deviations was 70.7 mg per 100 of body weight.

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