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Toxicity of Ochratoxin A and Penicillic Acid to Chicks

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The mycotoxins, ochratoxin A (OA) and penicillic acid (PA), are secondary metabolites of several fungal species and have been found as natural contaminants in a wide variety of foods and feedstuffs. OA has been found in barley (Fischback and Rodricks 1973), corn (Shotwell et al. 1969), oats (Krogh 1973), wheat (Scott et al. 1970; Prior 1976), and mixed feeds, dried white beans, and peanuts (Scott et al. 1972), while PA has been found in extremely high concentrations in corn (Kurtzman and Ciegler 1970). Experimental feeding of graded levels of OA in diets to broiler chicks has been demonstrated to have a deleterious effect on performance (Huff et al. 1974, 1975; Prior et al. 1980; Huff and Doerr 1981; and Kubena et al. 1983), whereas graded levels of PA fed up to 400 mg/kg of diet to broiler chicks produced no significant effects on growth or efficiency of feed utilization, suggesting that PA alone has little toxicity in chickens (Huff et al. 1980).

The simultaneous occurrence of more than one toxigenic fungus and/or mycotoxin might illicit toxic responses not encountered when present singly and therefore may present a serious problem to the poultry industry. Fungal strains have been isolated that can simultaneously produce OA and PA (Ciegler 1972; Bacon et al. 1973). Huff and Doerr (1981) observed a synergism between aflatoxin and OA in broiler chicks exposed over a 3 week period.

The purpose of the research reported herein was to investigate the toxicity of and describe the major effects of sublethal and multiple exposure of male white leghorn chicks to OA and PA singly and in combination.

MATERIALS AND METHODS

In 2 experiments, 7 day-old white leghorn (Hyline W-36) male chicks were individually weighed, wingbanded, and housed in electrically heated batteries under continuous lighting with feed and water available *ad libitum*. The commercial unmedicated starter diet used consisted of soybean meal, 29.0%; ground corn, 63.2%; dehydrated alfalfa meal, 3.5%; phosphorous supplement, 2.0%; oyster shell flour, 1.5%; salt, .5%; vitamin mix, .25%; manganese sulfate,

.025%; and zinc oxide, .025%. The diets contained or exceeded levels of critical nutrients recommended by the National Research Council (1977).

The OA and PA were purchased from Makor Ltd., Jerusalem, Israel. Purity of both mycotoxins was established using reverse phase high pressure liquid chromatography and thin layer chromatography. Solutions of OA, PA, and the OA-PA combination were prepared by dissolving the appropriate quantities of mycotoxins in 0.1M sodium bicarbonate. All solutions were prepared fresh in light-proof vials and maintained at -70°C until used for dosing. Solutions for dosing were calculated to provide 0.1 mg OA/ml and/or 6.0 mg PA/ml of solution. Chicks were dosed on alternating days for a period of 20 days (experiment 1) and 28 days (experiment 2) by gastric intubation using a ball tipped stainless steel needle.

Prior to dosing on alternating days, the chicks were individually weighed and feed consumption was recorded during the entire time of both studies. The chicks were checked at least twice daily for signs of overt toxicity and mortality was recorded as it occurred. Postmortem examinations for gross lesions were performed as soon after death as was feasible. At the termination of the experiments, all remaining chicks were killed by cervical fracture and the kidney, liver, lung, bursa, spleen, gizzard and proventriculus were examined for gross lesions.

In the first study, the chicks were randomly divided into 4 groups of 12 chicks each. The groups were dosed on alternating days as follows: control, none; solvent control, 1 ml of 0.1M sodium bicarbonate; .4 mg OA/kg of body weight; and .8 mg OA/kg of body weight. The doses were adminstered 10 times for a total dose of 4 mg OA/kg of body weight and 8 mg OA/kg of body weight.

In the second experiment, the chicks were randomly divided into 5 groups of 10 chicks each. The groups were dosed on alternating days as follows: control, none; solvent control, 1 ml of .1M sodium bicarbonate; 1 mg OA/kg of body weight and 60 mg PA/kg of body weight, and a combination dose of 1 mg OA/kg body weight + 60 mg PA/kg of body weight. The doses were administered 14 times for a total dose of 14 mg OA/kg of body weight and 840 mg PA/kg of body weight.

Data for body weight gains were compared statistically according to the general linear models procedure for analysis of variance and ranked by the Duncan's multiple range test (Barr et al. 1979).

RESULTS AND DISCUSSION

In experiment 1, the body weights of chicks in the group administered .4 mg OA/kg of body weight on alternating days did not differ significantly from the controls or solvent controls at any time during the experiment (Table 1). When the dosage was calculated as if it were present in the feed, the chicks administered .4 mg OA/kg of body weight would have consumed feed containing a range of .9 to 1.3 mg OA/kg of feed for the experimental period.

When compared to the controls and the solvent controls, there was a significant depression in body weights of chicks adminstered .8 mg OA/kg of body weight on alternating days. This depression in body weights occurred as early as 6 days after dosing began and continued for the remainder of the experiment. When the dosage was calculated as if it were present in the feed, the chicks would have consumed feed containing a range of 1.4 to 2.7 mg OA/kg of feed for the experimental period. These results agree with the report of Huff et al. (1974) that levels below 2.0 mg OA/kg of feed did not cause a significant depression in body weights. However, Prior et al. (1980) observed a significant depression in body weights of broiler chicks at levels as low as .5 mg OA/kg of feed. Caution should be exercised when comparing the data from this study and the data from the broiler studies by Huff et al. (1974) and Prior et al. (1980) since the chicks in our study were white leghorn and were adminstered the OA on alternating days which allowed time for metabolism of the OA before the next dose was adminstered. There were no differences in the efficiencies of feed utilization that could be attributed to treatment. No overt signs of toxicity were observed and no mortality occurred during the experiment. Post mortem examination of the chicks killed at the termination of the experiment revealed no gross lesions in the kidney, liver, lungs, bursa, spleen, gizzard or proventriculus.

Table 1. Effects of ochratoxin A on body weights and feed utilization (Study 1).

	Days post dosing				
Treatment	0	6	12	20	1-20
	B. W.[1] (g)	B. W. (g)	B. W. (g)	B. W. (g)	G. feed / G. gain
Control	71.1[a]	111.9[a]	166.5[a]	249.3[a]	3.80
Solvent Control	72.3[a]	108.4[a]	162.4[a]	248.7[a]	3.99
.4 mg OA/kg[2]	72.5[a]	106.8[ab]	156.3[a]	237.5[ab]	3.80
.8 mg OA/kg	71.5[a]	100.9[b]	144.8[b]	211.7[b]	3.81

[a,b]In the same vertical column of body weights, means followed by different letters are significantly different ($P<.05$) according to Duncan's multiple range test.

[1]B. W. = Body weight

[2]Chicks received 10 doses of either .4 mg or .8 mg OA/kg of B.W.

Table 2. Effects of ochratoxin A and penicillic acid on body weights and feed utilization (Study 2).

Treatment	Days post dosing						
	0	6	12	18	24	28	1-28
	B. W.[1] (g)	B. W. (g)	B. W. (g)	B. W. (g)	B. W. (g)	B. W. (g)	G. feed / G. gain
Control	70.6[a]	116.2[a]	179.0[a]	250.8[a]	330.2[a]	388.8[a]	3.44
Solvent Control	70.6[a]	113.0[a]	181.0[a]	255.6[a]	336.5[a]	392.3[a]	3.47
60 mg[2] PA/kg	70.3[a]	112.0[a]	175.1[a]	247.8[a]	331.1[a]	375.9[a]	3.19
1 mg OA/kg	70.1[a]	104.4[b]	152.9[b]	208.7[b]	279.4[b]	322.7[b]	3.89
1 mg OA/kg + 60 mg PA/kg	69.7[a]	98.3[c]	148.0[b]	204.6[b]	278.6[b]	320.7[b]	3.24

[a,b,c]In the same vertical column of body weights, means followed by different letters are significantly different ($P<.05$) according to Duncan's multiple range test.
[1]B. W. = Body weight
[2]Chicks received 14 doses of either 60 mg PA/kg of B. W., 1 mg OA/kg of B. W. , or 1 mg OA + 60 mg PA/kg of B. W.

In experiment 2, the body weights of chicks in the group administered 1 mg OA/kg of body weight on alternating days were significantly lower than the control, solvent control, and PA groups throughout the experiment (Table 2). When the dosage of OA and PA administered on alternating days was calculated as if it were present in the feed, the chicks administered OA singly or in combination with PA would have consumed feed containing a range of 1.9 to 3.0 mg OA/kg of feed. The chicks administered PA singly or in combination with OA would have consumed feed containing a range of 115 to 180 mg PA/kg of feed. The depression in body weight due to OA agrees with the reports of Huff et al. (1974, 1975), Prior et al. (1980), Huff and Doerr (1981), and Kubena et al. (1983) who observed that chicks fed diets containing OA at levels comparable to this experiment had reduced performance. The body weights of the chicks administered 60 mg PA/kg of body weight did not differ significantly from the controls or solvent controls at anytime during the experiment. These results agree with the report of Huff et al. (1980) who reported no deleterious effects when broilers were fed diets containing up to 400 mg/kg of diet. At 6 days post treatment, the chicks in the group administered the combination of

1 mg OA and 60 mg PA/kg of body weight had significantly lower body weights than any of the other groups, including the group administered OA singly. After 6 days post treatment, the combination group had body weights which were significantly lower than the controls, solvent controls and PA groups, but not lower than the group administered OA singly. These findings indicate that the initial simultaneous administration of OA and PA caused an increase in toxicity which was followed by recovery from the initial shock. These results support the report of Shepherd et al. (1981) who observed a recovery in daily weight gains and less renal damage at 21 days than at 10 days post treatment in the OA-PA combination groups. The only mortality occurred in the OA-PA combination group where one chick died on day 10 of the experiment. Post mortem examination of this chick and the chicks killed at the termination of the experiment revealed no gross lesions.

Growth retardation is an early sign seen in ochratoxicosis. Our results are consistent with this finding and show that the decrease in weight gain in the combination group was due to the effects of OA and not PA after 6 days post treatment. The efficiency of feed utilizaion was reduced in the chicks administered OA singly, but not when in combination with PA. The present data indicate the presence of OA and PA in combination may not present a more serious problem to the poultry industry than the presence of OA alone. However, OA alone does present a major problem and PA may occur at higher concentrations under field conditions than those administered in this experiment. The natural occurrence of PA at higher concentrations and in the presence of OA and other mycotoxins prevents the dismissal of the possible interactions of PA with other toxicants. The area of longer term subacute exposure to combinations of mycotoxins with each other and with other toxicants warrants further investigation.

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