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Ochratoxin A-Induced Iron Deficiency Anemia†

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Ochratoxin A at 8 μg per g of diet, but not at lower doses, fed to chickens from 1 day to 3 weeks of age resulted in significantly ($P < 0.05$) decreased packed blood cell volume and hemoglobin concentration without altering the number of circulating erythrocytes. Serum iron and percentage of transferrin saturation were lowered at 4 and 8 μg/g. Therefore, anemia was characteristic of severe ochratoxicosis of young chickens, and the anemia was categorized as a hypochromic-microcytic anemia of the iron deficiency type. These data indicate that ochratoxin A by itself does not cause hemorrhagic anemia syndrome of chickens and that an anemia caused by a nutritional deficiency can be elicited by a mycotoxin.

The ochratoxins are a family of mycotoxins produced primarily by the fungi *Aspergillus ochraceus* (31) and *Penicillium viridicatum* (32). Ochratoxin-producing fungi are ubiquitous, growing on a wide variety of food and feedstuffs including black and red peppers (2, 4), dry fish (30), rice (18), corn (4), and peanuts (8). Ochratoxin A is a potent nephrotoxin in experimental chicks (14), rats (21, 25), dogs (26), and swine (27). Furthermore, ochratoxin A has been implicated with abortion in dairy cattle (23), with nephropathy in swine (16), and with high mortality in turkeys (P. B. Hamilton, W. E. Huff, J. R. Harris, and R. D. Wyatt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, O21, p. 248). Ochratoxin A, based on 50% lethal dose determinations and minimal growth-inhibitory concentration, is the most potent mycotoxin studied in chickens to date (15).

The economically important hemorrhagic anemia syndrome of poultry is characterized by aplastic anemia with spontaneous hemorrhaging in various organs (11) and is caused by feed infested with any of several toxigenic fungi, including some that produce ochratoxins (9). In addition to this implication of fungi that produce ochratoxin as a possible cause of hemorrhagic anemia syndrome, it was reported later that ochratoxin A, which was not discovered until after the syndrome was described, caused an apparent suppression of hematopoiesis in the bone marrow of chicks (7, 20) and prolonged blood clotting in chickens (5). Thus, ochratoxin A seemed to be a reasonable candidate for the specific etiological agent of hemorrhagic anemia syndrome, whose cause continues to elude investigators of mycotoxicoses. This possibility was investigated by studying the anemia of ochratoxicosis in chickens.

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MATERIALS AND METHODS

Animal husbandry. Day-old male broiler chicks were obtained from the university farm. The chicks were housed in electrically heated batteries under continuous illumination, with feed and water available ad libitum. Ochratoxicosis was induced by incorporating known amounts of ochratoxin A into a commercial-type broiler starter ration from which all medications were omitted. Ochratoxin A was fed at the treatment levels of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 μg of toxin per g of feed. There were 4 groups of 10 birds for each treatment. The experimental design was completely randomized. The birds were fed from 1 day to 3 weeks of age, at which time the experiments were terminated.

Production of ochratoxin. Ochratoxin A was produced by growing *A. ochraceus* NRRL 3174 on wheat by the method of Trenk et al. (28). Ochratoxin A was extracted from the wheat by the method of Steyn and van der Merwe (22) and purified by thick-layer preparative chromatography on silica gel, using benzene-acetic acid (9:1) as the solvent. Ochratoxin A was removed from the silica gel by making a slurry with a hot benzene-acetic acid (9:1) solution; this slurry was filtered, and the procedure was repeated three times. The filtrates were combined, evaporated, and dissolved in benzene, from which ochratoxin A was crystallized. Crystalline ochratoxin A was dissolved in ethanol and mixed with a small portion of the diet, which was then dried and mixed with the remaining portion of the diet.

Hematology. When the birds reached 3 weeks of age, blood samples were collected from the brachial vein for hemoglobin determinations, packed cell volume analysis, and erythrocyte counts. Hemoglobin was determined by the method of Sunderman et al. (24), and packed cell volume was determined with a micro-hematocrit tube treated with heparin to prevent clotting. Erythrocyte counts of blood combined with

0.18 M sodium citrate (9:1) as the anticoagulant and pooled on a group basis were performed by the method of Natt and Herrick (19). From these values, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Blood samples from individual birds were allowed to clot, and the sera were collected and pooled on a group basis. Serum iron and total iron-binding capacity (TIBC) were measured by an independent laboratory (Rex Hospital, Raleigh, N.C.) by the DuPont automatic clinical analysis methodology.

Statistical analyses. The data obtained were subjected to an analysis of variance in which an *F*-ratio was calculated. If the *F*-ratio were significant ($P < 0.05$), the least significant difference among treatment means was calculated (1).

RESULTS

The effect of graded levels of dietary ochratoxin A on total erythrocyte count, packed cell volume, and hemoglobin concentration is shown in Table 1. The number of circulating erythrocytes was not altered significantly at any concentration, whereas the packed cell volume and hemoglobin concentration were decreased significantly ($P < 0.05$) at the highest concentration of ochratoxin used (8.0 μg/g). It should be noted that in this experimental system ochratoxin A at a level of 2.0 but not 1.0 μg/g inhibits growth (15). From these experimental values, the MCV and MCHC of the erythrocytes were calculated (Table 1). As expected, the values from birds fed 8 μg/g differed significantly ($P < 0.05$) from the control values, with the MCV and MCHC being decreased 25 and 19%, respectively. This means that the anemia in birds fed a high level of ochratoxin A is characterized by erythrocytes smaller than normal with a lower concentration of hemoglobin than normal.

To gain further insight into the anemia of ochratoxicosis, the iron content of serum and the TIBC of serum were determined (Table 2). Serum iron was significantly ($P < 0.05$) decreased at both 4.0 and 8.0 μg/g; however, TIBC, which is an indirect measure of serum transferrin content (34), was not significantly altered. From these experimental values was calculated (34) the percentage of transferrin saturation with iron (Table 2). Transferrin is the serum protein that binds and transports iron from entry and depot sites to utilization sites. These calculations showed that normal percentage saturation of transferrin was reduced by about one-half during the anemia caused by ochratoxin A (8 μg/g).

DISCUSSION

The anemia observed in broiler chickens during ochratoxicosis is characterized by a decrease in MCV and MCHC while erythrocyte counts remain constant (Table 1). Therefore, the anemia seen during ochratoxicosis is categorized as a type of hypochromic-microcytic anemia. Hypochromic-microcytic anemias are divided into three major groups: iron deficiency anemia, thalassemia, and sideroblastic anemia (34). These three groups of hypochromic-microcytic anemias can be distinguished by their differential effect on serum iron. Serum iron levels are normal to slightly elevated during thalassemia, which is a hereditary defect in peptide synthesis of hemoglobin, elevated during sideroblastic anemia, which is a hereditary defect in heme synthesis, and depressed during iron deficiency anemia, which is a nutritional disease (34). Ochratoxin A in the present experiments decreased serum iron and transferrin saturation (Table 2). Thus, ochratoxin A produces iron deficiency anemia, the most common type of hypochromic-microcytic anemia. The possibility

TABLE 2. *Effect of ochratoxin A on serum iron, TIBC, and transferrin saturation*

Ochratoxin A (μg/g)	Serum iron (μg/100 ml)	TIBC (μg/100 ml)	Transferrin saturation (%)
0.0	109 ± 5[a]	145 ± 2[a]	75.1 ± 2.5[a]
4.0	71 ± 2[b]	144 ± 6[a]	49.5 ± 3.0[b]
8.0	45 ± 3[b]	136 ± 11[a]	34.3 ± 5.6[b]

[a, b] The tabular values represent the mean of 4 groups of 10 birds with the standard error of the mean. The values in a column with different superscripts differ significantly ($P < 0.05$).

TABLE 1. *Effect of ochratoxin on parameters associated with anemia*

Ochratoxin (μg/g)	Total erythrocyte counts ($\times 10^5$/mm^3)	Packed cell vol (%)	Hemoglobin concn (g/100 ml)	MCV (μm^3)	MCHC (%)
0.0	29.6 ± 0.4	32.6 ± 0.5	9.7 + 0.2	110 ± 2	33.0 ± 1.3
0.5	28.1 ± 1.1	33.0 ± 0.4	10.7 + 0.3	118 ± 4	36.5 ± 2.1
1.0	29.8 ± 0.2	33.2 ± 0.6	9.6 ± 0.1	111 ± 1	32.0 ± 0.5
2.0	28.6 ± 1.0	32.7 ± 0.4	9.8 ± 0.1	115 ± 5	34.5 ± 1.3
4.0	28.5 ± 0.8	32.1 ± 1.1	9.3 ± 0.1	113 ± 2	32.5 ± 1.0
8.0	28.7 ± 1.2	25.8 ± 1.2[a]	7.0 ± 0.4[a]	89 ± 3[a]	24.5 ± 1.0[a]

[a] Values that differ significantly ($P < 0.05$) from the corresponding control values. All tabular values represent the mean of 4 groups of 10 birds with the standard error of the mean.

that the growth-inhibitory effect of ochratoxin A is a consequence of lowering the serum iron seems unlikely because iron deficiency anemia has been reported to occur independently of growth in chickens (12). The mechanism by which ochratoxin A induces iron deficiency anemia has not been determined, but the simplest explanation would appear to be an inhibition of iron absorption from the gastrointestinal tract. Malabsorption of dietary carotenoids during ochratoxicosis has been reported earlier (13; D. J. Osborne, W. E. Huff, and P. B. Hamilton, Poultry Sci. **55**:2075, 1976) and thus offers precedent. The production of iron deficiency anemia by ochratoxin A represents yet another way in which mycotoxins can be a health hazard. The anemia induced by ochratoxin also emphasizes the concern with which the nutritional aspects of mycotoxin toxicity should be regarded, particularly in populations of animals and humans that might be existing largely on diets that are nutritionally inadequate or marginally deficient for adequate nutrition (10).

It should be noted that the anemia observed in the present experiments in chickens does not agree with the report of elevated hemoglobin concentration and packed cell volume during ochratoxicosis in beagle dogs (26) and swine (27) and thus raises the question of whether the present data can be extrapolated correctly to mammals. Aside from possible species and toxin concentration differences, the observations on dogs and swine were made during an acute toxicosis and not during the more chronic toxicity studied here in young chicks. Szczech and coworkers (26, 27) attributed their observations in beagle dogs and swine to a hemoconcentration resulting from extensive diarrhea, which was not a symptom in our experimental chickens. The reported observation of suppressed hematopoiesis in newly hatched chicks (7, 20) during acute ochratoxicosis may be simply an acute transitory reaction of the neonatal chick and not comparable to the experimental system we used.

Hemorrhagic anemia syndrome of poultry, which was characterized by spontaneous hemorrhaging and aplastic anemia (11) and was provoked by feedstuffs infested with mycotoxigenic fungi (9), still has not been associated with a specific mycotoxin. Aflatoxin produced prolonged blood clotting times (6) and a hemolytic anemia (29). Rubratoxin, even at 1,000 μg/g, produced a barely significant anemia and impairment of capillary fragility without spontaneous hemorrhaging (35). T-2 toxin also did not cause spontaneous hemorrhaging, although it did prolong plasma recalcification times (5). From the present results, ochratoxin A, which appeared to be a good candidate based on reports of suppression of hematopoiesis (7, 20) and on prolonged prothrombin times (5), has been demonstrated to cause instead a hypochromic-microcytic anemia typical of a nutritional iron deficiency. These four toxins are toxic principles of fungi reported to cause the hemorrhagic anemia syndrome (9), yet none cause the characteristic aplastic anemia and spontaneous hemorrhaging. This suggests that other, as yet unisolated toxins are produced by these fungi or that several toxins act in concert to produce the syndrome. There is precedent for both possibilities, since *A. flavus* produces toxins other than aflatoxin (33) and the interactions between mycotoxins are extensive enough to warrant a review (17). The historical and economic implications of hemorrhagic anemia syndrome would appear to warrant establishing an experimental model and systematically determining the cause.

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