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Effect of Ochratoxin and Aflatoxin on Serum Proteins, Complement Activity, and Antibody Production to *Brucella abortus* in Guinea Pigs

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The effect of ochratoxin alone and in combination with aflatoxin and *Brucella abortus* antigen on complement activity, serum proteins, and antibody response in guinea pigs was investigated. Ochratoxin did not affect complement activity or antibody response and there was no interaction between ochratoxin and aflatoxin on any of the responses tested. Ochratoxin significantly lowered the level of β-globulin in serum of guinea pigs. There was no significant interaction between aflatoxin and antigen on lowering of the serum albumin levels of guinea pigs.

Among the varied effects of aflatoxins and rubratoxin on animal species is that they have been demonstrated to depress complement activity in guinea pigs (14, 19). Aflatoxin did not affect the antibody response of guinea pigs to *Brucella abortus* antigen, although complement activity was depressed (20). Electrophoretic analysis of guinea pig serum indicated that those given aflatoxins possessed elevated levels of gamma-globulin, depressed levels of α_2-globulin, and frequent depression of total protein levels (20).

Some effects of aflatoxins are potentiated by simultaneous feeding of rubratoxin B (3, 14). Since it is possible that other mycotoxins may interact with aflatoxin, we investigated the effect of ochratoxin alone and in combination with aflatoxin and *B. abortus* antigen on complement activity, serum proteins, and antibody response.

MATERIALS AND METHODS

Guinea pigs. Female guinea pigs weighing between 475 to 525 g were randomly distributed into eight groups of six animals each. They were fed a commercially prepared diet and water ad libitum throughout the experiment. Chloroform extracts of a 200-g sample of the guinea pig feed were assayed for aflatoxin and ochratoxin by thin-layer chromatography.

Toxins. Partially purified aflatoxin (PPA), produced by using *Aspergillus flavus* NRRL 2999, was taken from the same lot used in previous studies (12, 14, 19, 20). The PPA was 34.8% B_1, 1.9% B_2, 23.8% G_1, and 1.1% G_2. For purposes of this study, aflatoxin G_1 was considered one-half as toxic as B_1 and aflatoxin B_2 and G_2 were negated in computing toxic equivalents of aflatoxin B_1. Therefore, all doses are expressed in milligrams of toxic equivalents of aflatoxin B_1.

The ochratoxin was produced in shaker flasks of moistened, cracked wheat (15), inoculated with spores of *Aspergillus ochraceus* NRRL 3174. After incubating for 7 days at 28 C, the molded wheat was extracted with chloroform and filtered; the filtrate was mixed with hexane to precipitate the ochratoxins. The hexane precipitate was redissolved in chloroform and extracted with 0.5 M aqueous sodium bicarbonate (7). After the sodium bicarbonate was acidified, the solution was added to hexane; the solution was held for 24 h at 5 C and a crystalline fraction was recovered by filtration. Fluorescent determination by thin-layer chromatography (16) showed that the crystalline material contained ochratoxin A, 89%, and ochratoxin B, 5%.

Antigen. The antigen used was *B. abortus* strain 1119-3 antigen consisting of 10^{11} heat-killed, whole cells suspended in saline solution.

Administration of toxins to guinea pigs. Daily doses for each guinea pig given aflatoxin was 0.01 mg of toxin equivalents B_1 per day. To prepare doses, the desired quantity of PPA was dissolved in chloroform and the appropriate amount of solution was placed in no. 5 gelatin capsules (Eli Lilly and Co., Chicago). After allowing 4 h for the chloroform to evaporate, the capsules were filled with crystalline lactose to enhance taste, and stored in the dark at 4 C. The daily oral dose for each guinea pig given ochratoxin was 0.45 mg. This dose level produced occasional deaths in a 4-week period in preliminary studies. Doses were prepared with ochratoxin using the same solvent and methods as for PPA. Sufficient doses were prepared to provide daily oral doses for a 4-week period. Administration of doses was accomplished by placing the capsules in the mouths of each guinea pig. Care was taken to insure that each dose had been swallowed before returning the guinea pigs to their respective cages. The treatment groups are shown in Table 1.

The control group was given lactose only. Those animals in groups given *B. abortus* antigen were given 0.5 ml injected subcutaneously on the first day of each week beginning 1 week after toxin administration commenced and continuing for two more injections. Blood was taken from all animals at the termination of the experiment.

Serological determinations. Complement determinations (19) were conducted on serum from blood samples obtained from each guinea pig at the conclusion of the experiment.

Total serum protein was determined by the biuret method (5) and differential serum proteins were determined by microzonal electrophoresis conducted in accordance with the manufacturer's procedures (model R101, Microzonal Electrophoresis Instruments, Fullerton, Calif.).

Serological procedures for *B. abortus* were conducted as described (20).

RESULTS AND DISCUSSION

Observations of the mean values indicated an increase in all serum proteins except albumin in the guinea pigs given antigen when compared to the control group (Table 1). The group given ochratoxin possessed lower levels of all proteins than the control group. The group given aflatoxin had an increase in albumin and gamma-globulin and a decrease in α_1, α_2- and β-globulin when compared to the control group (Table 1).

Unlike rubratoxin, aflatoxin, and other hepatotoxic substances from previous studies (11, 14, 19), ochratoxin given to guinea pigs at the rate of 0.45 mg per day did not affect complement activity, nor did it affect antibody response to *B. abortus* antigen, and there was no interaction on any of the responses tested in an analysis of variance test. Perhaps ochratoxin is primarily a nephrotoxin in the guinea pig and has little effect on the liver as has been the case with ochratoxicosis in beagle dogs (17, 18). Ochratoxin significantly lowered the level of β-globulin in serum of the guinea pigs, whereas the remainder of the serum proteins were not significantly changed (Table 2). In a search of the literature, the effects of ochratoxin on serum proteins in animals was not found.

Suppression of antibody formation by aflatoxin has been described in mice given typhoid vaccine (4) and in chickens given injections of sheep erythrocytes (P. Thaxton and P. B. Hamilton, Poult. Sci. **50:** 1936, 1971). In the study reported here, there was no effect of either aflatoxin or ochratoxin (or both) on antibody formation by guinea pigs to *B. abortus* antigen. Although aflatoxin has impaired resistance to *Pasteurella multocida* infection in turkeys vaccinated against fowl cholera, it was apparently not associated with agglutinating antibody since the impairment in resistance could be overcome by giving injections to vaccinated turkeys of either normal or immune serum before challenge inoculation (9, 10). Also, there were no apparent differences in agglutinin titers between vaccinated birds on aflatoxin and vaccinated birds on normal diet.

Although aflatoxin significantly decreased total serum protein and complement activity there was no significant increase in gamma-globulin levels in guinea pigs and no increase in agglutinating antibody to *B. abortus* antigen (Table 2). Other studies have shown an increase in gamma-globulin levels in guinea pigs (14, 20), but no increase in agglutinating antibody to

TABLE 1. *Mean values of serum proteins and complement titers of guinea pigs given aflatoxin, ochratoxin and Brucella abortus antigen alone or in combination*

Treatment	Total serum protein (g/100)	Electrophoresis (serum proteins g/100 ml)					Complement titer ($\log CH_{50}$[a])
		Albumin	α_1-globulin	α_2-globulin	β-globulin	Gamma-globulin	
Control	5.33	2.960	0.398	1.041	0.444	0.490	2.31
Aflatoxin	4.80	3.054	0.189	0.756	0.276	0.524	2.12
Ochratoxin	5.00	2.898	0.281	0.986	0.360	0.475	2.34
Antigen	5.49	2.706	0.412	1.107	0.473	0.803	2.44
Ochratoxin + aflatoxin	4.69	3.062	0.189	0.730	0.195	0.525	2.08
Aflatoxin + antigen	4.87	2.464	0.304	0.858	0.343	0.911	2.26
Ochratoxin + antigen	5.60	2.865	0.429	1.101	0.478	0.727	2.44
Aflatoxin + ochratoxin + antigen	4.75	2.685	0.292	0.790	0.275	0.719	2.25

[a] Log CH_{50}, Log of the complement titer utilizing 50% hemolysis end point.

TABLE 2. *Mean differences and results of analysis of variance*

Source	Total serum protein	Electrophoresis (serum proteins g/100 ml)					Complement titer
		Albumin	α_1-globulin	α_2-globulin	β-globulin	Gamma-globulin	
Aflatoxin	−0.58[a]	−0.019	−0.142[a]	−0.279[a]	−0.170[a]	+0.033	−0.214[a]
Ochratoxin	−0.11	+0.075	−0.029	−0.038	−0.056[b]	−0.066	−0.003
Antigen	+0.22[b]	−0.304[a]	+0.100[a]	+0.098[a]	+0.081[a]	+0.284[a]	+0.142[a]

[a] Significant effect $P < 0.01$.
[b] Significant effect $P < 0.05$.

B. abortus antigen (20). These differences may be due to differences in dosage levels or regimens used in the studies. In swine given aflatoxin (1) and in animals given other hepatotoxic substances or with chronic liver disease (2, 6), there was an increase in gamma-globulin levels. In studies of *A. fumigatus* infection and aflatoxicosis in turkey poults, the birds on aflatoxin had significantly greater levels of serum gamma-globulin than did the birds on normal diet (13). In the latter study, only the birds on aflatoxin diet possessed precipitating antibody to *A. fumigatus* antigen. The rapidity of transport of aflatoxin into liver cells and the rate of metabolism of aflatoxin varies between species (8). Therefore, the differences observed among these studies could reflect differences between animal species or perhaps differences in kinds of antibodies affected by aflatoxin.

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