NOTES

Interaction Between Aflatoxicosis and a Natural Infection of Chickens with *Salmonella*

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Broiler chicks with a natural congenital infection of *Salmonella worthington* required a lower concentration of dietary aflatoxin (0.625 μg/g) to depress growth than uninfected chicks (2.50 μg/g).

The possible interaction of aflatoxicosis with infectious diseases has been a question since the discovery of aflatoxin. One of the first reports of aflatoxicosis (14) described the isolation of *Salmonella* from the internal organs of turkeys that had the disease. Brown and Abrams (4) consistently isolated *Salmonella* from ducklings and chickens with typical aflatoxicosis. They also discovered a hypoproteinemia, which included the globulin fractions, and proposed that birds were more susceptible to *Salmonella* during aflatoxicosis. Abrams (1) extended this hypothesis to other bacterial and viral diseases.

Experiments designed to prove this hypothesis have yielded contradictory results. Aflatoxicosis and infection with *Salmonella gallinarum* exerted their effects on body weight and mortality independently and without interaction (16); however, an interaction on body weights and crop weights was found in chickens consuming aflatoxin and infected with *Candida albicans* (6). Pier et al. (10) reported that aflatoxin did not impair acquired resistance to Newcastle Disease virus, and Adinarayanaiah et al. (2) reported that aflatoxin had no effect on antibody formation against *S. pullorum* antigen. Richard et al. (12) found no interaction of aflatoxin and *Aspergillus fumigatus* on mortality, histopathologic lesions, or growth rate in turkey poults despite the two factors together causing the formation of precipitating antibodies against *A. fumigatus*. In an interesting study Pier and Heddleston (9) found that aflatoxin consumed by turkey poults and young chickens during or after immunization with *Pasteurella multocida* interfered with the development of resistance to a lethal challenge with the organism. These contradictory results are even more inexplicable in view of the findings that dietary aflatoxin can cause a dose-related inhibition of the reticuloendothelial system (7) and immunosuppression in chickens (17). Thus, natural aflatoxicosis has been associated with the occurrence of *Salmonella* in birds, whereas laboratory experiments have generally failed to find interactions between aflatoxicosis and infectious agents. One possible explanation for these contradictory results is that the conditions used in the laboratory experiments were too far removed from field conditions. We want to report a laboratory experiment arising from a field case which demonstrates an interaction between aflatoxicosis and salmonellosis.

The opportunity to investigate possible interactions arose when an unusual field case was referred to us. It was characterized by a slightly increased mortality rate and greatly depressed growth rate in certain flocks of young broiler chickens. Analysis (11) of feed (broiler starter mash) samples from the affected houses revealed aflatoxin B1, in quantities ranging from trace amounts to 0.600 μg/g. These observations suggested aflatoxicosis, since these symptoms are associated with the disease (15). Further inquiry revealed that the affected birds came from a single flock of breeder hens. Cloacal swabs from 15 affected birds selected at random were incubated in tetraionate broth (BBL) for 24 h at 37 C. Then a loopful of the broth was streaked on brilliant green agar (BBL), which was observed for typical *Salmonella* colonies after incubation. The affected chicks were uniformly infected with *S. worthington* (identified by the Center for Disease Control, Atlanta, Ga.). The affected birds also had a slight diarrhea and dehydration, which are the primary symptoms usually associated with uncomplicated paratyphoid infections in chickens (3). Before the breeder flock was discarded, we were able to acquire some chicks
from it and from an uninfected flock of the same age and genetic stock (Indian River × Indian River) on a nearby farm.

The infected and uninfected chicks were brought to the laboratory and divided into groups of 10. They were housed in electrically heated batteries with feed and water available ad libitum. The feed was a commercial broiler starter ration to which graded amounts of aflatoxin (0, 0.625, 1.25, 2.5, 5.0, and 10.0 μg/g of feed) were added. The aflatoxin was produced by growing Aspergillus parasiticus NRRL 2999 on rice by the method of Shotwell et al. (13). The moldy rice was steamed, dried, and ground to a fine powder, which was analyzed for total aflatoxin content by the spectrophotometric method of Nabney and Nesbitt (8) with the modification of Wiseman et al. (19). The percentages of aflatoxin B1, B2, G1, and G2 (70, 9, 16, and 5%, respectively) were determined spectrophotometrically (8) after separation on thin-layer chromatograms (11). There were four groups of 10 infected birds and four groups of 10 uninfected birds per aflatoxin level, and the experimental design was completely randomized. The birds were fed for 3 weeks and their body weights were determined. The data were submitted to an analysis of variance, and the least significant difference between treatment means was determined (5).

The statistical analyses showed that both aflatoxin and a natural infection with S. worthington had a significant (P < 0.05) effect on body weight and that there was a significant interaction between them (Table 1). The interaction was most clearly revealed in the threshold dose of aflatoxin required to exert an effect on body weight. In the uninfected birds 2.5 μg/g or greater was required to depress growth rate. (This same threshold dose in uncompromised broiler chicks has been obtained without exception in over 100 different experiments in our laboratory.) In infected birds even the smallest dose (0.625 μg/g) caused a significant inhibition of growth. The mortality records revealed that there was no similar interaction on mortality.

The observations in this experiment and field case indicate that there can be an interaction between aflatoxicosis and an infection with S. worthington on body weight. In addition, we observed a similar interaction in two other instances in which the commercially obtained chicks were not known to be infected with Salmonella until the laboratory experiments were ended; unfortunately, the epidemiological efforts in the field failed because the feed had been consumed and because the placement of the chicks could not be accurately traced. These observations suggest that the failure of many investigators to find an interaction between aflatoxicosis and infectious diseases may be the result of their failure to create the necessary but as yet unknown conditions in the laboratory. These observations also suggest that interactions exist under field conditions and that the symptoms observed may not necessarily be those expected. This consideration is reinforced by reports that birds with aflatoxicosis (6) and salmonellosis (18) are more susceptible to below normal temperatures, such as frequently occur under field conditions. Together, the interactions make for a difficult epidemiology and point out some of the insidious aspects of mycotoxicoses confronting the animal industries.

| Aflatoxin (μg/g) | Body wt (g) | Control | Infected |
|------------------|-------------|---------|----------|
| 0                | 504         | 310     |          |
| 0.625            | 492         | 259     |          |
| 1.25             | 526         | 284     |          |
| 2.50             | 460         | 252     |          |
| 5.00             | 348         | 228     |          |
| 10.0             | 269         | 207     |          |

* Each value is the mean body weight of four groups of 10 birds at 3 weeks of age.
* These values differ significantly (P < 0.05) from the corresponding zero aflatoxin value.

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