Correlation of Aflatoxin Contamination With Zinc Content of Chicken Feed†

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Feed samples from chicken houses in five commercial chicken operations were analyzed for Zn, Mn, Fe, Cu, Cd, and aflatoxin content. Mean aflatoxin content of these samples was 14 ppb (14 ng/g) as opposed to 1.2 ppb in samples taken when the feed was made. Aflatoxin content of the feed samples correlated ($r = 0.325$) significantly ($P < 0.05$) with Zn content but not with Mn, Fe, or Cu, all of which correlated significantly with Zn. Zn content of unamended feed (<20 ppm [$20 \mu\text{g/g}$]) is normally supplemented with a mineral premix containing Zn, Mn, Fe, and Cu to meet the nutrient requirements of chickens (40 ppm of Zn). The mean zinc concentration of the feed samples (117 ppm) was about threefold greater than the nutrient requirement and ranged from 58 to 162 ppm in individual samples. These field survey results parallel earlier reports of augmented production of aflatoxin in monocultures of aflatoxigenic fungi in corn and other ingredients supplemented with Zn. These results suggest that stricter control of Zn levels during manufacture could reduce aflatoxin contamination of feed consumed by chickens.

Knowledge that zinc has a striking effect on aflatoxin production resulted from in vitro studies showing that zinc stimulated aflatoxin production in liquid media (1, 14). Deletion of molybdenum, boron, copper, iron, manganese, and zinc one at a time and in combinations led Mateles and Adye (12) to conclude that zinc was required for the production of aflatoxin. Lee et al. (8) reported that zinc was specifically required for the production of aflatoxins by *Aspergillus flavus*, but Davis et al. (5) concluded that the influence of zinc was indirect, perhaps through its essentiality for growth. Marsh et al. (11) in a more definitive study found that adding low levels of zinc salts to cultures of *Aspergillus parasiticus* in a defined basal medium increased aflatoxin up to 1,000-fold while increasing mycelial weight less than 3-fold. The levels of zinc reported for optimal production of aflatoxin have ranged from 0.4 μg (12) to 50 μg (10) per ml of medium. This wide divergence is thought to be the result of strain differences (10, 15), of various endogenous levels of zinc in the basal media (10, 15), and of the low levels of aflatoxin produced in chemically defined media (5, 18).

The possibility that zinc might be a controlling factor for aflatoxin production in natural materials such as grains was implicit in the proposal of Schroeder (19) that the enhanced production of aflatoxin in media enriched with corn steep liquor was the result of zinc in the corn extract. In a direct test of this hypothesis, Lillehoj et al. (9) found no significant difference in the zinc content of corn kernels infected with or free from *A. flavus*; however, the very high levels of zinc in corn germ (208 $\mu\text{g/g}$) apparently were not readily available because the addition of zinc ions significantly increased the aflatoxin yield in a dose-related fashion. These authors concluded that it would be difficult to establish a clear cause-effect relationship between zinc and aflatoxin in corn because zinc is largely present as the phytate, which is not readily available biologically (16). Bassir and Adekunle (3) surveyed 13 tropical foodstuffs for their ability to support aflatoxin production and found zinc in quantities thought not be limiting. Another study of 10 tropical foodstuffs (15)

designed to explain resistance or susceptibility of commodities to aflatoxin production concluded that aflatoxin production was not correlated linearly with the zinc concentrations of the foodstuffs and that the optimal zinc requirement for maximal aflatoxin production was substrate specific.

The foregoing studies, which failed to provide a clear cause-effect relation between zinc content and aflatoxin production in natural products, utilized fungal monocultures on materials which had been sterilized and amended with culture media and ingredients before inoculation and incubation under laboratory conditions. A recent study (7) which associated productivity losses in commercial broiler chicken operations with low levels of aflatoxin produced in feed during and after its manufacture suggested that the relationship of zinc to aflatoxin production could be tested more realistically with unaltered natural products. This study reports on the correlation between aflatoxin production and zinc content of samples of chicken feed taken from feed containers at chicken farms.

MATERIALS AND METHODS

Sample collection. Five independent broiler chicken companies located in North Carolina participated in this study. Six growers who received their 1-day-old birds within 1 week of each other were chosen from each company. The growers were classified as good, mediocre, or poor with the aid of an arbitrary productivity index (7), and two growers from each company were selected in each classification.

Feed samples were collected weekly from each feed mill and each grower during the grow-out period of a flock (ca. 7 weeks). A portable vacuum sampler (model no. 9320/8970; Black and Decker Manufacturing Co., Towson, Md.) was used to collect ca. 75 g of feed from alternate feeder pans in the chicken house or from about every 9 m in trough-type feeders. The portions from within a house at each sampling time were combined and mixed by pouring them on a sheet of clean plastic and rolling the sample by lifting alternate corners of the sheet.

Measurements. Aflatoxin content of the feed was measured by method 26.031 of the Association of Official Analytical Chemists (6). Zinc, manganese, iron, copper, and cadmi-

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um content in the feed was measured by atomic absorption spectrophotometry on 2.5-g samples of feed ashed at 500°C for 8 h, dissolved in concentrated HCl, and diluted to 0.5 N HCl before analysis.

Statistical analyses. Data were evaluated by the multiple regression procedures of Barr et al. (2). Normality of the distribution of the aflatoxin and zinc concentrations was tested by calculating the *D* statistic of Kolmogorov when $n \geq 50$ and the *W* statistic of Shapiro and Wilks (17) when $n < 50$. A normal distribution of the aflatoxin concentration was obtained by log transformation. The association between zinc and aflatoxin in the untransformed data set was also tested by a chi-square frequency analysis (21).

RESULTS

The mean level of aflatoxin in the feed as it left the mills where it was manufactured was 1.2 ppb (1.2 ng/g), and the mean aflatoxin concentration of samples taken from the farms was 14 ppb. Regression analysis of the relationship between aflatoxin concentration and zinc concentration of feed samples containing aflatoxin ($n = 51$; of the 222 samples taken from chicken houses, 171 were negative for aflatoxin) yielded a significant positive correlation ($r = 0.325$; $P < 0.05$) between the two variables (Fig. 1). The aflatoxin concentrations in the 51 positive samples were not normally distributed, according to a Kolmogorov *D* statistic. A normal distribution could be restored (W [normal] = 0.9625; $P < W = 0.435$) by eliminating the 21 points below 10 ppb, which represented trace but detectable amounts of aflatoxin (thus, not accounting for any variation in zinc), and doing a log transformation of the remaining 30 points. Analysis of the transformed data revealed that $r = 0.559$ with $P = 0.0013$ for the concordance between aflatoxin and zinc. Chi-square frequency analysis of the original data segregated into high, medium, and low concentration classes of aflatoxin and zinc gave $\chi^2 = 13.72$ with $P = 0.033$.

It should be pointed out that the aflatoxin concentrations found were generally low, with the highest concentration

FIG. 1. Regression of aflatoxin concentration on zinc concentration of feed samples contaminated with aflatoxin.

TABLE 1. Correlation coefficients between contents of aflatoxin and selected metals in chicken feed

Sample content	Correlation coefficient with:				
	Mn	Cu	Zn	Fe	Aflatoxin
Mn	1.000	0.501 ^a	0.683 ^a	0.382 ^a	0.215
Cu		1.000	0.588 ^a	0.214	0.194
Zn			1.000	0.399 ^a	0.325 ^b
Fe				1.000	-0.197
Aflatoxin					1.000

^a Significant at $P < 0.01$.

^b Significant at $P < 0.05$.

being 192 ng/g. The concentrations of zinc associated with feed samples which were positive for aflatoxin ranged from 72 to 162 $\mu\text{g/g}$. These zinc concentrations are markedly higher than the 22 $\mu\text{g/g}$ reported in whole corn (9) and the range of 9 to 37 $\mu\text{g/g}$ found in 10 tropical foodstuffs (15).

A correlation matrix derived from multiple regression analyses for the associations between aflatoxin, zinc, manganese, iron, and copper revealed (Table 1) that only zinc was significantly ($P < 0.05$) correlated with aflatoxin. On the other hand, zinc was significantly correlated with manganese, copper, and iron. Correlation analysis with the log-transformed aflatoxin concentrations also failed to show a significant correlation of aflatoxin with iron, copper, or manganese. Cadmium was not found in any sample within the detection limit of 0.2 ppm (0.2 $\mu\text{g/g}$). The mean Zn concentration for all 222 feed samples was 117 ppm, and the range was 54 to 189 ppm.

DISCUSSION

The significant ($P < 0.05$) correlation ($r = 0.325$) between the concentrations of zinc and aflatoxin in samples of chicken feed contaminated with aflatoxin (Fig. 1) suggests a possible cause-effect relationship in which aflatoxin production is limited by the zinc concentration in feed or by factor(s) which control the zinc concentration in feed. This hypothesis agrees with the thrust of previous investigations into the relationship of aflatoxin production to minerals (9, 11, 15).

The sources of zinc in the feed samples analyzed in this study could be (i) the zinc indigenous to the ingredients, (ii) the zinc added to the diet in a mineral premix to meet the nutrient requirements of broiler chickens, and (iii) the zinc imparted to the feed as a result of its flow across the surfaces of feed-handling equipment composed of galvanized (zinc-coated) steel. The zinc content of ingredients (13) comprising the bulk of common chicken diets (e.g., corn has ca. 10 ppm, and soybean meal has ca. 45 ppm) is much lower than the levels found in feed samples analyzed in this study ($\bar{x} = 117$ ppm; range, 54 to 189 ppm). Indeed, the levels indigenous to ingredients are generally lower than the nutrient requirement of chickens which is 40 ppm (13, 20). To meet the nutrient requirements of chickens for zinc, it is recommended that a minimum of 35 ppm be added to practical diets as zinc sulfate (22). Obviously, the source of the high levels of zinc which are correlated with aflatoxin production is the mineral premix added deliberately to the diet or the galvanized coating of the feed-handling equipment, which would be an inadvertent source of zinc. The galvanized coating does not seem to be a likely source because it also contains cadmium (Prime Western Grade galvanized coating contains the following: Zn, >98%; Pb, <1.6%; Cd, <0.5%; and Fe, <0.05% [4]), and cadmium was not found in any feed sample (detec-

tion limit, 0.2 ppm). The mineral premix was confirmed as the source of the zinc associated with aflatoxin production by the correlation of Fe, Mn, and Cu with Zn but not with aflatoxin (Table 1). The naturally occurring concentrations of Fe, Mn, and Cu in feed ingredients limit chicken growth (13, 20), and like Zn, these metals are added commonly to chicken diets in the mineral premix which contains Zn.

If it is assumed that the high levels of zinc correlating with aflatoxin arise in the mineral premix and that the site of toxin formation is in the corn or soybean components, then the situation observed in chicken feed parallels the previously reported augmentation of aflatoxin production when monocultures of aflatoxigenic fungi on corn, beans, and other ingredients were supplemented with zinc (9, 15). This parallel suggests that aflatoxin formation in feed can be partially controlled by limiting the addition of Zn to the diets. The mean concentration of Zn in feed was 117 ppm, which is about threefold greater than the minimal nutritional requirement (1, 3, 20) and about twofold greater than the level recommended in practical diets to provide a margin of safety (22). The intercept of the regression line with the x axis at 55 ppb of Zn (Fig. 1) then provides a target concentration of zinc which should not be greatly exceeded if aflatoxin production is to be limited. The range of concentration of zinc found in feed in this study varied from 54 to 189 ppm, which suggests a serious problem with quality control as well as with overformulation. The quality control failure would appear to be the inconsistent addition of premix, the inconsistent mixing of the feed, or both. Stricter control of the feed manufacturing process would give more uniform feed better suited to the nutritional needs of poultry as well as possibly lessening the aflatoxin contamination of feed.

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