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Mortality in broiler chicks on feed amended with *Fusarium proliferatum* culture material or with purified fumonisin B_1 and moniliformin

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Abstract. Two hundred twenty-eight male chicks (Columbia × New Hampshire) were given feed amended with autoclaved culture material (CM) of *Fusarium proliferatum* Containing fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and moniliformin in 3 separate feeding trials. Purified FB_1 and moniliformin were given separately and in combination in a fourth feeding trial. Birds were given amended rations at day 1 (Trial 1 and 4), day 7 (Trial 2), and day 21 (Trial 3) and their respective ration was given for 28 days (Trial 1), 21 days (Trial 2), 7 days (Trial 3), and 14 days (Trial 4). FB_1 concentrations were 546, 193, and 61 ppm; FB_2 were 98, 38 and 14 ppm; and moniliformin were 367, 193, and 66 ppm in the first 3 feeding trial regimens. Chicks in Trial 4 were given dietary concentrations of purified FB_1 at 274 and 125 ppm, and moniliformin at 154 and 27 ppm. FB_1 and moniliformin, both alone and in combination, produced dose-responsive clinical signs, reduced weight gains and mortality in chicks. Age of birds given amended feeds had little difference in the clinical response; however, those given the rations from days 7 or 21 were slightly less susceptible than those given rations beginning at 1 day of age. Additive effects were noted when the toxins were given in combination. When toxins were given separately, adverse effects took longer to occur. A system to monitor pattern and rate of defecation (RD) was developed for assessing the chicks' approach to feed, water and heat source as illness progressed. Our results indicate that chicks fed corn heavily infected with *F. proliferatum* under field conditions could suffer acute death similar to that described for 'spiking mortality syndrome' during the first 3 weeks of age.

Key words: Chicken, Fumonisin, *Fusarium moniliforme*, *Fusarium proliferatum*, Moniliformin, Mycotoxin

Introduction

Incidents of mycotoxins in feed ingredients reported over the past 25 years have led to an increased awareness of potential mycotoxicoses in poultry. The worldwide prevalence of fumonisins in corn mandates that research be conducted to determine their acute and chronic effects in poultry. The potential for decreased productivity in the broiler industry due to mycotoxicoses and concomitant secondary infections resulting from mycotoxin-induced immunosuppression underscore the relevance of the research described herein.

Fumonisins B_1 and B_2 are members of a recently discovered class of mycotoxins produced by *Fusarium moniliforme* [1–3], and closely related species, such as *Fusarium proliferatum* [4–6]. *F. moniliforme* is one of the most prevalent fungal contaminants of corn worldwide [5]; how-

ever, the misidentification of *F. proliferatum* as *F. moniliforme* has exaggerated the reported prevalence of the latter species [5, 6]. Both of these species have been shown to produced fumonisins; however, *F. proliferatum* also produces moniliformin, while many *F. moniliforme* do not [5]. Fumonisin contamination of corn is common, although concentrations vary greatly with geographic location and climatic conditions [7]. The high incidence of fumonisin-associated diseases in livestock indicates that the 1989 US corn crop was heavily contaminated [8, 9]. High concentrations of fumonisins were generally reported in corn screenings; however, the fungus is frequently found in apparently healthy kernels [10].

Fumonisin B_1 (FB_1) has been implicated as a cause of equine leukoencephalomalacia (ELEM), an acutely fatal neurological disease of horses and donkeys [9, 11, 12]. The toxin has also been associated with a fatal disease in swine characterized by pulmonary edema and pancreatic and liver lesions [7, 13, 14]. Most of these studies were conducted with corn screenings that had been analyzed for FB_1, but not moniliformin. If the fungus was misidentified as *F. moniliforme*, but was actually *F. proliferatum*, moniliformin and other toxic metabolites could have been present.

FB_1 has caused liver tumors in rats [15, 16]. Epidemiological studies in South Africa have indicated a relationship between exposure to fumonisin-contaminated corn and esophageal cancer in humans [17]. Cattle appear to be relatively resistant to FB_1, but preliminary data indicate a possible adverse effect on the rate of weight gain and function of the immune system (M Kehrli, personal communication).

Little is known about FB_1 toxicity in domestic fowl. Acute deaths have been reported in ducklings fed culture material from a number of *F. moniliforme* isolates [1, 18–20]. Toxic effects may have been due to the production of moniliformin by some of these isolates, but in at least two studies moniliformin was not detected in the culture material [5, 19], and deaths were attributed to an unidentified toxin.

Moniliformin-producing isolates of *Fusarium* spp have been reported to be acutely toxic to young chickens [21]. Unidentified *Fusarium* mycotoxins also have been associated with acute deaths [22], thiamine deficiencies [23], decreased weight gain and feed efficiency, and leg deformities in chicks [24]. Recently, in vitro studies showed that FB_1 can affect avian macrophage function and morphology [25].

Experimental studies in poultry fed known amounts of purified FB_1 have not been reported. Field observations have suggested that FB_1 contaminated corn (10–25 ppm FB_1) may be associated with the 'spiking mortality syndrome' of broilers [DE Green, personal communication]. This syndrome was characterized by significantly increased mortality in chicks between 10 and 16 days of age. Clinical signs of this type of syndrome as induced by feeding culture material of *F. moniliforme* var. *subglutinans* included ataxia, paralysis, dyspnea, weakness and stunted growth [26]. Postmortem lesions included coagulative necrosis and hemorrhages in the liver, and necrosis of bile ducts, lymphoid organs and pancreatic acinar cells.

This report characterizes a toxic syndrome produced by feeding broiler chicks diets amended with *F. proliferatum* culture material (CM) having known concentrations of FB_1, FB_2 and moniliformin. Additionally, broilers were fed diets amended with pure FB_1 and moniliformin to compare the effects of these individual mycotoxins with the effects of the combined toxins observed in the CM-amended diets.

Materials and methods

Laboratory culture for toxin(s) production. The *Fusarium* isolate used in this study was *Fusarium proliferatum* M-5991, obtained in lyophilized form, from the Fusarium Research Center, The Pennsylvania State University. Mass inoculum was prepared by dissolving the lyophilized culture in 2 ml sterile, distilled water and dispensing ca. 0.2 ml of culture suspension onto each of 10 pre-

pared plates (15 cm diameter) containing 50 ml V-8 juice agar medium. The plates were incubated for 7–8 days at 25 °C in the dark. The plates were then flooded with 20 ml sterile, distilled water and the conidia suspended with a glass rod. The suspensions from 10 plates were combined in a 250-ml culture bottle and serial dilution plates inoculated to determine viable cell count (cfu) for the combined preparation.

Cultures were prepared by steeping 300 g polished, parboiled rice in 300 ml water in 2.8 L Fernbach flasks for 2–3 h at room temperature. Flasks were capped with two standard milk filters held in place with rubber bands. The steeped rice was autoclaved for 30 min at 15 psi. Two ml of *F. proliferatum* suspension 3×10^7 cfu/ml) were added to each flask and mixed. Inoculated flasks were incubated in the dark (flasks covered with aluminium foil) at 25 °C for 21–28 days. After incubation, CM was autoclaved for 30 min and dried in stainless steel pans for 18 h at 90 °C. The dried CM was ground in a Quaker City Model 4-E grain mill (Straub Co., Philadelphia, PA), and mixed for 20 min in a Hobart Model A-200 blender (Hobart Manufacturing Co., Troy, OH). The CM was stored at 0 °C.

Analyses of CM and prepared feeds. Ten g of ground, blended CM or prepared feed (in duplicate) was placed in a 250-ml glass-stoppered Erlenmeyer flask and 100 ml methanol-water (80 + 20) was added. The mixture was shaken for 60 min on a wrist action shaker (Burrell Model 75) and the extract was decanted and filtered through rapid flow paper (Schleicher & Schuell #588; S&S, Keene, NH). CM/feed residue was re-extracted with a second 100 ml solvent and filtered. Two ml of each extract were diluted with 4 ml deionized, distilled water, mixed and fumonisins (FB_1 and FB_2) were partially purified on a C_{19} solid phase extraction (SPE) column (Analytichem Mega Bond Elut, Varian, Harbor City, CA) as described by Bennett et al. [31]. The naphthalene dicarboxaldehyde (NDA) derivatives of reference standard and extracts were prepared [27] and the concentrations of FB_1 and FB_2 determined by HPLC with fluorescence detection (excitation, 250 nm; emission, 418 nm cut-off filter). Fumonisins were separated using a gradient of 60% A [acetonitrile-acetic acid (100 + 1)] plus 40% B [water-acetic acid (100 + 1)] for 8 min at 1.0 ml/min and then to 80%A + 20%B for 10 min. Retention times for FB_1 and FB_2 were 6 and 12 min, respectively. The level of each toxin was determined by comparing the peak height of standards and peak height of samples. The concentrations found in the first and second extracts were added to obtain total toxin in each sample.

Moniliformin in CM extracts (5 ml) was partially purified on a C_{18} (1 g) SPE column [27]. The concentration of moniliformin was determined by HPLC on a RP C_{19} column (4.6 mm × 25 cm) with ion-pair mobile phase of acetonitrile-water-tetrabutyl ammonium hydroxide (100 + 900 + 10) and diode array detection at 229 nm [28].

Isolation and purification of FB_1 *and moniliformin.* CM from 21–28 days incubation of 300 g rice with *F. proliferatum* M-5991 was pre-extracted with ethyl acetate (2–500 ml volumes) to remove pigments produced by this isolate. Fumonisins and moniliformin were extracted by mixing the CM (ca. 150 g) with 1 liter methanol-water (80 + 20) and steeping overnight. The extract was filtered and the residue was re-extracted. Solvent was reduced to ca. 200 ml on a rotary evaporator. Fumonisins and moniliformin were partially purified on an XAD-2 column (100 g) [29]. After the sample was added, the column was washed with water (3L) to remove moniliformin. Fumonisins were eluted with methanol (1L). The methanol solution was concentrated to near dryness and partitioned in the following three phase solvent system: acetonitrile-hexane-dichloromethane-water (50 + 50 + 10 + 20) [30]. The lower phase was concentrated and dissolved in water (1L). Purification of FB_1 was done by preparative high performance liquid chromatography (HPLC) on a Dynamax C_{19} column and eluted with the following mobile phase: methanol-water-trifluoroacetic acid (65 + 35 + 1). FB_1

elution was monitored by refractive index and UV detector so that pure FB_1 could be collected that was not contaminated with a UV absorbing material that elutes with the leading edge of the FB_1 peak. The fraction containing the purified FB_1 was lyophilized in a tared vial for dry weight determination. The resulting material was off-white in color and hygroscopic. Purity was determined by comparing the HPLC peak of the NDA-derivative with that of reference standard FB_1 (Sigma Chemical Co., St. Louis, MO).

Moniliformin from the water-wash of the XAD-2 column was concentrated to near dryness and purified by the procedure of Burmeister [31]. Instead of preparative TLC, moniliformin was purified by chromatography on a C_{19} SPE column. Purity was determined by HPLC (diode array detection) of sample and reference standard (Sigma).

Chickens and housing. One-day old male chicks (Columbia × New Hampshire) were obtained from the Avian Research Center, Department of Animal Sciences, College of Agriculture, University of Illinois, and housed in multi-tiered battery-type brooders. Treatment group replicates were assigned to different levels in the batteries to eliminate bias due to position. Birds were housed in an enclosed, thermally controlled building. Room temperature, lighting, water and feeding space were uniform for all replicates. Feed and water were given ad libitum.

Diets. Fusarium proliferatum M-5991 autoclaved CM containing FB_1, FB_2 and moniliformin was added to nutritionally balanced broiler ration No. 4 [32] formulated by the UI Avian Research Center. The unamended ration did not contain FB_1, FB_2 or moniliformin. Aflatoxin, trichothecenes and zearalenone were not detected in the ration or the CM. The CM was ground 3 times in a Bunn Model G-3 grinder and added to the broiler ration in a Hobart Model M-802 mixer. The amount of CM (ca. 1200 ppm FB_1, as determined by HPLC analysis) added to each diet was considered as a replacement for corn. Amino acids, vitamins and minerals were added to balance the 3 experimental diets with the control diet in Trials 1 through 3. Diets T2, T3 and T4 contained 5.1%, 16.0% and 45.5% CM, respectively. The concentrations of FB_1, FB_2 and moniliformin in the various diets, as determined by HPLC analysis, are shown in Table 1.

Purified FB_1 (ca. 90%) and moniliformin (>91%) were mixed into experimental diets for Trial 4 as follows. The calculated amount of 90% pure FB_1 needed for 1300 gm of FB_1 'high' concentration diet was dissolved in 10 ml distilled water and mixed into the basal ration. The 'high' concentration diet was mixed with the basal ration to prepare the 'low' concentration diet. Concentrations of FB_1 were 274 ppm (P3) and 125 ppm (P2) (Table 1). The two moniliformin diets were prepared in a similar manner. The concentrations of moniliformin were 154 ppm (P5) and 27 ppm (P4). The combination (P6) diet (FB_1 and moniliformin) was prepared by mixing equal parts of P3 and P5 diets. The concentrations were calculated to be 137 ppm FB_1 and 77 ppm moniliformin (Table 1).

Experimental protocols. Two-hundred twenty-eight male chicks were randomized by body weight and assigned to a feed treatment group (Table 1). Birds were monitored daily for clinical signs, group activity and mortality; weight gains were measured weekly. Birds that died or were euthanized in a moribund condition during the trial period were examined postmortem. Survivors were killed and examined at the end of their respective feeding trials.

Defecation index method of monitoring group activity. Cardboard sheets (2.5 × 2.5 feet) were marked into 4 distinct triangles (△) (Fig. 1) and placed on the floor of each battery. The sheets were examined every 12 h and the number of droppings per triangle was recorded. The number of droppings in the triangles adjacent to feed, water or heat source reflected differences in activity of birds given different concentrations of mycotoxin(s). The Rate of Defecation (RD) re-

Table 1. Experimental feeding protocols for trials 1–4

Trial No.	Treatment groups*	No of chicks	FB_1 (ppm)	FB_2 (ppm)	Moniliformin (ppm)	Days of exposure
1	T1	30	0	0	0	1–28
1	T2	30	61	14	66	1–28
1	T3	30	193	38	193	1–28
1	T4	30	546	98	367	1–28
2	PF1	6	0	0	0	7–28
2	PF2	6	61	14	66	7–28
2	PF3	6	193	38	193	7–28
2	PF4	6	546	98	367	7–28
3	R1	6	0	0	0	21–28
3	R2	6	61	14	66	21–28
3	R3	6	193	38	193	21–28
3	R4	6	546	98	367	21–28
4	P1	10	0	0	0	1–14
4	P2	10	125	0	0	1–14
4	P3	10	274	0	0	1–14
4	P4	10	0	0	27	1–14
4	P5	10	0	0	154	1–14
4	P6	10	137	0	77	1–14

* Toxin levels in diets of each treatment group determined by HPLC analysis described in text.

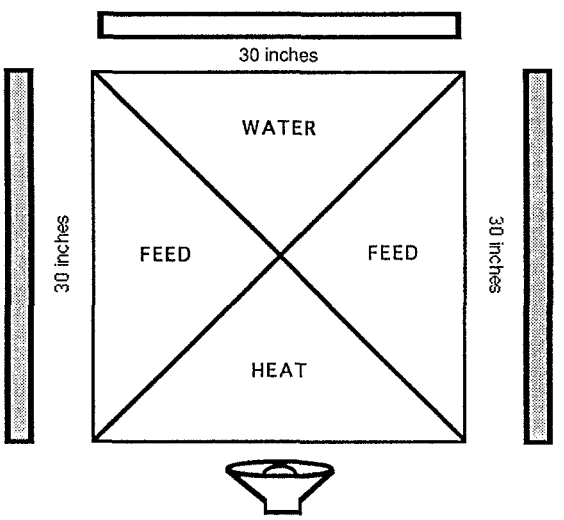

Fig. 1. Pattern of triangle compartments marked on cardboard for defecation counts.

flected clinical onset and degree of severity in a group of affected birds. A relative RD was established by the equation:

$RD = \Sigma D$ in a $\Delta/B \times T$,

where

D = number of droppings in a triangle adjacent to water ($\Delta 1$), feed (Δs 2 and 3) or heat source ($\Delta 4$); B = number of birds per group, and T = time interval (12 h in these studies)

Statistical analyses. Data on body weights, defecation indices and mortality were analyzed for intratreatment and intertreatment differences within each of the 4 trials. Mean body weights, standard deviation (SD) and standard error of the mean (SEM) were calculated. Analysis of variance and mean comparisons among age groups, mycotoxin concentrations, weight gains and mortality were performed using Dunnet Double Sided tests [33].

Results and discussion

Clinical findings Trial 1. Dose responses to FB_1, FB_2 and moniliformin were noted in all treatments with 1-day old birds. All birds on CM-amended diets were adversely affected during the 4-week trial; there was 100% mortality in treatment group T4, 87% in T3 and 37% in T2. No clinical signs or deaths occurred in controls (Fig. 3). Birds in T4 (highest concentrations) were clinically affected first (after 2 days), followed by

Fig. 2. Three-day old chick, Trial 1, treatment 4, with flexion of hock joints, fed CM-amended diet 48 h.

those in T3 (after 5 days) and T2 (after 7 days), respectively. Clinical responses included hyperexcitation and ataxia, with backward motion to gain balance. Next, birds became markedly depressed, weak and usually died within 24 h. The typical syndrome was characterized by partial flexion of the hock joints (Fig. 2) that progressed to complete flexion and peracute immobility. Birds became exhausted, assumed a 'sailing duck' position resting on their sternum and were unresponsive to external stimuli. Torticollis and head tremors occurred in a few birds but were not uniform features. As the syndrome progressed, feed and water intake gradually decreased; however, their gastrointestinal tracts contained undigested feed at necropsy, indicating that birds did not die of starvation. Body temperatures typically decreased (from a normal of 40.5 °C to 35.5 °C before death) causing birds to huddle under the heat source. Birds closed their eyes, stretched their legs in opposite directions and rested one or both wings and beaks on the floor. Finally, birds had transient dyspnea and gasping terminating in flaccidity and death.

Birds in treatment T4 (546 ppm FB_1; 98 ppm FB_2; 367 ppm moniliformin) first decreased feed intake on day 2, T3 (193 ppm FB_1; 38 ppm FB_2; 193 ppm moniliformin) on day 4 and T2 (61 ppm FB_1; 14 ppm FB_2; 66 ppm moniliformin) on day 6. In general, the decreased feed intake was followed by a transient increase in water intake and seeking the heat source (within 24 h). Subsequently, water and feed intake decreased. Birds surviving 2 weeks developed some tolerance to the mycotoxins and deaths decreased. However, weight gains remained poor in comparison to the birds on control (T1) diet (Table 2). Surviving birds on diets T2 and T3 developed rough feathering and rudimentary combs and wattles by 4 weeks of age. Testes in these birds were consistently smaller than those on the control diet.

Trial 2. Similar to Trial 1, 7-day old birds given the same diet were clinically affected after 48 h on the CM-amended feed (PF4). The PF3 birds started showing clinical signs on the fourth day, and the PF2 birds on the 6th day. The clinical picture was identical to that of Trial 1 except the progression from hock flexion to immobility was not peracute. All birds on CM-amended diets were clinically affected during the 21-day feeding trial. Mortality was 100% in PF4, 67% in PF3 while 0 in PF2 and controls (Fig. 4). Survivors had poor weight gain (Table 2), rough feathering, and cachexia. Combs and wattles were undeveloped compared to the controls.

Trial 3. Twenty-one-day old birds were given the same CM-amended diets for 7 days. As in the first two trials, those on the highest levels of mycotoxins (R4: 546 ppm FB_1; 98 ppm FB_2; 367 ppm moniliformin) became clinically affected after 48 h. However, the clinical syndrome was somewhat different. Affected birds huddled together, were off feed, had ruffled feathers, and several had whitish diarrhea which appeared to be predominantly uric acid and bile. The diarrhea progressed and birds became comatose and died within 24 h. There was 100% mortality in R4, 33% in R3 and 0 in R2 and controls (Fig. 5). R3 birds became mildly affected at 72 h and the first death occurred on the 6th day. Birds in R2 became slightly depressed on day 7, but diarrhea was not common. Markedly reduced weight gain (Table 2) and un-

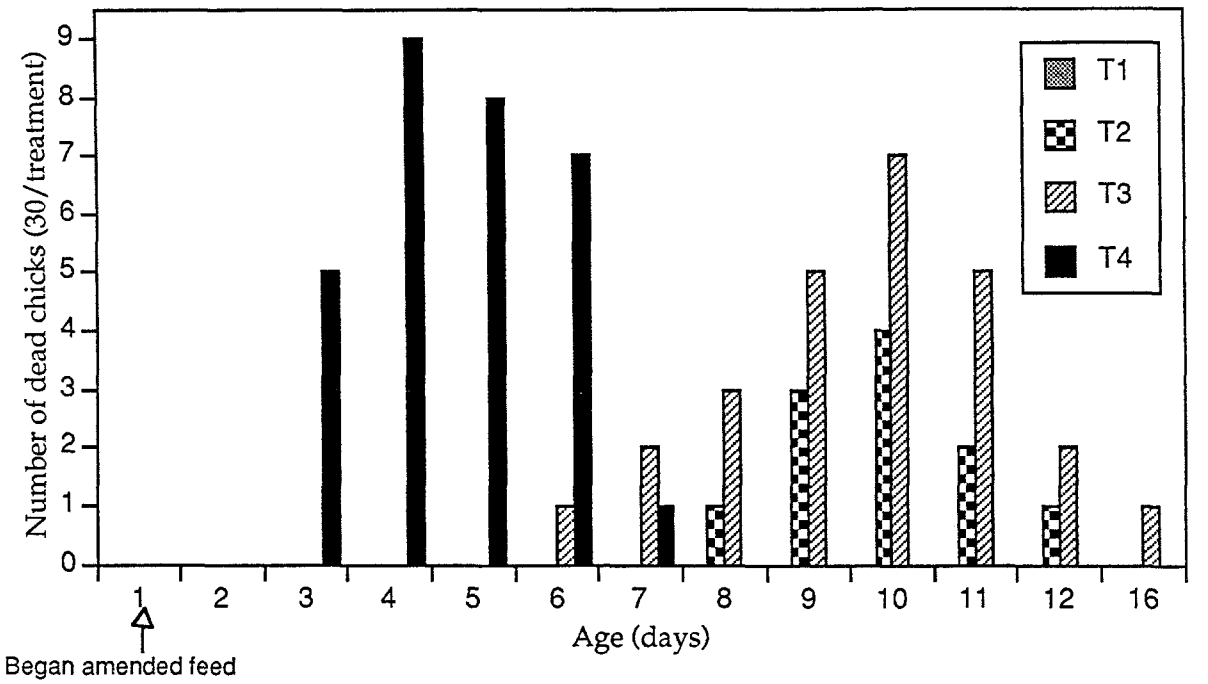

Fig. 3. Mortality pattern for chicks in treatments T1-T4, Trial 1.

Table 2. Weekly mean body weight of chicks in trials 1–4

Trial	Treatment	Weekly mean body weight + SEM of chicks (gm)				
		Day 1	Day 7	Day 14	Day 21	Day 28
1	T1	42.34 ± 0.27[a]	86.96 ± 0.54[a]	176.75 ± 1.32[a]	356.48 ± 0.43[a]	635.62 ± 0.488[a]
1	T2	■41.44 ± 1.29[a]	53.92 ± 5.96[b]	99.86 ± 0.98[b]	207.24 ± 0.91[b]	368.00 ± 09.34[b]
1	T3	■43.37 ± 0.31[a]	33.52 ± 1.25[c]	48.50 ± 2.52[c]	71.50 ± 0.450[c]	+
1	T4	■40.43 ± 1.28[a]	30.50 ± 0.50[c]	+	–	–
2	PF1	43.42 ± 0.29[e]	86.92 ± 0.55[e]	173.70 ± 1.47[e]	352.63 ± 2.14[e]	621.57 ± 1.92[e]
2	PF2	42.33 ± 0.27[e]	■ 87.43 ± 0.62[e]	124.5 ± 0.96[f]	230.63 ± 2.14[f]	359.43 ± 8.54[f]
2	PF3	41.42 ± 1.30[e]	■ 86.67 ± 0.45[e]	75.33 ± 0.96[g]	–	–
2	PF4	43.42 ± 0.29[e]	■ 86.93 ± 0.53[e]	*45.67 ± 1.01[h]	+	–
3	R1	42.28 ± 0.23[j]	87.37 ± 0.53[j]	177.62 ± 0.91[j]	356.33 ± 1.98[j]	628.87 ± 0.88[j]
3	R2	43.17 ± 0.26[j]	84.08 ± 0.44[j]	173.42 ± 1.45[j]	■357.33 ± 2.01[j]	535.88 ± 8.34[k]
3	R3	41.02 ± 1.20[j]	83.00 ± 0.51[j]	176.87 ± 2.01[j]	■358.30 ± 3.30[j]	239.20 ± 4.41[m]
3	R4	43.28 ± 0.271[j]	85.20 ± 0.54[j]	172.67 ± 1.43[j]	■354.67 ± 1.97[j]	▲222.50 ± 5.94[n]
4	P1	42.20 ± 0.51[p]	104.40 ± 2.04[p]	197.59 ± 3.73[p]	–	–
4	P2	■43.18 ± 0.51[p]	85.21 ± 3,04[q]	95.00 ± 8,97[q]	–	–
4	P3	■41.15 ± 0.59[p]	61.59 ± 5.29[r]	77.20 ± 6.09[r]	–	–
4	P4	■42.315 ± 0.59[p]	78.70 ± 4.35[q]	73.74 ± 3.60[r]	–	–
4	P5	■41.16 ± 1.58[p]	63.13 ± 3.56[r]	53.72 ± 5.96[s]	–	–
4	P6	■43.23 ± 0.48[p]	40.30 ± 5.41[s]	+	–	–

■ Indicates the day amended feed was initiated.

[a-s] Values with similar superscripts within a trial on given day do not differ statistically, while values with different superscripts differ significantly ($p < 0.05$).

+ Indicates 100% mortality.

* Weights were taken at the time of death (9–11 days of age).

▲ Weights were taken at the time of death (24–27 days of age).

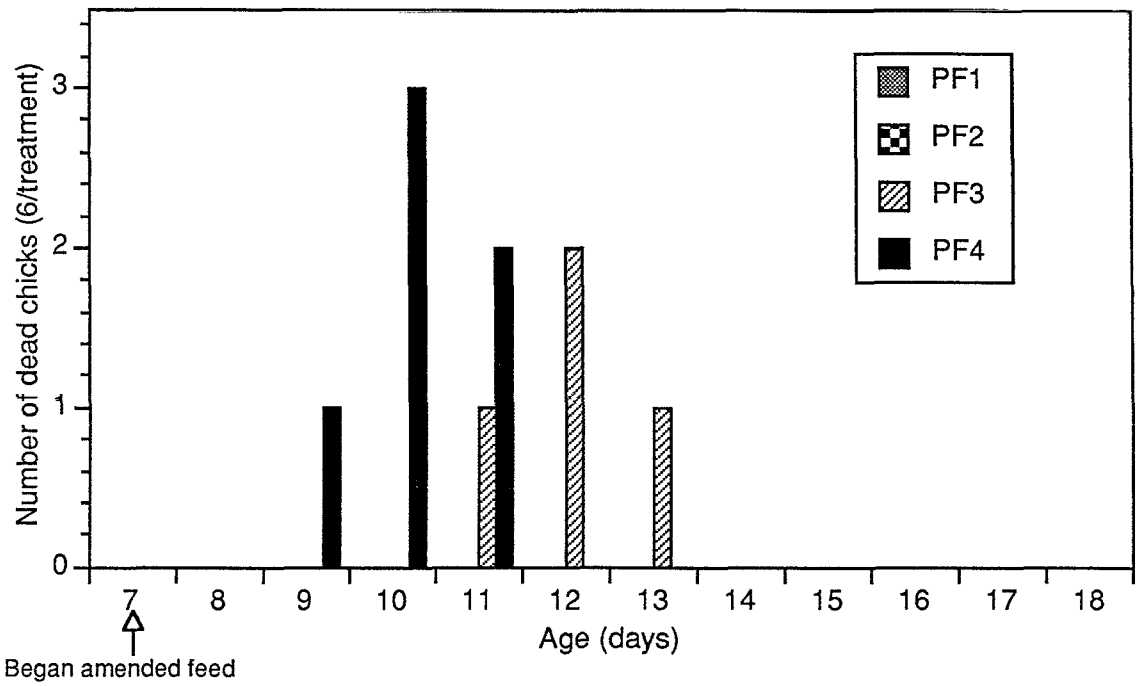

Fig. 4. Mortality pattern for chicks in treatments PF1-PF4, Trial 2.

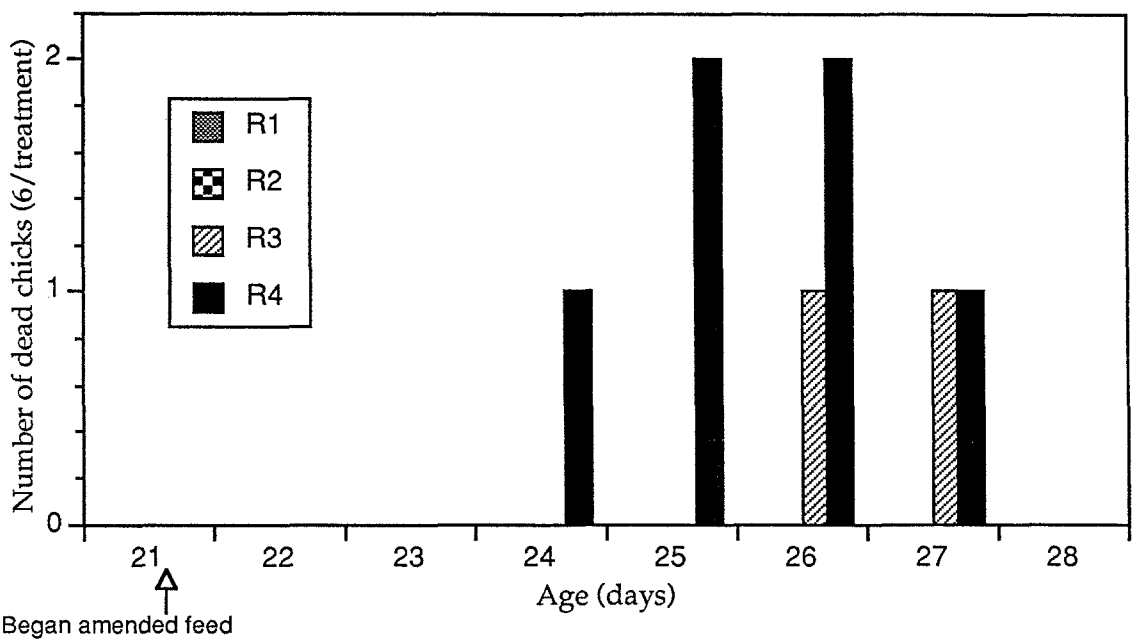

Fig. 5. Mortality pattern for chicks in treatments R1-R4, Trial 3.

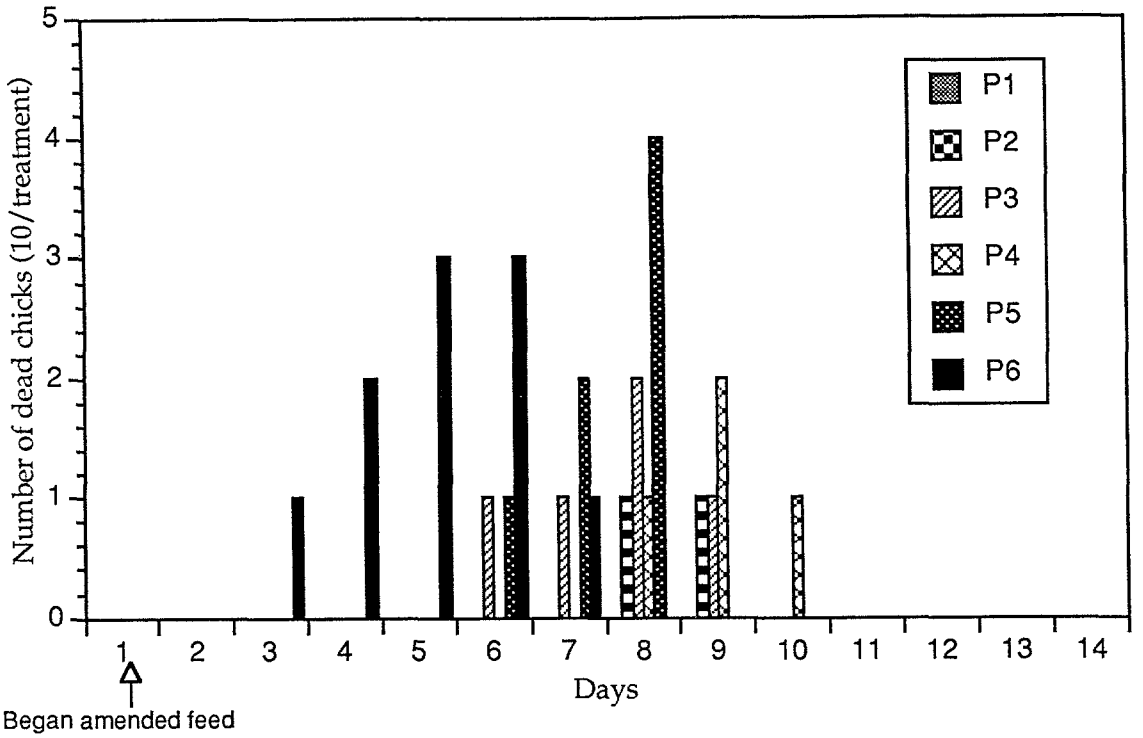

Fig. 6. Mortality pattern for chicks in treatments P1-P6, Trial 4.

developed combs, wattles and testes were characteristic.

Trial 4. Birds in treatment P6 (combined mycotoxins: 137 ppm FB_1; 77 ppm moniliformin) were first affected after 48 h. The clinical syndrome was identical to treatment group T4 (Trial 1). Birds in P5 (154 ppm moniliformin) and P3 (274 ppm FB_1) were affected on day 5. Clinical signs were similar in both treatment groups (P5; P3), but were less pronounced than those in P6. Birds in P4 (27 ppm moniliformin) and P2 (125 ppm FB_1) were affected by day 7. Controls remained normal during the 14 day trial. Mortality was 100% in P6 (combination), 70% in P5 (higher moniliformin), 50% in P3 (higher FB_1), 40%, in P4 (lower moniliformin), and 20% in P2 (lower FB_1) (Fig. 6). Weight gains were significantly depressed in all treatment groups compared to controls (Table 2). Results indicated that moniliformin may be more toxic to young broilers than FB_1; however, their toxicities appeared to be additive.

Weight gains. The initial weights of birds in all treatment groups were uniform on day 1 of all 4 trials. Dose-response reduced weight gains occurred in birds after 7 or more days on their respective treatments compared to controls (Table 2). There were significant ($p < 0.05$) decreases in weight gains (60–80%) in birds at the two higher feed concentrations of mycotoxins (combination of 546 or 193 ppm FB_1; 98 or 38 ppm FB_2; 367 or 193 ppm moniliformin, respectively), and reduced weight gains (38–44%) in birds on the lower concentrations (61 ppm FB_1; 14 ppm FB_2; and 66 ppm moniliformin). Surviving birds were apparently normal except for poor weight gains. Reduced weight gains may have been due to reduced feed intake; however, birds that died had feed in their digestive tracts, indicating that consumption continued through the

early stages of toxicosis. Poor weight gains may have been due to lesions in the gastrointestinal tract and pancreas that are reported in a related paper [34]. Insufficient pancreatic activity and reduced liver function have been reported to cause stunted growth in chicks [35]. Survival and proliferation of turkey peripheral blood lymphocytes have been shown to be adversely affected by in vitro exposure to FB_1 or FB_2 [36], using an MTT bioassay [37]. Mycotoxins can exert an effect as immunomodulators at low levels [38].

In Trial 4, purified FB_1 and moniliformin were given separately and in combination; weight gains were significantly less than controls in all groups. Differences in weight gain were dose-related in all groups, with the lowest weight gain in the group fed the combined FB_1 (137 ppm) and moniliformin (77 ppm) (P6). However, moniliformin-fed groups (P5 and P4) had lower weight gains ($p < 0.05$) than FB_1-fed groups (P3 and P2) (Table 2).

Defecation index. The Rate of Defecation (RD) patterns in control birds were predominantly in those triangles near feed and water sources (Fig. 7). Representative RD indices for controls were: $\Delta 1$ = water 0.43; $\Delta 2$ = feed, 0.40, $\Delta 3$ = feed 0.41 and $\Delta 4$ = heat source 0.18, with a cumulative RD index of 1.42. As birds became adversely affected on the amended diets, their RDs shifted towards the water, then toward the heat source after they were severely affected (Fig. 8). A representative RD index for a severely affected group of birds (T4 at 144 h on highest mycotoxin diet) was: $\Delta 1$ = water 0.10, $\Delta 2$ = feed 0.14, $\Delta 3$ = feed 0.14 and $\Delta 4$ = heat source 0.58, with a cumulative RD index of 0.96. Ninety percent of birds that became moribund or immobile were in $\Delta 4$ (heat source).

There were significant differences ($p < 0.05$) in RD indices among the 4 treatment groups in Trial 1 once birds began to be clinically affected; however, the patterns of RD changes were comparable in all groups on amended feeds as the syndrome progressed. Figs. 8–10 present representative RD indices of each treatment group beginning at the time when birds became clinically affected. The RD indices remained uniform in the controls. Except for the temporal differences, the RD indices in clinically affected birds in Trials 2 and 3 were dose-responsive and similar to Trial 1.

Measuring the RD indices was effective in monitoring the activity of chicks in their approach to feed, water and heat source. It was sensitive to the progression of clinical signs of disease leading to death. This model was a quantitative indicator of feed and water intake and fecal output. The data provided a graphic picture of birds shifting from one area to another as illness progressed.

General discussion. Some *Fusarium* isolates commonly identified as *F. moniliforme* have been shown to be *F. proliferatum* [5, 6]. Furthermore, *F. proliferatum* cultures can produce moniliformin as well as fumonisins, whereas many *F. moniliforme* do not produce moniliformin [5]. Under field conditions the incidence of *F. proliferatum* infecting feedstuffs may be more common than previously thought. Therefore, moniliformin may be a common contaminant along with fumonisins [5]. This occurrence could affect the accuracy of attributing fusariotoxicoses in horses, pigs and chickens to fumonisins without testing for moniliformin [7, 9, 13, 14, 39]. This may account for varied results reported by investigators using animals fed corn contaminated with fumonisins. The levels of fumonisin B_1, B_2 and moniliformin in diets of each of the treatment groups (Table 1) were determined by HPLC analyses of each diet preparation. Some discrepancy in the values obtained in these assays become apparent when a calculated level is compared with the level obtained by analysis. This occurrence results from the fact that fumonisins and moniliformin are not completely extracted by our methods even though two extractions were performed on each diet preparation. The level of extractable fumonisins in culture material has been reported to be significantly reduced when the culture material is dried under different conditions (L.G. Rice, personal

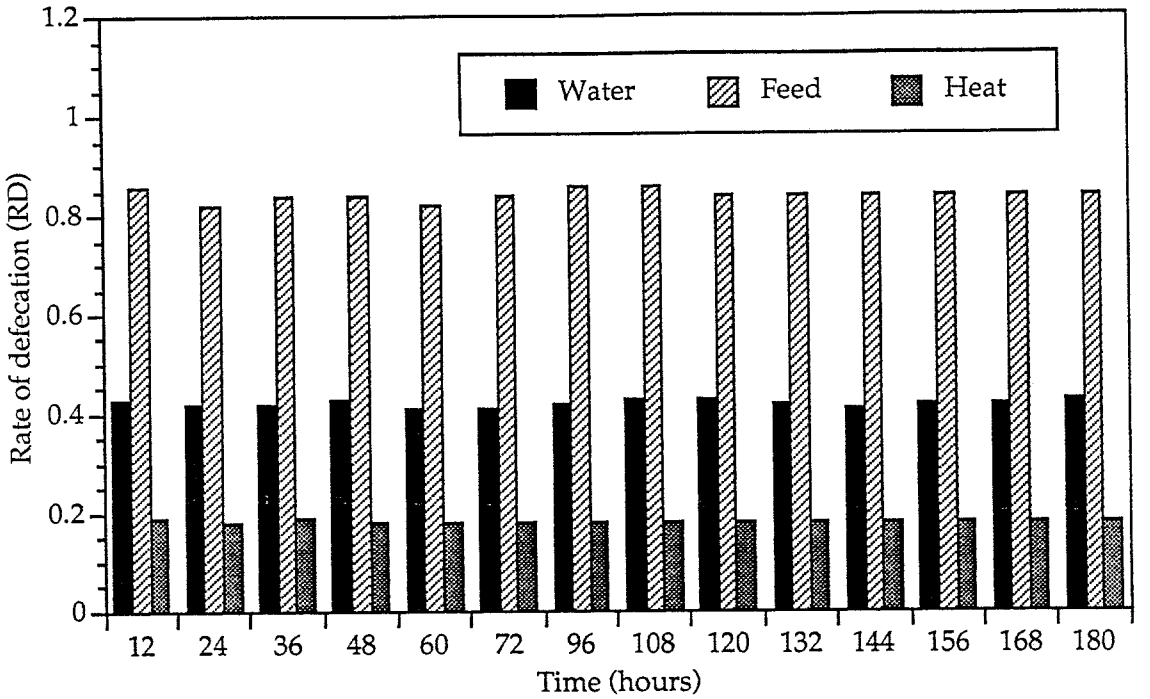

Fig. 7. Rate of defecation in triangles near feed, water and heat source in Trial 1 (T1) (control). Feces were discarded without counts being made at 84 h.

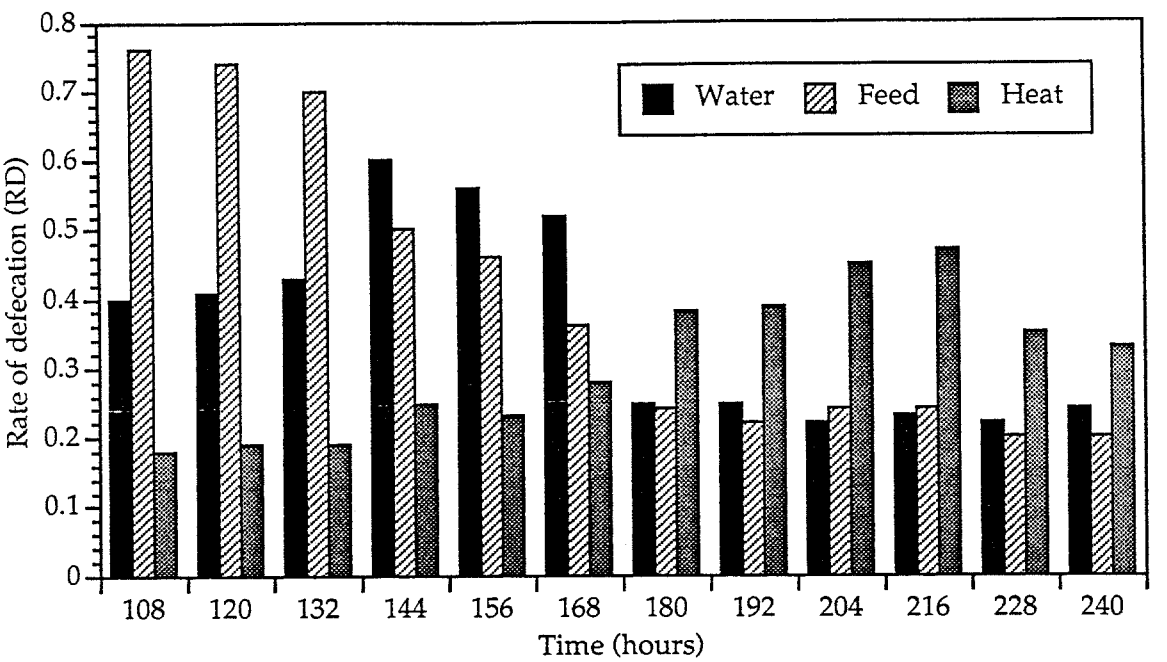

Fig. 8. Rate of defecation in triangles near feed, water and heat source in Trial 1 (T2) (61 ppm FB_1, 14 ppm FB_2, and 66 ppm moniliformin).

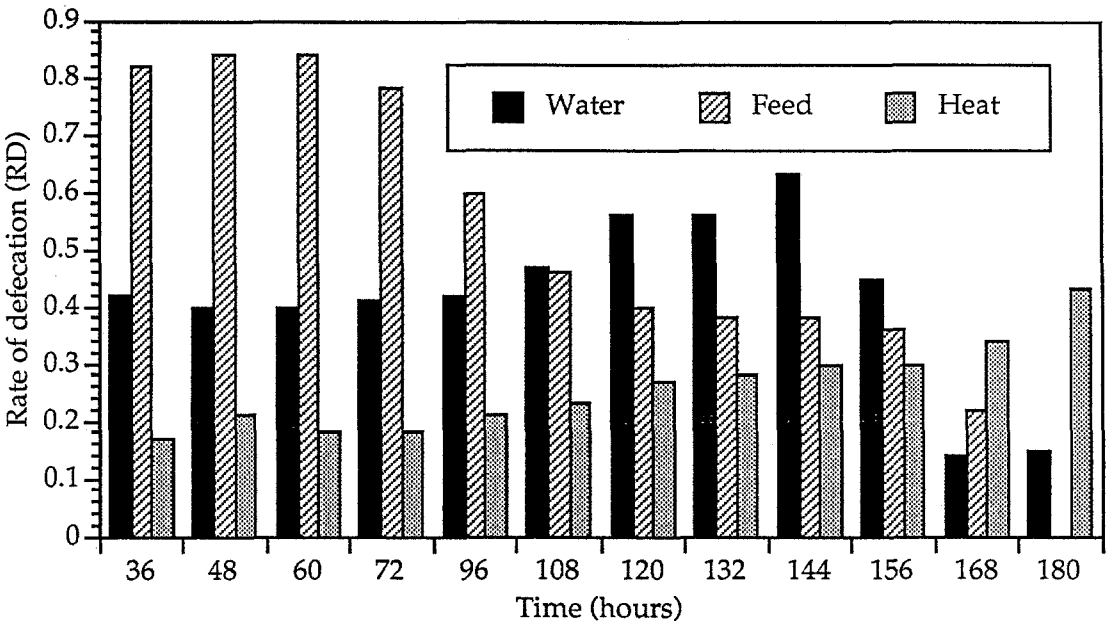

Fig. 9. Rate of defecation in triangles near feed, water and heat source in Trial 1 (T3) (193 ppm FB_1, 38 ppm FB_2 and 193 ppm moniliformin). Feces were discarded without counts being made at 84 h.

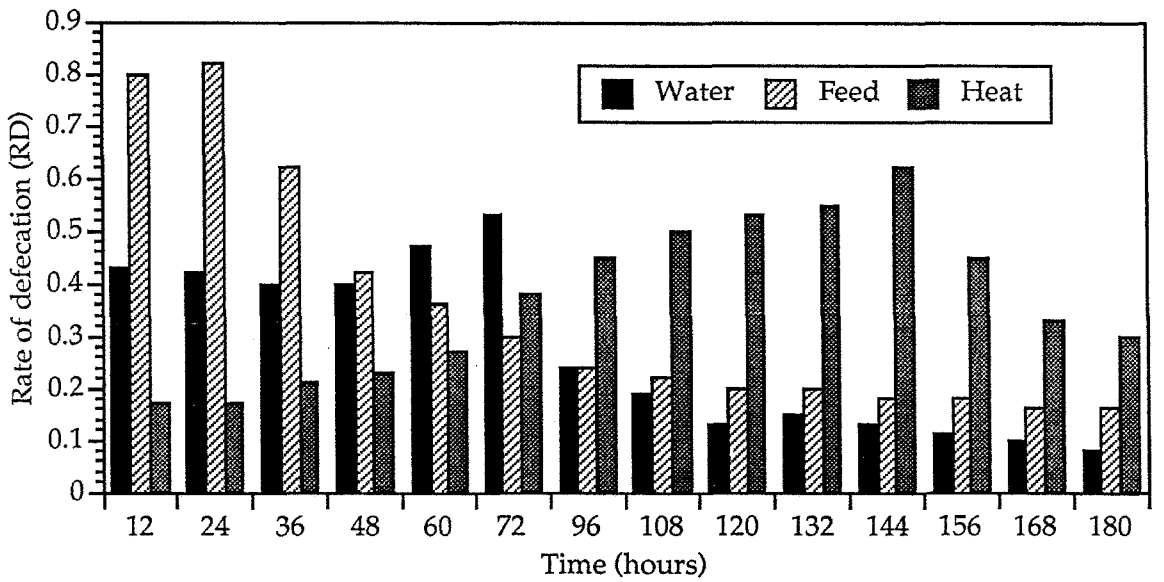

Fig. 10. Rate of defecation in triangles near feed, water and heat source in Trial 1 (T4) (546 ppm FB_1, 98 ppm FB_2 and 367 ppm moniliformin). Feces were discarded without counts being made at 84 h.

communication). It appears that moniliformin behaves in a similar manner.

These studies showed that FB_1 and moniliformin, both alone and in combination, produced dose-responsive clinical signs, reduced weight gains and mortality in chicks. Age of birds when started on amended feeds made little difference in the clinical picture; however, those beginning

on 7 and 21 days of age were slightly less susceptible than those started on day 1.

These studies did not establish the maximum no-effect level (NOEL) of fumonisins and moniliformin in chicken rations, although one may approximate the NOEL by statistical extrapolation. We have not attempted this because of the age variations of birds in the 4 trials. Additional feeding studies should be done to establish the maximum no-effect concentrations of fumonisins and moniliformin. Although high levels of toxins were used in the studies, chicks fed corn infected with *F. proliferatum* under field conditions could suffer the 'spiking mortality syndrome' of acute death in the first 3 weeks of age.

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