Individual and Combined Effects of Fumonisin B_1 Present in *Fusarium moniliforme* Culture Material and T-2 Toxin or Deoxynivalenol in Broiler Chicks[1]

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ABSTRACT The individual and combined effects of feeding diets containing 300 mg fumonisin B_1 (FB_1), and 5 mg T-2 toxin (T-2)/kg of diet, or 15 mg/kg deoxynivalenol (DON, vomitoxin) from naturally contaminated wheat were evaluated in two studies in male broiler chicks from day of hatch to 19 or 21 d of age in Experiments 1 and 2, respectively. When compared with controls, body weight gains were reduced 18 to 20% by FB_1, 18% by T-2, 2% by DON, 32% by the FB_1 and T-2 combination, and 19% by the FB_1 and DON combination. The efficiency of feed utilization was adversely affected by FB_1 with or without T-2 or DON. Mortality ranged from none for the controls to 15% for the FB_1 and T-2 combination. Relative weights of the liver and kidney were significantly increased by FB_1 with or without T-2 or DON. Serum concentrations of cholesterol were increased in chicks fed FB_1 with or without T-2 or DON. Activities of aspartate aminotransferase, lactate dehydrogenase, and gamma glutamyltransferase were increased in chicks fed FB_1 at 300 mg/kg alone and in combination with T-2 or DON, indicating possible tissue damage and leakage of the enzymes into the blood. Results indicate additive toxicity when chicks were fed diets containing 300 mg FB_1 and 5 mg T-2/kg of diet and less than additive toxicity when chicks were fed 300 mg FB_1 and 15 mg DON/kg of diet. Of importance to the poultry industry is the fact that toxic synergy was not observed for either of these toxin combinations and the likelihood of encountering FB_1 at this concentration in finished feed is small. However, under field conditions with additional stress factors, the toxicity of these mycotoxins could be altered to adversely affect the health and performance of poultry.

(*Key words*: fumonisin, T-2 toxin, deoxynivalenol, toxicity, chicken)

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INTRODUCTION

Some common soil fungi may contaminate grains that may be consumed by humans or animals. *Fusarium* spp. are in this category and may produce mycotoxins known as fumonisins (FB). These include FB_1, FB_2, FB_3, and FB_4, with FB_1 being the major metabolite (Gelderblom *et al*., 1992). Fumonisin B_1 has been shown to be the causative mycotoxin for some major toxicological effects in animals, including leucoencephalomalacia in horses (Marasas *et al*., 1988; Kellerman *et al*., 1990; Wilson *et al*., 1990), pulmonary edema in swine (Harrison *et al*., 1990; Ross *et al*., 1990; Colvin *et al*., 1993), and hepatotoxic and carcinogenic effects in rats (Gelderblom *et al*., 1991). Culture material from *Fusarium moniliforme* (Brown *et al*., 1992; Ledoux *et al*., 1992, 1994; Weibking *et al*., 1993a; Kubena *et al*., 1996) and *Fusarium proliferatum* (Javed *et al*., 1993) containing FB_1 has been associated with poor performance, increased relative organ weights, and hepatitis in broilers. Reduced performance, increased relative organ weights, and alterations in serum constituents and enzyme activities (Weibking *et al*., 1993b, 1994; Kubena *et al*., 1995a,b, 1996). Hepatocellular hyperplasia and myocardial alterations (Weibking *et al*., 1993b) have been reported in turkeys. Although relatively high concentrations of FB_1 were fed in studies with chickens and turkeys, toxicity was observed, thus precluding the dismissal of FB_1 as not being a mycotoxin of importance to the poultry and livestock industries.

The T-2 toxin is a naturally occurring mycotoxin produced by several species of fungi in the genus

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Fusarium (Bamburg *et al.*, 1970) that are found in many grains and feeds. The T-2 toxin causes reduced performance and severe oral lesions in poultry (Wyatt *et al.*, 1972, 1973a,b; Chi *et al.*, 1977; Chi and Mirocha, 1978; Hoerr *et al.*, 1981a,b, 1982a,b; Huff *et al.*, 1988a,b; Kubena *et al.*, 1989a,b, 1990, 1994a,b, 1995a), abnormal behavior (Wyatt *et al.*, 1973a), altered feathering (Wyatt *et al.*, 1975), and a coagulopathy (Doerr *et al.*, 1981).

Deoxynivalenol (DON, vomitoxin) is a mold metabolite produced by *Fusarium* species of fungi (Vesonder *et al.*, 1976) and causes feed refusal and emesis in swine (Vesonder *et al.*, 1973, 1976; Forsyth *et al.*, 1977). Deoxynivalenol has been found in many areas of the world (Vesonder *et al.*, 1978; Mirocha *et al.*, 1979; Pathre and Mirocha, 1979; Côté *et al.*, 1984; Hagler *et al.*, 1984). Several reports in the literature indicate that poultry may be relatively insensitive to DON (Hulan and Proudfoot, 1982; Moran *et al.*, 1982, 1983; Hamilton *et al.*, 1983, 1985a,b; Trenholm *et al.*, 1983; Kubena *et al.*, 1985, 1987, 1988; Lun *et al.*, 1986). Huff *et al.* (1986) and Kubena *et al.* (1988) observed reduced performance and changes in hematological and serum chemistry parameters in male broilers fed diets containing 16 mg DON/kg from wheat naturally contaminated with DON. Kubena and Harvey (1988) observed an anemia, which was transitory in nature, in male Leghorn chicks fed a diet containing 18 mg DON/kg from wheat naturally contaminated with DON. Feeding the combination of T-2 and DON to broiler chicks resulted in the significant alteration of several parameters not significantly altered by T-2 or DON individually (Kubena *et al.*, 1989b).

To the authors' knowledge, FB_1 and T-2 or FB_1 and DON have not been observed to occur simultaneously in a single potential feed grain source. However, because multiple grain sources are used in poultry and livestock diets, the possibility exists for diets to be co-contaminated with FB_1 and T-2 or FB_1 and DON. The toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities (Huff *et al.*, 1988b). The effects of these combinations of mycotoxins have not been previously reported in chickens. Therefore, the purpose of this research was to investigate and describe the major effects of feeding male broiler chicks diets containing FB_1 and T-2 or FB_1 and DON in combination from day of hatch to 19 or 21 d of age.

MATERIALS AND METHODS

Two experiments were conducted using day-old male broiler chicks obtained from a commercial hatchery. The chicks were individually weighed, wing-banded, and maintained in electrically heated batteries under continuous fluorescent lighting with feed and water provided for *ad libitum* consumption. A factorial design was used with diets containing FB_1 (0 or 300 mg/kg) and T-2 (0 or 5 mg/kg) in Experiment 1 and FB_1 (0 or 300 mg/kg) and DON (0 or 15 mg/kg) in Experiment 2. In Experiment 1, there were four replicates of five chicks per dietary treatment and the chicks were grown to 19 d of age. In Experiment 2, there were six replicates of six chicks per dietary treatment and the chicks were grown to 21 d of age. In Experiment 1, the chicks were fed a commercial corn-soybean meal diet. In Experiment 2, the chicks were fed a wheat-soybean meal diet. In both experiments, the diets were formulated without added antibiotics, coccidiostats, or growth promoters and contained or exceeded the levels of nutrients recommended by the National Research Council (1994). The basal diet was analyzed for mycotoxins and was below the detection limits for aflatoxin, DON, zearalenone, and cyclopiazonic acid, as established by the methods given by Clement and Phillips (1985). Ground *F. moniliforme* (M-1325) culture material containing 4,700 mg FB_1, 1,400 mg FB_2, and 430 mg FB_3/kg of material, produced by methods previously described by Weibking *et al.* (1993b), was substituted for ground corn to obtain the desired level of FB_1 (300 mg/kg of diet). The diet also contained approximately 89 mg/kg FB_2 and 27 mg/kg FB_3 of these less toxic metabolites of *F. moniliforme.* By nuclear magnetic resonance and mass spectrometry, the T-2 was determined to be greater than 99% pure. For the diets containing FB_1, the culture material was mixed directly into the diets. The T-2 was incorporated into the diet by dissolving it in 95% ethanol and then mixing the appropriate quantities with 2 kg of the diet. After drying, these 2-kg quantities of diet were mixed with the rest of the basal diet to produce the treatments containing these mycotoxins. The DON treatments were produced by substituting wheat naturally contaminated with DON for the control wheat. Contaminated and uncontaminated wheat samples were analyzed for DON by the method of Tacke and Casper (1996). The DON-contaminated wheat was found to contain 27 mg DON/kg and no detectable level of zearalenone (detection limit = 100 μg/kg).

Chicks were weighed on an individual basis and feed consumption for each replicate was recorded weekly. When the chicks reached 19 d of age (Experiment 1) and 21 d of age (Experiment 2), 12 chicks (4 replicates of 3 chicks each in Experiment 1) and 18 chicks (6 replicates of 3 chicks each in Experiment 2) from each treatment were bled by cardiac puncture for serum biochemical analyses and for hematological determinations.

After blood samples were taken, these chicks were killed by cervical dislocation and the liver, kidney, heart, spleen, pancreas, proventriculus, gizzard, and bursa of Fabricius were removed and weighed. In Experiment 1, heads were removed from all chicks and visually scored for oral lesions (using a four-point scoring system ranging from 1 to 4) by the same individual without knowledge as to treatment groups. A lesion score of 1 indicated no visible lesions; a score of 2 was seen as one or two mouth lesions clearly visible on either the lower or upper mandible; a lesion score of 4 was seen as large lesions occurring at several sites within the mouth, principally on the upper and lower mandibles, the

TABLE 1. Effects of feeding diets containing fumonisin (FB_1) from *Fusarium moniliforme* culture material and/or T-2 toxin (T-2) on BW gain, efficiency of feed utilization, oral lesions, and mortality at 19 d, Experiment 1[1]

Treatment		BW gain				19-d BW change from control	19-d feed consumption	Feed:gain	Oral lesion scores[2]	Mortality
T-2	FB_1	1 to 7 d	8 to 14 d	15 to 19 d	1 to 19 d					
(mg/kg)		(g)				(%)	(g/bird)	(kg:kg)	(no.)	(%)
0	0	55^{a}	173^{a}	182^{a}	411^{a}	0	524^{a}	1.53^{b}	1.00^{c}	0^{b}
5	0	45^{b}	143^{b}	149^{b}	339^{b}	–18	446^{ab}	1.63^{b}	2.23^{a}	5^{ab}
0	300	51^{ab}	136^{bc}	149^{b}	335^{bc}	–18	488^{ab}	1.77^{a}	1.00^{c}	10^{ab}
5	300	42^{b}	116^{c}	123^{c}	281^{c}	–32	407^{b}	1.78^{a}	1.43^{b}	15^{a}
LSD[3]		10	26	26	56		84	0.14	0.28	14

[a–c]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values represent the mean of four groups of five chicks each per treatment minus mortality.

[2]Significant antagonistic interaction between FB_1 and T-2.

[3]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

corners of the mouth, and the back of the tongue; lesions scored as 3 were intermediate in appearance to lesions scored 2 or 4. Hemoglobin was measured as cyanmethemoglobin. Erythrocyte count and mean corpuscular volume were determined with a Coulter[3] Model ZM Counter equipped with a Model C 256 channelyzer and acucomp software. Hematocrits were measured by the microhematocrit centrifugation method. The mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations were calculated. Serum concentrations of uric acid, creatinine, urea nitrogen, glucose, calcium, inorganic phosphorus, total protein, albumin, cholesterol, triglycerides, and activities of alkaline phosphatase, aspartate aminotransferase, glutamyltransferase, lactate dehydrogenase, and creatine kinase were determined on a clinical chemistry analyzer[4] according to the manufacturer's recommended procedure.

Data (pen means) for all response variables in each experiment were subjected to ANOVA (Snedecor and Cochran, 1967) as a 2 × 2 factorial using the General Linear Models procedure in the PC-SAS® version 6.02 statistical software (SAS Institute, 1987). Variable means for treatments showing significant differences in the ANOVA were compared using the Fischer's protected least significant difference procedure (Snedecor and Cochran, 1967). All statements of significance are based on the 0.05 level of probability.

RESULTS

Experiment 1

The individual and combined effects of feeding FB_1 from *F. moniliforme* and T-2 on chick performance are presented in Table 1. By the end of Week 1, BW gains were significantly reduced in chicks fed diets containing T-2 alone or FB_1 and T-2 in combination. By the end of Week 2, BW gains were reduced by all toxin treatments, when compared with controls. When compared with controls, feed consumption per bird was significantly lower in chicks fed the diet containing FB_1 and T-2 in combination and the efficiency of feed utilization was reduced in chicks fed the diets containing FB_1 with or without T-2. When compared with controls, oral lesion scores were significantly increased in chicks fed the diets containing T-2 with or without FB_1. However, oral lesion scores were lower in the chicks fed the FB_1 and T-2 combination diets. Mortality was significantly increased in chicks fed the diet containing FB_1 and T-2 in combination, when compared with controls.

Relative weights of the liver and kidney were increased in the chicks fed the diet containing 300 mg FB_1/kg with or without T-2, whereas the relative weights of the pancreas and gizzard were increased only in chicks fed the diet containing FB_1 and T-2 in combination (Table 2). The relative weight of the spleen was increased only in the chicks fed the diet containing 300 mg FB_1/kg alone. The relative weights of the heart, bursa of Fabricius, and proventriculus were not significantly altered by any of the treatments (data not shown).

Data presented in Table 3 show that, when compared with controls, serum concentrations of cholesterol and calcium were significantly increased in chicks fed the diet containing FB_1, with or without T-2, whereas serum concentrations of total protein and albumin were increased only in the chicks fed the diet containing 300 mg FB_1/kg alone. Serum concentrations of uric acid, urea nitrogen, creatinine, triglycerides, glucose, and inorganic phosphorus were not altered by any of the toxin treatments (data not shown). Serum enzyme activities of aspartate aminotransferase and gamma glutamyltransferase were significantly increased in chicks fed the diet containing FB_1 alone, whereas the activity of lactate dehydrogenase was increased in chicks fed the diets containing FB_1, with or without T-2. The activities of alkaline phosphatase, creatine kinase, and alanine trans-

[3]Coulter Electronics, Hialeah, FL 33012.

[4]Gifford Impact 400E, Ciba Corning Diagnostics Corp., Gifford Systems, Oberlin, OH 44774.

TABLE 2. Effects of feeding diets containing fumonisin (FB_1) from *Fusarium moniliforme* culture material and/or T-2 toxin (T-2) on relative organ weights at 19 d, Experiment 1[1]

Treatment						
T-2	FB_1	Liver	Kidney	Spleen[2]	Pancreas	Gizzard
(mg/kg)		(g/100 g BW)				
0	0	4.14[b]	0.66[b]	0.10[b]	0.50[b]	3.45[b]
5	0	4.13[b]	0.68[b]	0.09[b]	0.49[b]	3.76[ab]
0	300	5.19[a]	0.83[a]	0.13[a]	0.53[ab]	3.76[ab]
5	300	5.07[a]	0.79[a]	0.10[b]	0.56[a]	4.09[a]
LSD[3]		0.42	0.07	0.03	0.05	0.40

[a–b]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values represent the mean of four groups of three chicks each per treatment.

[2]Significant antagonistic interaction between FB_1 and T-2.

[3]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

ferase and hematological values were not altered by any of the toxin treatments (data not shown).

Experiment 2

The individual and combined effects of feeding 300 mg FB_1/kg from *F. moniliforme* culture material and 15 mg DON/kg from naturally contaminated wheat on chick performance are presented in Table 4. At the end of Week 1 and continuing throughout the experiment, BW gains of chicks fed the diets containing FB_1 alone or in combination with DON were significantly lower than those of controls. Feed consumption per bird was not significantly altered; however, efficiency of feed utilization was reduced in chicks fed the diets containing FB_1 alone or in combination with DON. When compared with controls, mortality was significantly higher in the chicks fed the diet containing FB_1 and DON in combination.

The relative weights of the liver, kidney, proventriculus, and gizzard were significantly increased in chicks fed the diets containing FB_1 alone or in combination with DON (Table 5). The relative weight of the gizzard was also increased in chicks fed the diet containing DON alone. The relative weights of the bursa of Fabricius and heart were significantly increased only in chicks fed the diet containing DON alone. When compared with controls, no significant changes were observed in relative weights of the spleen and pancreas (data not shown).

Data presented in Table 6 show that serum concentrations of total protein and cholesterol and serum activities of aspartate aminotransferase, lactate dehydrogenase, and gamma glutamyltransferase were significantly increased in chicks fed diets containing FB_1, with or without DON. Serum urea nitrogen was increased only in chicks fed the diet containing FB_1 and DON in combination. There were no significant alterations in serum concentrations of albumin, uric acid, creatinine, triglycerides, glucose, calcium, and inorganic phosphorus or activities of alkaline phosphatase, creatine kinase, and alanine aminotransferase, or hematological values (data not shown).

DISCUSSION

The occurrence of co-contamination of grains and feeds is being reported frequently by analytical laboratories. In fact, the occurrence of single mycotoxin contamination seems to be rare. Many combinations of mycotoxins have been studied in poultry, as indicated by Kubena *et al.* (1994a, 1996). Fortunately for the poultry and livestock industries, additive or less than additive

TABLE 3. Effects of feeding diets containing fumonisin (FB_1) from *Fusarium moniliforme* culture material and/or T-2 toxin (T-2) on serum biochemical values and serum enzyme activities at 19 d, Experiment 1[1]

Treatment								
T-2	FB_1	Total protein	Albumin	Cholesterol	Calcium	Aspartate amino-transferase	Lactate dehydrogenase	Gamma glutamyl-transferase
(mg/kg)		(g/dL)		(mg/dL)		(IU/L)		
0	0	3.25[b]	1.28[bc]	150[b]	11[b]	158[b]	475[b]	11.7[bc]
5	0	3.12[b]	1.27[c]	142[b]	11[b]	149[b]	478[b]	10.0[c]
0	300	3.73[a]	1.49[a]	173[a]	13[a]	273[a]	1,381[a]	14.4[a]
5	300	3.40[ab]	1.40[ab]	177[a]	13[a]	227[ab]	1,108[a]	13.6[ab]
LSD[2]		0.34	0.12	22	1.9	104	566	2.4

[a–c]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values represent the mean of four groups of three chicks each per treatment.

[2]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

TABLE 4. Effects of feeding diets containing fumonisin (FB_1) from *Fusarium moniliforme* culture material or deoxynivalenol (DON) from contaminated wheat or both on body weight gain, efficiency of feed utilization, and mortality at 21 d, Experiment 2[1]

Treatment		BW gain				21-d BW change from control	21-d feed consumption	Feed:gain	Mortality
DON	FB_1	1 to 7 d	8 to 14 d	15 to 21 d	1 to 21 d				
(mg/kg)		(g)				(%)	(g/bird)	(kg:kg)	(%)
0	0	133[a]	235[a]	281[a]	649[a]	0	978[ab]	1.51[b]	0[b]
15	0	123[ab]	225[a]	286[a]	634[a]	–2	975[ab]	1.54[b]	3[ab]
0	300	107[c]	176[b]	232[b]	520[b]	–20	910[b]	1.76[a]	6[ab]
15	300	110[bc]	180[b]	231[b]	526[b]	–19	1,014[a]	1.93[a]	11[a]
LSD[2]		15	25	27	52		93	0.18	9

[a–c]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values represent the mean of six groups of six chicks each per treatment minus mortality.

[2]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

toxicities have been reported for the majority of combinations studied. This means the response observed with the combination of two mycotoxins was similar to or less than the response predicted from the addition of the response of the individual toxins. However, the response of the toxins cannot be predicted based on their individual toxicities. The interactions resulting from simultaneous feeding of two mycotoxins have ranged from synergistic for aflatoxin and ochratoxin A (Huff and Doerr, 1981; Huff *et al.*, 1983), aflatoxin and T-2 toxin (Huff *et al.*, 1988a,b), and aflatoxin and diacetoxyscirpenol (Kubena *et al.*, 1993) to antagonistic for ochratoxin A and deoxynivalenol (Kubena *et al.*, 1988). Fumonisin B_1, T-2, and DON are important mycotoxins due to their prevalence in feedstuffs that are used in poultry and livestock diets. There is considerable information on the individual toxicity of T-2 and DON, as indicated by Kubena *et al.* (1989b). This publication also documented the effects of the combination of DON and T-2. Recently, information on the toxicity of culture material containing FB_1 in poultry has been reported by several researchers (Brown *et al.*, 1992; Ledoux *et al.*, 1992; Javed *et al.*, 1993, 1995; Weibking *et al.*, 1993a,b, 1994, 1995; Kubena *et al.*, 1995a,b, 1996). The results obtained when FB_1, T-2, or DON were fed alone in two experiments reported herein are consistent with previous reports on the effects of these toxins in poultry.

This study defined, for the first time in chickens, the toxicity of the combination of FB_1 and T-2 (Experiment 1) and FB_1 and DON (Experiment 2). In Experiment 1, the toxicity of the combination of FB_1 and T-2 was characterized by reduced BW gains, decreased feed consumption, decreased efficiency of feed utilization, oral lesions (induced by T-2), increased mortality, increased relative weights of the liver and kidney (induced by FB_1), increased relative weights of the pancreas and gizzard, increased serum concentrations of cholesterol and calcium, and increased activity of lactate dehydrogenase. There was a significant antagonistic interaction between FB_1 and T-2 for relative weight of the spleen, because, when fed together, T-2 interfered with the action of FB_1 for increased spleen weight, which meets the criteria for an antagonistic effect as stated by Klaassen and Eaton (1991). There was a significant antagonistic interaction between FB_1 and T-2 for oral lesion scores because, when fed together, FB_1

TABLE 5. Effects of feeding diets containing fumonisin (FB_1) *Fusarium moniliforme* culture material or deoxynivalenol (DON) from contaminated wheat or both on relative organ weights at 21 d, Experiment 2[1]

Treatment		Liver	Kidney	Proventriculus	Gizzard	Bursa of Fabricius	Heart[2]
DON	FB_1						
(mg/kg)		(g/100 g BW)					
0	0	2.94[b]	0.50[b]	0.61[b]	2.16[c]	0.30[b]	0.80[b]
15	0	2.98[b]	0.52[b]	0.62[b]	2.48[b]	0.35[a]	0.93[a]
0	300	4.47[a]	0.62[a]	0.83[a]	2.68[ab]	0.29[b]	0.83[ab]
15	300	4.34[a]	0.63[a]	0.90[a]	2.80[a]	0.28[b]	0.79[b]
LSD[3]		0.52	0.07	0.15	0.27	0.04	0.12

[a–c]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values represent the mean of six groups of three chicks each per treatment.

[2]Significant antagonistic interaction between FB_1 and DON.

[3]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

TABLE 6. Effects of feeding diets containing fumonisin (FB_1) from *Fusarium moniliforme* culture material or deoxynivalenol (DON) from contaminated wheat or both on serum biochemical values and serum enzyme activities at 21 d, Experiment 2[1]

Treatment							
DON	FB_1	Total protein	Urea nitrogen	Cholesterol[2]	Aspartate amino-transferase[2]	Lactate dehydro-genase	Gamma glutamyl-transferase
(mg/kg)		(g/dL)	(mg/dL)		(IU/L)		
0	0	2.68[b]	1.77[b]	137[c]	162[c]	598[c]	11.9[c]
15	0	2.74[b]	1.83[b]	131[c]	160[c]	698[c]	14.0[bc]
0	300	2.98[a]	2.06[ab]	168[b]	230[b]	948[b]	15.8[ab]
15	300	3.08[a]	2.16[a]	207[a]	305[a]	1,203[a]	18.3[a]
LSD[3]		0.17	0.32	26	55	193	4

[a–c]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Values represent the mean of six groups of three chicks each per treatment.

[2]Significant synergistic interaction between FB_1 and DON.

[3]LSD = Least significant difference as determined by Fisher's protected LSD procedure.

interfered with the action of T-2 for increased oral lesion scores.

In Experiment 2, the toxicity of the combination of FB_1 and DON was expressed as reduced BW gain and decreased efficiency of feed utilization (induced by FB_1), increased relative weights of the liver, kidney, proventriculus, and gizzard (induced by FB_1), increased serum concentrations of urea nitrogen and cholesterol, and increased serum activities of aspartate, lactate dehydrogenase, and gamma glutamyltransferase. There was a significant antagonistic interaction between FB_1 and DON for relative heart weight, as FB_1 interfered with the action of DON for increased heart weights. There was a significant synergistic interaction between FB_1 and DON for serum concentration of cholesterol and serum activity of aspartate aminotransferase, as the increases in these two parameters were significantly greater than would be predicted from the combined increases of the individual toxins.

The decreased BW gains of 32% in Experiment 1 and 19% in Experiment 2 (best described as additive between FB_1 and T-2 in Experiment 1 and less than additive between FB_1 and DON in Experiment 2) and the decreased efficiency of feed utilization observed in both experiments are most likely associated with disruption of sphingolipid biosynthesis by FB_1 (Wang *et al.*, 1991; Riley *et al.*, 1993) because values were similar to those of FB_1 alone. In Experiment 1, the inhibition of protein synthesis by the toxins (Tung *et al.*, 1975; Kubena *et al.*, 1993, 1994a, 1995a,b) was also a probable contributing factor. The reduced BW gain from the combination in Experiment 2 appears to be caused by FB_1 and DON does not appear to be a contributing factor. This result is not surprising because previous studies have shown that a similar concentration of DON from contaminated wheat is borderline in terms of toxicity to young growing chicks (Kubena *et al.*, 1985, 1988, 1989b; Kubena and Harvey, 1988; Huff *et al.*, 1986).

The increased relative weights of the liver might be associated with alterations in lipid metabolism, primarily as a result of impaired lipid transport as suggested by Kubena *et al.* (1994a). The increased relative weights of the liver in chicks fed diets containing FB_1 agrees with previous reports in chicks (Ledoux *et al.*, 1992; Wiebking *et al.*, 1993a) and in turkey poults (Wiebking *et al.*, 1993b, 1994, 1995; Kubena *et al.*, 1995a,b, 1996). The increased relative weights of the kidneys observed in both experiments agrees with the reports of Ledoux *et al.* (1992) in chicks fed diets containing the FB_1 and the FB_1 and ochratoxin A experiments of Kubena *et al.* (1996) in turkeys. Brown *et al.* (1992) did not observe a difference in kidney weights of broiler chicks fed FB_1. The increased relative weights of the proventriculus and gizzard have been previously reported in chicks fed diets containing FB_1 by Ledoux *et al.* (1992) and in turkey poults fed diets containing FB_1 by Kubena *et al.* (1995a,b, 1996) and is most likely due to the overall irritative properties of the mycotoxins, typically described as focally reddened mucosa (Hoerr *et al.*, 1982a). The increased relative weights of the pancreas in chicks fed FB_1 and T-2 in combination agree with the reports in turkeys when FB_1 and T-2 or FB_1 and aflatoxin were fed in combination (Kubena *et al.*, 1995a) but not when FB_1 and DAS were fed in combination to turkeys (Kubena *et al.*, 1996) or with Experiment 2, reported herein when FB_1 and DON were fed in combination.

Oral lesions were present in all chicks fed the T-2 diet (average score of 2.23) and the combination diet (average score of 1.43). The significant decrease in the lesion score for chicks fed the combination diet when compared to the chicks fed the T-2 diet was caused partially by the decreased feed consumption and thus reduced intake of T-2. The decreased feed consumption does not totally account for the decrease in lesion score and statistical analysis indicates a significant antagonistic interaction between FB_1 and T-2.

Serum concentrations of cholesterol were increased by the toxin combinations in both experiments, whereas serum concentrations of total protein and urea nitrogen were increased only by the FB_1 and DON combination and the serum concentration of calcium was increased only by the FB_1 and T-2 combination. The increased activities of aspartate aminotransferase and lactate dehydrogenase in both experiments and gamma glutamyltransferase in the FB_1 and DON combination experiment agrees with the previous report of Javed *et al.* (1995) in chicks and the report of Kubena *et al.* (1995a,b, 1996) in turkeys. Ledoux *et al.* (1992) observed an increase in activity of only aspartate aminotransferase in chicks. These increased serum enzyme activities most likely reflect tissue damage and leakage of the enzymes into the blood (Tietz, 1976; Kubena *et al.*, 1995a,b, 1996).

These data indicate that FB_1, T-2, and DON and the FB_1 and T-2 combination and the FB_1 and DON combination can affect the health and performance of growing broiler chicks. These data also show that the effects of these two combinations may be more severe than the individual toxins for several variables, as evidenced by the fact that several variables not significantly altered by the individual mycotoxins were adversely affected by one or both mycotoxin combinations. Poultry and livestock may be more susceptible to these toxins, as well as to other mycotoxins, if nutritional, health, or other stress factors are involved; differences due to breed or strain might also occur. These factors make it difficult to dismiss the potential effects of these mycotoxins when present in combination.

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