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The Individual and Combined Effects of Fusaric Acid and T-2 Toxin in Broilers and Turkeys

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Abstract: Two experiments were conducted to evaluate the individual and combined effects of fusaric acid (FA) and T-2 toxin (T-2) in broiler chicks and turkey poults. In each experiment, 80 day-old birds were allotted randomly to a 2×2 factorial arrangement with treatments of 0 and 250 mg FA/kg feed and 0 and 4 mg T-2/kg feed. Diets were fed to 4 pen replicates of 5 birds each for 21 days. Feed intake and body weight gain of poults were reduced ($p<0.05$) by the T-2 and the FA\T-2 combination diets. Poults fed T-2 and the FA\T-2 combination diets were also less efficient ($p<0.05$) in converting feed to gain. There were no treatment effects ($p>0.05$) on performance of broilers. Poults fed FA and the FA\T-2 combination diets had increased ($p<0.05$) heart weights, whereas chicks fed FA and the FA\T-2 combination diets had increased ($p<0.05$) kidney weights. Poults fed the combination FA\T-2 diet had higher ($p<0.05$) serum AST values compared to all other treatments. Poults fed the T-2 and FA\T-2 combination diets had increased ($p<0.05$) serum Na but reduced ($p<0.05$) serum Mg. Uric acid concentrations were higher ($p<0.05$) in chicks fed the FA and FA\T-2 combination diets. Oral lesions were present in chicks (68%) and poults (100%) fed T-2 with or without FA. Data indicate no toxic synergy when FA and T-2 were fed simultaneously to broilers and turkeys at these dietary concentrations.

Key words: Broilers, poults, fusaric acid, T-2 toxin

Introduction

Fusaric Acid (FA) is one of several mycotoxins produced by the fungus *Fusarium verticillioides* (formerly *moniliforme*) that is ubiquitous on corn throughout the world (Burmeister *et al*., 1985). Fusaric acid is a hypotensive agent and is moderately toxic when compared to other *Fusarium* mycotoxins (Smith and Sousadias, 1993). In the only studies reported to date that evaluated the effects of FA in poultry, Chu *et al*. (1993) found that levels up to 150 mg FA/kg diet did not negatively affect chick performance, but did suppress cell mediated immunity in chicks. Fairchild *et al*. (2005) reported no differences in growth performance of poults fed up to 300 mg FA/kg diet for 18 days, whereas Ogunbo *et al*. (2005) reported no differences in growth performance in chicks and poults fed up to 400 mg FA/kg diet from hatch to 21 days.

T-2 toxin (T-2) is a metabolite produced by several species of fungi in the genus *Fusarium* (Bamburg *et al*., 1970) and has been shown to cause reductions in feed intake and weight gain (Wyatt *et al*., 1973; Chi *et al*., 1977; Huff *et al*., 1988). There are also several reports of T-2 contaminated feed causing dose related oral lesions in chickens (Kubena *et al*., 1989; Wyatt *et al*., 1972).

Co-contamination of FA and T-2 has been reported in corn and finishing swine rations (Smith and Sousadias, 1993). Fusaric acid has been reported to synergize the toxicity of some trichothecenes in a bioassay with caterpillars (Dowd, 1988) and has also been reported to synergize the toxicity of fumonisin B_1 in a fertile chicken egg embryo bioassay (Bacon *et al*., 1995). The objectives of this research were to determine the individual and combined effects of FA and T-2 in broiler chicks and turkey poults from hatch to 21 days of age.

Materials and Methods

Eighty day-old birds (broilers in experiment 1 and poults in experiment 2) were weighed and allotted randomly to pens in a stainless steel chick battery. Birds were maintained on a 24 h constant light schedule and allowed *ad libitum* access to feed and water. A 2×2 factorial arrangement of treatments was used with 4 pen replicates of 5 birds, assigned randomly to each of 4 dietary treatments, from hatch to day 21. Dietary treatments were: 1) 0 mg FA/kg, 0 mg T-2/kg (Control); 2) 250 mg FA/kg, 0 mg T-2 /kg; 3) 0 mg FA/kg, 4 mg T-2/kg; and 4) 250 mg FA/kg, 4 mg T-2/kg. Birds were fed a typical corn-soybean meal-based diet that contained or exceeded the levels of nutrient recommended by the NRC (1994). The T-2 was obtained from G.E. Rottinghaus, Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO. The crystalline T-2 was dissolved in an excess of acetone and mixed with 500 g of ground corn.

Table 1: Individual and combined effects of fusaric acid (FA) and T-2 on performance of broiler chicks and turkey poults from 1 to 21 days of age[1]

FA	T-2 Toxin	Feed Intake	Body Weight Gain	Feed Conversion
(mg/kg)		(g)		(g:g)
Chicks				
0	0	776	602	1.29
250	0	788	608	1.30
0	4	773	600	1.29
250	4	740	587	1.26
Pooled SEM		11	10	0.02
Source of variation		Probability		
T-2		0.0625	0.2568	0.3694
FA		0.3949	0.7145	0.5766
T-2 x FA		0.0861	0.3447	0.3972
Turkeys				
0	0	653[a]	438[a]	1.49[a]
250	0	624[a]	419[a]	1.49[a]
0	4	468[b]	287[b]	1.63[b]
250	4	432[b]	258[b]	1.68[b]
Pooled SEM		19	13	0.03
Source of variation		Probability		
T-2		0.0001	0.0001	0.0004
FA		0.1185	0.0944	0.4227
T-2 x FA		0.8511	0.7393	0.4083

[1]Data are means of four replicate pens of five birds each, [ab]Values in column, within species, with different superscripts are significantly different ($p<0.05$)

The final mixture was placed on a rotovap and the acetone evaporated to dryness, before being mixed with the rest of the diet to produce the T-2 treatments. Fusaric acid was purchased from Sigma Chemical Company, St Louis, MO. Crystalline FA was mixed with a small portion of ground corn and then mixed directly with the rest of the diet to produce the FA treatments. Diets were screened by the methods of Rottinghaus *et al.* (1982, 1992) and found to be below detection limits for the following mycotoxins: aflatoxin, fumonisin, citrinin, sterigmatocystin, zearalenone, ochratoxin A, vomitoxin and diacetoxyscirpenol.

On day 21, birds were weighed individually and feed consumption was determined for each pen of birds. Twelve birds (four replicates of 3 birds each) from each treatment were euthanatized with CO_2 gas and blood samples were collected via cardiac puncture for serum chemistry analysis. Serum biochemical values were determined using an autoanalyzer[1]. Following blood sampling, 12 birds per treatment were killed by cervical dislocation. The liver, kidney, spleen, heart, bursa of Fabricus and pancreas were removed and weighed. At the termination of the experiment, all birds were examined for the presence of oral lesions.

Data were analyzed by the General Linear Model procedure of SAS software (SAS Institute, 1985) as a 2×2 factorial. Means for treatments showing significant differences in the analysis of variance were compared using Duncan's new multiple range procedure. Statistical significance was accepted at $p<0.05$.

Results

The individual and combined effects of FA and T-2 on chick and poult performance are presented in Table 1. No interactive effects between FA and T-2 on performance were evident with respect to either the chick or turkey study. Feed intake, body weight gain and feed conversion of broilers were not affected ($p>0.05$) by dietary treatments and averaged 769 g, 599 g and 1.28 g/g, respectively. Feed intake and body weight gain were reduced ($p<0.05$) in poults fed dietary treatments containing T-2. Poults fed diets containing T-2 were also less efficient ($p<0.05$) in converting feed to gain.

No interactive effects between FA and T-2 on organ weights were evident with respect to either the chick or turkey study (Table 2). Absolute kidney weight was heavier ($p<0.05$) in chicks fed dietary treatments containing FA, whereas absolute spleen weight was lower ($p<0.05$) in chicks fed dietary treatments containing T-2 (Table 2). Absolute heart weight was significantly heavier in poults fed dietary treatments containing FA (Table 2).

Serum uric acid and aspartate aminotransferase (AST) were higher ($p<0.05$), whereas serum sodium was lower ($p<0.05$) in chicks fed diets containing FA (Table 3). Serum Na and AST was higher ($p<.05$), whereas serum Mg was lower ($p<0.05$) in poults fed diets containing T-2 (Table 3). Poults fed diets containing FA or T-2 alone had similar serum AST as the controls, however poults fed the combination FA\T-2 diet had higher serum AST in comparison to poults fed T-2 or FA

Table 2: Individual and combined effects of fusaric acid (FA) and T-2 on absolute organ weights of 21 day-old broiler chicks and turkey poults[1,2]

FA	T-2 toxin	Heart	Liver	Kidney	Spleen	Bursa
(mg/kg)		(g)				
Chicks						
0	0	4.32	17.0	5.65[b]	0.72[ab]	1.89
250	0	4.47	18.2	6.19[a]	0.80[a]	2.05
0	4	4.05	17.9	6.03[ab]	0.58[b]	1.90
250	4	4.15	18.0	6.42[a]	0.63[b]	1.69
Pooled SEM		0.22	0.5	0.15	0.11	0.05
Source of variation		Probability				
T-2		0.2029	0.5383	0.0655	0.0181	0.1139
FA		0.5690	0.2383	0.0108	0.2743	0.8126
T-2 x FA		0.9209	0.2994	0.6275	0.7560	0.1222
Turkeys						
0	0	2.66[b]	12.33	4.79	0.45	1.05
250	0	2.85[a]	12.32	4.88	0.47	1.09
0	4	2.82[b]	12.35	4.57	0.42	1.00
250	4	2.96[a]	12.90	4.54	0.40	0.97
Pooled SEM		0.07	0.23	0.10	0.02	0.08
Source of variation		Probability				
T-2		0.2953	0.4351	0.1078	0.1573	0.5489
FA		0.0465	0.2284	0.7124	0.8525	0.9611
T-2 x FA		0.6690	0.1876	0.4857	0.2460	0.6169

[1]Data are means of four replicate pens of three birds each, [2]Absolute organ weights adjusted for final body weight by analysis of covariance, [ab]Values in column, within species, with different superscripts are significantly different ($p<0.05$)

Table 3: Individual and combined effects of fusaric acid (FA) and T-2 on serum biochemistry of 21 day-old broiler chicks and turkey poults[1]

FA	T-2 toxin	TP	UA	Na	Mg	AST
(mg/kg)		(g/dL)	(mg/dL)	(mmol/L)	(mg/dL)	(U/L)
Chicks						
0	0	2.13	12.53[b]	135[ab]	4.04	198[b]
250	0	2.28	17.27[a]	132[b]	4.00	279[a]
0	4	2.19	12.66[b]	138[a]	3.87	229[ab]
250	4	2.03	17.36[a]	133[b]	4.02	257[ab]
Pooled SEM		0.08	0.97	1	0.18	24
Source of variation		Probability				
T-2		0.3019	0.9132	0.0768	0.6918	0.8402
FA		0.9229	0.0009	0.0060	0.7574	0.0449
T-2 x FA		0.1067	0.9866	0.4526	0.5984	0.2864
Turkeys						
0	0	2.91	14.1	133[b]	6.76[a]	296[bc]
250	0	2.92	15.2	133[b]	6.49[a]	251[c]
0	4	2.99	13.0	135[a]	5.74[b]	348[b]
250	4	2.91	13.5	135[a]	5.39[b]	453[a]
Pooled SEM		0.09	0.9	0.6	0.18	26
Source of variation		Probability				
T-2		0.7013	0.1511	0.0017	0.0003	0.0008
FA		0.7013	0.4061	0.9420	0.1282	0.2724
T-2 x FA		0.6400	0.7622	0.7171	0.8260	0.0184

[1]Data are means of four replicate pens of three birds each, [abc]Values in column, within species, with different superscripts are significantly different ($p<0.05$), TP = total protein; UA = uric acid; Na = sodium; Mg = magnesium; AST = aspartate aminotransferase

alone, resulting in a significant T-2 by FA interaction for AST (Table 3). This was the only T-2 by FA interaction observed for serum biochemical and mineral values in both broilers and turkeys.

Oral lesions were present in 75% and 60% of the chicks fed T-2 and the combination T-2\FA diet, respectively. Oral lesions were present in 100% of the poults receiving diets containing T-2 and the lesions were more severe than those observed in the chicks.

Discussion

The trichothecenes are a group of potent mycotoxins, with T-2 being among the most toxic of this group. Depressions in body weight gains of poults observed in the T-2 treatment group is consistent with a previous study in which turkey poults were fed a diet containing 2 mg/kg T-2 from day 8 to day 28 (Richard *et al.*, 1978). The lack of a T-2 effect on body weight gains of broiler chicks is in contrast to several reports indicating

reduced weight gains in broilers fed this level of T-2 (Wyatt *et al*., 1972, 1973; Chi *et al*., 1977; Kubena *et al*., 1989). However, these results are consistent with a previous study by Kubena *et al*. (1989) who also observed no negative effects on body weight gains of broilers fed 4 mg/kg T-2 from hatch to 21 days. Wyatt *et al*. (1973) suggested that severe oral lesions in birds, caused by T-2, impair their ability to eat resulting in reduced weight gains. In the present study, only 60-75% of the chicks had oral lesions compared to 100% of the turkey poults.

In these experiments, the lack of a FA effect on performance of broilers and poults is similar to that observed in previous studies with FA with chicks (Chu *et al*., 1993; Ogunbo *et al*., 2005) and poults (Fairchild *et al*., 2005; Ogunbo *et al*., 2005). These results confirm previous reports indicating that FA acid is only moderately toxic to animals and is the reason why FA has been classified as a phytotoxin rather than a mycotoxin (Burmeister *et al*., 1985; Matsuo, 1983).

The increase in kidney weights of chicks fed diets containing FA is inconsistent with a previous report (Ogunbo *et al*., 2005) in which FA decreased absolute kidney weights of broiler chicks. The increase in heart weights of turkeys fed FA was not observed in a previous study in which poults were fed FA at levels up to 400 mg/kg (Ogunbo *et al*., 2005).

The decrease in spleen size in chicks fed diets containing T-2 have been observed previously in chicks (Wyatt *et al*., 1973) fed diets containing 4 mg/kg T-2. Wyatt *et al*. (1973) reported that, in chicks, the spleen and pancreas were the two organs that were most sensitive to T-2. A similar decrease in spleen weight was not observed in turkeys in the present study or in a previous study by Richard *et al*. (1978) and may reflect a species difference.

The failure of T-2 to affect total serum protein in chicks is consistent with some reports (Wyatt *et al*., 1973; Chi *et al*., 1977; Richard *et al*., 1978), but inconsistent with other reports indicating that T-2 decreased serum total protein (Kubena *et al*., 1989, 1990).

There are fewer reports with respect to T-2 effects on serum protein in turkey poults. In contrast to the present report, Richard *et al*. (1978) observed an increase in serum total protein in turkeys fed 10 mg/kg T-2 for 4 weeks. These results are difficult to interpret since the tricothecenes (including T-2) are known to be the most potent inhibitors of eukaryotic protein synthesis (Leeson *et al*., 1995).

A slight increase in uric acid observed in chicks fed FA suggests that renal function was mildly affected at this dietary FA concentration. A similar increase in uric acid was not observed in turkeys. Although FA decreased serum sodium concentrations in chicks and T-2 increased serum sodium in turkeys the values were not greatly different from controls and they all fell within the normal physiological range (Ritchie *et al*., 1994). Decreases in serum Mg in turkeys fed FA have not been reported previously and a similar decrease was not observed in chicks.

In chicks, serum AST was slightly increased by FA inclusion in the diet and this was not observed in turkeys. There was a significant FA/T-2 interaction for AST in turkeys. Turkeys fed FA alone or T-2 toxin alone had AST values that were not different from controls, however chicks fed the combination FA/T-2 diet had significantly higher AST values than controls. The magnitude of all AST level changes among treatments was small and may not be indicative of significant hepatic disease.

The results of this study support earlier works on oral lesions in birds receiving T-2 diets. While no lesions were seen in birds receiving FA diets, oral lesions were present in those fed T-2 toxin alone and in combination with FA in similar proportions. This suggests the oral lesions to be a direct effect of T-2 toxin alone, as severe oral lesions due to the consumption of T-2 have been reported by several researchers (Wyatt *et al*., 1972, 1973; Huff *et al*., 1988; Kubena *et al*., 1989). The fact that 100% of poults fed diets containing T-2 had oral lesions and the lesions were more severe, suggests that poults were unable to consume a normal amount of feed, resulting in poor growth performance.

Dowd (1988) had suggested that FA can synergize the toxicity of the trichothecenes and T-2 is one of the most potent mycotoxins in this group. However, the above findings indicate no synergistic effects when FA and T-2 were fed in combination to broiler chicks and young turkey poults at the dietary concentrations of 250 mg FA/kg and 4 mg T-2/kg. Based on the results of these experiments, turkey poults appear to be more susceptible to T-2 than chicks. The greater susceptibility of turkeys to T-2 when compared to chicks has been reported previously (Richard *et al*., 1978). In addition, these data indicate that T-2 is more toxic to poultry than FA.

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