Chronic Effects of Fumonisin B<sub>1</sub> in Broilers and Turkeys Fed Dietary Treatments to Market Age<sup>1</sup>

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ABSTRACT Floor pen studies were conducted with 270 broiler chicks and 144 turkey poults, all 1 wk old, to evaluate the chronic effects of fumonisin B<sub>1</sub> (FB<sub>1</sub>). A completely randomized design was used in both studies with six pen replicates of 15 chicks or eight pen replicates of six poults assigned to each of three dietary treatments from Weeks 1 to 7 (broilers) or to Week 14 (turkeys). Fusarium moniliforme (M-1325) culture material was added to a typical corn-soybean basal diet to supply 0, 25, or 50 mg FB<sub>1</sub>/kg diet. Feed intake, body weight gain, and feed conversion of chicks were not affected (P > 0.05) by FB<sub>1</sub>. Turkeys fed 50 mg FB<sub>1</sub>/kg had significantly (P < 0.05) lower feed intake than the controls. Compared with controls, chicks and turkeys fed FB<sub>1</sub> diets had significantly higher liver sphinganine to sphingosine ratios (P < 0.05). Relative organ weights of chicks were not affected (P > 0.05) by FB<sub>1</sub>, other than those chicks fed 25 mg FB<sub>1</sub>/kg, which had lower (P < 0.05) relative proventriculus weights than the chicks fed 0 or 50 mg FB<sub>1</sub>/kg. Broilers fed 50 mg FB<sub>1</sub>/kg had decreased serum calcium and increased serum chloride when compared to broilers fed 0 or 25 mg FB<sub>1</sub>/kg. Hematology was not affected (P > 0.05) by dietary FB<sub>1</sub>. No lesions were present in any organ examined microscopically. Results indicate that 50 mg FB<sub>1</sub>/kg diet is detrimental to turkeys but is not toxic to broilers fed to market age.

(Key words: poult, chick, fumonisin B<sub>1</sub>, chronic toxicity, Fusarium moniliforme)

INTRODUCTION Fusarium moniliforme has been shown to produce the mycotoxin fumonisin B<sub>1</sub> (FB<sub>1</sub>). Fumonisins are a new group of mycotoxins that were first isolated and chemically characterized by Gelderblom et al. (1988) and the structures elucidated by Bezuidenhout et al. (1988). Two disease syndromes that are caused by fumonisin toxicity are equine leukoencephalomalacia (Marasas et al., 1988b) and porcine pulmonary edema (Harrison et al., 1990). Fumonisin B<sub>1</sub> has been shown to be carcinogenic (Gelderblom et al., 1988) and hepatoxic (Voss et al., 1989) in rats and has been associated with esophageal cancer in humans (Marasas et al., 1988a). Broilers and turkeys are relatively resistant to FB<sub>1</sub> toxicity but do exhibit poor performance, increased organ weights, and increased sphinganine to sphingosine (SA:SO) ratio. Turkeys also exhibit hepatic hyperplasia (Weibking et al., 1994), and broilers exhibit multifocal hepatic necrosis (Weibking et al., 1993a) at high levels of FB<sub>1</sub>.

The SA:SO ratio is the most sensitive biomarker of FB<sub>1</sub> toxicity in many animal species (Merrill et al., 1993; Riley et al., 1993). Sphingolipid inhibition has been proposed as the mechanism of toxicity of FB<sub>1</sub> (Wang et al., 1991). Fumonisin B<sub>1</sub>, at levels as low as 20 mg/kg, has been shown to cause a significant increase of SA:SO ratios in broilers fed purified FB<sub>1</sub> (Henry et al., 2000). Increases in SA:SO ratios have been observed in turkey poult fed 25 mg FB<sub>1</sub>/kg (Ledoux et al., 1996), in broilers and turkeys fed 75 mg FB<sub>1</sub>/kg (Weibking et al., 1993a; Weibking et al., 1994), and in catfish fed 20 mg FB<sub>1</sub>/kg (Yildirim et al., 2000).

Chronic effects of FB<sub>1</sub> have been studied in turkey poult fed 75 mg FB<sub>1</sub>/kg diet for 18 wk (Bermudez et al., 1996) and laying hens fed 100 or 200 mg FB<sub>1</sub>/kg for 420 d (Kubena et al., 1999). Decreased body weight gain and enlarged livers were present in turkeys fed 75 mg FB<sub>1</sub>/kg (Bermudez et al., 1996). However, 100 and 200 mg FB<sub>1</sub>/kg did not have an effect on body weight gain (at 420 d) or relative liver weight (at 112 d) in laying hens (Kubena et al., 1999). A FB<sub>1</sub> survey of corn samples from...
1992 to 1999, collected from University of Missouri yield test plots, indicated that 29.4 and 4.7% of the samples were positive for FB1 at levels ranging from 1 to 5 and 5 to 10 mg FB1/kg, respectively. Some (2.3%) of the samples contained over 10 mg FB1/kg (Rottinghaus, 1999, College of Veterinary Medicine, University of Missouri, Columbia, MO 65205, unpublished data). Results of this survey suggest that FB1 is a common contaminant of corn and that there is the potential for much higher levels of FB1 to be present in corn, especially in poorer grades.

The objective of the present study was to investigate the chronic effects of feeding low levels of FB1 to broilers and turkeys, raised on litter to market age. Response variables used to evaluate toxicity included performance, relative organ weights, serum chemistry, liver SA:SO ratios, hematology, and histopathology.

**MATERIALS AND METHODS**

**Experimental Design and Birds**

Two experiments were conducted using 270 1-wk-old male Cornish cross broiler chicks and 144 1-wk-old Nicholas turkey pouls. Birds were raised on litter in floor pens (5 ft. × 4 ft.) in a commercial-type facility. A complete randomized design was used in both studies with six pen replicates of 15 chicks or eight pen replicates of six pouls allotted randomly to each dietary treatment. Day-old chicks and pouls were purchased from commercial hatcheries and fed an NRC-type diet (National Research Council, 1994) until Day 7. In the chick study, birds were fed experimental diets from Days 7 to 49, whereas in the turkey study, birds were fed diets from Days 7 to 98. Birds were monitored daily for signs of morbidity and mortality, and birds that were obviously sick were removed and euthanized. The animal care and use protocol was reviewed and approved by the University of Missouri Animal Care and Use Committee.

**Fumonisin Production and Diet Preparation**

Dietary treatments were prepared by substituting ground *F. moniliforme* M-1325 culture material for Celulose® (nonnutritive filler) in a typical corn-soybean meal basal diet, with Coban 60® supplying monensin at 90 g/ton feed. Production of fumonisin culture material was reported previously by Weibking et al. (1993a). Culture material was analyzed for FB1, FB2, and FB3 by the HPLC procedure of Wilson et al. (1990). Fumonisin culture material contained 6,500 mg FB1/kg by analysis and made up 0, 0.38, and 0.77% of the diets and supplied 0, 25, and 50 mg FB1/kg diet, respectively. Dietary FB1 concentrations were confirmed by analysis. Diets were formulated to be isocaloric and isonitrogenous and met or exceeded the nutrient requirements of broilers and turkeys as recommended by the National Research Council (1994). Diets were screened by the method of Rottinghaus et al. (1982) and found to be free of aflatoxin, citrinin, vomitoxin, zearalenone, ochratoxin A, T-2 toxin, diacetoxyscirpenol, and moniliformin (Ledoux et al., 1995).

**Sample Collection**

At the end of Weeks 3, 6, and 7 (broilers) or Weeks 3, 6, 9, 12, and 14 (turkeys) of the experiments, birds were weighed and feed consumption was determined for each pen. On Day 49 (broilers) or 98 (turkeys), birds were anesthetized with carbon dioxide gas, and blood samples were collected via cardiac puncture from three birds per replicate (18 and 24 per treatment from broilers and turkeys, respectively) for serum biochemistry (in 4.5-mL serum tubes), and hematochemical determinations (in 4.5-mL lithium-heparin tubes). Serum was analyzed for glucose, calcium, phosphorus, uric acid, cholesterol, albumin, total protein, globulin, sodium, potassium, chloride, aspartate aminotransferase (AST), alkaline phosphatase (ALKP), and γ-glutamyltransferase (GGT) by using an autoanalyzer. Hemoglobin was measured as cyanmethemoglobin, using spectrophotometry. Red blood cell (RBC) counts, mean corpuscular volumes (MCV), and hematocrits were analyzed with a coulter counter using instrument settings described by Steel et al. (1977). Mean corpuscular hemoglobin concentrations (MCHC) and mean corpuscular hemoglobin concentrations (MCHC) were calculated. After blood sampling, the same 18 broilers and 24 turkeys per treatment were euthanatized by cervical dislocation, and liver, heart, kidney, spleen, bursa of Fabricius, gizzard, pancreas, and proventriculus were excised and weighed. Liver samples were immediately put on dry ice for later determination of SA:SO ratios. Livers were analyzed for sphinganine and sphinosine levels by HPLC, using procedures described by Merrill et al. (1988).

**Histopathology**

Postmortem examinations were performed on five broilers and turkeys from each treatment group at 7 (broilers) and 14 (turkeys) wk of age. After the birds were euthanatized with carbon dioxide, samples of brain, liver, heart, lung, kidney, spleen, bursa of Fabricius, thymus, esophagus, ventriculus, proventriculus, pancreas, and jejunum were excised from each bird and fixed in 10% neutral-buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin. Tissue sections from all treatment groups were examined microscopically.
TABLE 1. Chronic effects of fumonisin B₁ (FB₁) on performance of 7-wk-old broilers

<table>
<thead>
<tr>
<th>FB₁ level (mg/kg)</th>
<th>Feed intake (g)</th>
<th>Body weight gain (g)</th>
<th>Feed conversion (g:g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5831</td>
<td>3052</td>
<td>1.91</td>
</tr>
<tr>
<td>25</td>
<td>5841</td>
<td>3027</td>
<td>1.93</td>
</tr>
<tr>
<td>50</td>
<td>5807</td>
<td>3002</td>
<td>1.93</td>
</tr>
<tr>
<td>SEM</td>
<td>42</td>
<td>33</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data are means of six replicate pens of 15 broilers each.

Statistical Analysis

Data were analyzed by one-way analysis of variance using the general linear models procedure of SAS (SAS Institute, 1985). Mean differences were determined using Fisher’s protected least-significant difference test. Statistical significance was accepted at \( P < 0.05 \), unless stated otherwise.

RESULTS AND DISCUSSION

Overall, 1-to-7-wk feed intake, body weight gain, and feed conversion were not affected by dietary FB₁ in broilers (Table 1). There was no significant effect of dietary FB₁ on body weight gain, feed intake, or feed conversion in broilers fed any treatment, during any time period. As in the present study, no significant differences in overall body weights were observed in previous chronic studies with laying hens fed diets containing 100 or 200 mg FB₁/kg for 15 laying cycles of 28 d (Kubena et al., 1999) or for male turkeys fed 75 mg FB₁/kg for 18 wk (Bermudez et al., 1996).

The effects of dietary FB₁ on turkey performance are presented in Table 2. Compared to controls and turkeys fed 25 mg FB₁/kg, significant decreases in feed intake were observed in turkeys fed 50 mg FB₁/kg at Weeks 10 to 12 and for the overall 1-to-14-wk period. At Weeks 4 to 6 and 10 to 12, body weight gains were significantly reduced in turkeys fed 50 mg FB₁/kg when compared to controls. Reduction in overall (1-to-14-wk) body weight gain observed in turkeys fed 50 mg FB₁/kg was not significant (\( P < 0.06 \)) when compared to controls. Bermudez et al. (1996) also reported decreases in body weights, of male turkeys fed FB₁ for 18 wk, during certain periods. However, overall 18-wk body weights were not affected by FB₁. During Weeks 4 to 6 in the present study, feed conversion was poorer in turkeys fed 50 mg FB₁/kg when compared to controls. However, during Weeks 7 to 9, controls had a poorer feed conversion than turkeys fed 50 mg FB₁/kg. Levels of FB₁ used in the present study have not been reported to affect performance of broiler chicks or turkey pouls when fed for shorter periods. In acute titration studies with broilers (Weibking et al., 1993a) and turkeys (Ledoux et al., 1996), the levels of FB₁ that caused a decrease in feed intake and body weight gain was 450 and 325 mg FB₁/kg, respectively. Diarrhea was not observed in this study or in previous acute FB₁ chick (Weibking et al., 1993a) and turkey (Ledoux et al., 1996) studies in which birds were fed low levels (<10%) of culture material.

Liver SA:SO ratios are summarized in Table 3. Compared with controls, increased liver SA:SO ratios were observed in broilers and turkeys fed diets that supplied 25 and 50 mg FB₁/kg, with turkeys fed 50 mg FB₁/kg having higher SA:SO ratios than turkeys fed 25 mg FB₁/kg. Similar levels of FB₁ have also been shown to cause an increase in SA:SO ratios in broilers fed purified FB₁ (20, 40, or 80 mg/kg) for 21 d (Henry et al., 2000). Increases in SA:SO ratios have also been observed previously in turkey pouls fed 25 and 75 mg FB₁/kg (Ledoux et al., 1996) and broilers and turkeys fed 75 mg FB₁/kg (Weibking et al., 1993a; Weibking et al., 1994) from culture material for shorter periods of time. Wang et al. (1991) found that FB₁ inhibition of de novo sphingolipid biosynthesis resulted in increased SA:SO ratios and proposed...
that this inhibition may be the initial molecular target of
these toxins. The SA:SO ratios increased as a consequence
of the accumulation of SA in tissues and serum resulting
from FB1 inhibition of N-acyltransferase. Wang et al.
(1992) hypothesized that the depletion of complex sphin-
golipids (resulting from FB1 inhibition of biosynthesis)
and accumulation of sphinganine may well be the cause
of pathology observed in FB1 toxicosis, because these
sphingolipids are involved in regulation of cell surface
receptors, ion pumps, and other systems vital for cell
function and survival. However, the mechanism by which
these alterations in sphingolipids cause tissue damage is
still not known.

No significant differences were observed in the relative
liver, heart, kidney, pancreas, bursa of Fabricius, gizzard,
or spleen weights of broilers and turkeys fed FB1 (data
not shown). Relative proventriculus weights were lower
in chicks fed 25 mg FB1/kg compared with controls or
birds fed 50 mg FB1/kg but were not affected in turkeys
fed either level of FB1 (Table 3). Similar to the present
study, Henry et al. (2000) did not observe an effect on
relative organ weights by feeding chicks 20, 40, or 80 mg/
kg of purified FB1 for 21 d. In titration studies with broilers
(Weibking et al., 1993a), the levels of FB1, from culture
material, that caused increases in relative liver, kidney,
and proventriculus weights were 450, 375, and 525 mg
FB1/kg, respectively. In a 21-d titration study with tur-
keys, levels of FB1 that caused increases in relative liver
weights were 25, 50, or ≥175 mg FB1/kg; however, relative
liver weights of turkeys fed 75 or 100 mg FB1/kg were
not significantly different from the controls (Ledoux et
al., 1996).

Increases in relative liver weights have been observed in
turkeys fed 75 mg FB1/kg (Weibking et al., 1994) or
200 mg FB1/kg (Bermudez et al., 1997) for 21 d and 75
mg FB1/kg for 18 wk (Bermudez et al., 1996). With results
of this study and that of Bermudez et al. (1996), the level
of FB1 that would probably cause a consistent effect on
relative liver weight in turkeys is between 50 and 75 mg
FB1/kg. In a chronic FB1 toxicity study with laying hens,
no significant increase in relative liver weights were ob-
served in hens fed 100 or 200 mg FB1/kg for 112 d (Kubena
et al., 1999). However, significant increases in relative
liver and spleen weights have been reported in broilers
fed 10 mg/kg purified FB1 for 8 d and then fed uncontamin-
ated feed until Day 42, and in broilers fed 300 mg FB1/
kg from culture material for 10 d (Espada et al., 1994).

Histopathology results indicated that no lesions were
found in the brain, liver, heart, lung, kidney, spleen, bursa
of Fabricius, thymus, esophagus, ventriculus, proventric-
ulus, pancreas, or jejunum of controls or birds fed dietary
FB1. Liver lesions and increased liver weights observed in
previous studies indicate that the liver is the primary
site of FB1 toxicity in poultry (Weibking et al., 1993a;
Weibking et al., 1994; Ledoux et al., 1996; Bermudez et
al., 1997). Results of this study indicate that these levels
of FB1 (≥250 mg/kg) did not cause liver pathology when
fed for 18 wk.

Serum chemistry results are presented in Table 4. Tur-
keys fed 25 and 50 mg FB1/kg had increased serum so-
dium levels when compared to the controls. Chicks fed 50
mg FB1/kg had decreased serum calcium and increased
serum chloride levels when compared to the controls and
birds fed 25 mg FB1/kg. An increase in serum sodium has
previously been reported in broilers fed 125 or 274
mg/kg purified FB1 for 14 d (Javed et al., 1995). Unlike
the present broiler experiment, there was no effect on
serum calcium or chloride in broilers fed 20, 40, or 80 mg/
kg purified FB1 for 21 d (Henry et al., 2000). In contrast to
the present chick experiment, an increase in serum cal-
cium and a decrease in serum sodium were reported in
broilers fed 300 mg FB1/kg for 10 d (Espada et al., 1994).

Fumonisin B1 did not significantly affect serum glucose,
phosphorus, uric acid, cholesterol, albumin, total protein,
globulin, potassium, AST, ALKP, or GGT (data not
shown). ALKP was also not affected in previous experi-
ments with turkeys (Bermudez et al., 1997) and laying
hens (Kubena et al., 1999) fed 200 mg FB1/kg or broilers
fed 20, 40, or 80 mg/kg purified FB1 (Henry et al., 2000).
An increase in AST and liver lesions and a decrease in
ALKP have been observed in turkeys fed 100 mg FB1/kg
(Weibking et al., 1993b). Increases in the serum enzymes
GGT, lactate dehydrogenase, and AST that occurred in
broilers fed 30 or 300 mg FB1/kg from cultural material
were suggested to be linked to liver damage (Espada et
al., 1994). The lack of effect of FB1 on serum enzymes, in

### Table 3. Chronic effects of fumonisin B1 (FB1) on liver sphinganine to sphingosine (SA:SO) ratios and relative proventriculus weights of 7-wk-old broilers and 14-wk-old turkeys

<table>
<thead>
<tr>
<th>FB1 level (mg/kg)</th>
<th>Broiler</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SA:SO ratio</td>
<td>Proventriculus (g/100 g BW)</td>
</tr>
<tr>
<td>0</td>
<td>0.19±</td>
<td>0.28±</td>
</tr>
<tr>
<td>25</td>
<td>0.52±</td>
<td>0.25±</td>
</tr>
<tr>
<td>50</td>
<td>0.59±</td>
<td>0.29±</td>
</tr>
<tr>
<td>SEM</td>
<td>0.03</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**aValues within columns with no common superscript differ significantly (P < 0.001).**
1Data are means of eight livers per treatment.
2Data are means of six replicate pens of three broilers each.
3Data are means of five livers per treatment.
4Data are means of eight replicate pens of three turkeys each.
the present studies, is in agreement with histopathology, in that no liver lesions were observed in chicks or turkeys fed FB1.

No significant effects were observed in hemoglobin, RBC counts, hematocrit, MCV, MCH, or MCHC in broilers or turkeys (data not shown). In a previous broiler study, RBC count, hematocrit, hemoglobin, and MCV were not affected by ≤525 mg FB1/kg (Weibking et al., 1993a). However, broilers fed 450 and 525 mg FB1/kg had increased MCH and MCHC (Weibking et al., 1993a). Javed et al. (1995) observed a decrease in hemoglobin in broilers fed 125 or 274 mg/kg purified FB1; at Days 7 and 14. In a previous turkey study, no effects were observed in MCV, MCH, and MCHC and RBC count, hematocrit, and hemoglobin effects did not occur until turkeys were fed >400 mg FB1/kg (Ledoux et al., 1996). However, Weibking et al. (1993b) have shown decreases in MCV and MCH in turkeys fed 200 mg FB1/kg but no effect on RBC count, hemoglobin, hematocrit, and MCHC. Hemoglobin has been observed to increase in turkeys fed lower levels of FB1 (75 mg/kg) for 21 d (Weibking et al., 1994). Ledoux et al. (1996) concluded that the inconsistency in hematology results among experiments suggests that hematology is not a sensitive indicator of FB1 toxicity.

In conclusion, based on performance, 50 mg FB1/kg is detrimental to turkeys but is not toxic to broilers fed to market age. Liver sphingolipid results indicate that 25 and 50 mg FB1/kg causes an increase in the SA:SO ratio, but no significant toxic effect was observed with this change in the SA:SO ratio. Changes in the SA:SO ratio with no concurrent pathology suggests that this ratio is an extremely sensitive biomarker for exposure to FB1 but may not be a good indicator of clinically significant disease in poultry.

Based on the response variables evaluated, FB1 (alone) does not appear to pose a threat to the poultry industry. This conclusion was made after a review of worldwide FB1 surveys of commodities for human or animal consumption. With the exception of a few corn screening and moldy corn samples that contained up to 330 mg/kg FB1, most feed, food, and corn samples contained FB1 ranging from 0 to 19 mg/kg, with 78% of the samples evaluated ranging from 0 to 10 mg/kg (Broomhead, 2000).

According to FDA guidance levels (U.S. Food and Drug Administration, 2001), the recommended maximum allowable level of total fumonisins in a poultry diet is 50 mg/kg. According to the present research, the maximum allowable FB1 level in turkeys diets should be between 25 and 50 mg FB1/kg, which corresponds to between 33 and 66 mg/kg total fumonisins. The FDA guidance level was determined using data from 21-d studies, whereas the present research was conducted for a longer period (until market age). However, it should be noted that with the volume of feed produced and consumed in modern poultry operations it is highly unlikely that any poultry flock would be fed rations containing such high levels of FB1 for extended time periods (>3 wk).

### REFERENCES


containing moniliformin, supplied by Fusarium fujikuroi culture material, and fumonisin, supplied by Fusarium moniliforme culture material, to laying hens. Poultry Sci. 78:1499–1505.


