Chronic Effects of Fumonisin B₁ on Ducks

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ABSTRACT

Partially purified fumonisin B₁ (FB₁) was orally administrated for 77 d to 5 groups of 8 mule ducks starting at 7 d of age; the concentrations corresponded to 5 diets containing 0, 2, 8, 32, and 128 mg of FB₁/kg of feed. No mortality was observed, and no effects on feed consumption and body weight gain were observed at the end of the treatment period. But, surprisingly, FB₁ ingested at 32 and 128 mg/kg led to decreased body weight from d 28 to 63 and from d 7 to 63, respectively. FB₁ had no effect on the relative weight of heart and breast muscle, whereas a significant increases in the relative weights of gizzard, spleen, and liver were measured in ducks receiving 32 and 128 mg of FB₁/kg of feed without evidence of detectable microscopic modification of these organs. FB₁ had no significant effect of the serum aspartate aminotransferase and γ-glutamyltransferase levels but increased serum total protein, cholesterol, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase levels when 128 mg of FB₁/kg of feed was given. Serum, liver, and kidney sphinganine to sphingosine ratio was significantly increased in ducks fed 8 to 128 mg of FB₁/kg of feed. The biggest increase was observed in kidneys, suggesting that this organ is the most sensitive to detect FB₁-induced disruption of sphingolipid metabolism.

(The key words: fumonisin B₁, mycotoxin, duck, sphinganine, sphingosine)

INTRODUCTION

Fumonisin B₁ (FB₁) is the major mycotoxin produced by Fusarium verticillioides and Fusarium proliferatum fungi that are widely found to contaminate corn and corn screenings (Gelderblom et al., 1992). This mycotoxin has been linked to human esophageal cancer (Yoshizawa et al., 1994; Marasas, 1995; Ueno et al., 1997; Groves et al., 1999) and is reported to be carcinogenic to rodents (Gelderblom et al., 1991). In animals, 2 syndromes caused by FB₁ are equine leukoencephalomalacia (Marasas et al., 1994; Marasas, 1995; Ueno et al., 1997; Groves et al., 1999) and porcine pulmonary edema (Harrison et al., 1990). Hepatic and renal toxicity are also observed in several species, including lambs, rats, broilers, turkeys, and ducks (Brown et al., 1992; Ledoux et al., 1992; Weibking et al., 1993a,b, 1995; Espada et al., 1994, 1997; Bermudez et al., 1995, 1996, 1997).

Avian species are relatively resistant to fumonisin toxicity, and subacute exposure to high levels of FB₁ has been associated with poor performance, increased relative organ weights, and alterations in serum constituents and enzyme activities in broiler chicks, turkeys, and ducks (Weibking et al., 1993a, 1994; Kubena et al., 1995a, 1997a,b; Bailly et al., 2001; Raynal et al., 2001; Tran et al., 2003; Tardieu et al., 2004). These effects are associated with altered sphingolipid biosynthesis and increased sphinganine to sphingosine (Sa/So) ratio. This ratio is now considered as the most sensitive biomarker for FB₁ exposure in all species investigated, including avians (Weibking et al., 1993a; Ledoux et al., 1996; Henry et al., 2000; Bailly et al., 2001; Broomhead et al., 2002; Tran et al., 2003; Tardieu et al., 2004). Unfortunately, the link between FB₁-toxicity and FB₁-increased Sa/So ratio is not obvious at least in avian species.

Although subacute toxicity of FB₁ has been frequently investigated in avian species, the chronic effects of FB₁ are less understood. Previous studies conducted with turkeys for 14 and 18 wk demonstrated that 50 and 75 mg of FB₁/kg of feed, respectively, are detrimental to animal performance (Bermudez et al., 1996; Broomhead et al., 2002). By contrast, FB₁ does not have an effect on body weight gain of broilers fed 50 mg of FB₁/kg to market age (7 wk; Broomhead et al., 2002) or of laying hens fed 200 mg of FB₁/kg for 420 d (Kubena et al., 1999).

The objective of the present study was to investigate the chronic effects of feeding different levels of FB₁ to mallard ducks from 1 to 12 wk of age. Four levels of FB₁ were investigated to take into account the large distribution of the contamination of feeds. Response variables used to evaluate toxicity included body weight, feed con-
sumption, relative organ weights, serum chemistry, histopathology, and serum, liver, and kidney Sa/So ratios.

MATERIALS AND METHODS

Chemicals and Fumonisin Production

Fumonisins were produced as previously described by using a highly toxicogenic strain of F. verticillioides (NRRL-3428) isolated from corn associated with an acute case of equine leukoencephalomalacia (Bailly et al., 1996). Briefly, autoclaved rice was inoculated with 1 cm² of 1-week subculture on PDA. Flasks were incubated for 5 wk at 20°C. Rice cultures were extracted by mechanical agitation overnight with acetonitrile/water (vol/vol). The extracts were filtered, concentrated by acetonitrile evaporation, and analyzed for fumonisins by HPLC according to Rice et al. (1995). The reference standard for FB₁ was purchased. The purity of the crude extract was of 54% FB₁, 8% FB₂, and 9% FB₃. Twenty-nine percent of the extracts were constituted by rice pigments, and they were present for control and treated animals. This extract was diluted in water before administration to birds. The absence of other fusariotoxins was tested (moniliformin, fumarsin C). Aflatoxin B₁, ochratoxin A, zearalenone, T₂ toxin, and deoxynivalenol were tested using a Veratox quantitative test kit. All other chemicals and reagents were of the highest grade available. They were purchased from Scharlau⁴ and Sigma Chemical Co. In all studies, distilled deionized water was used.

Treatments of Birds and Sample Collection

All experimental procedures with birds were in accordance with the French National Guidelines for the care and use of animals for research purposes. Forty mule ducks, 1 d of age, were allowed to acclimate with free access to feed and water for 1 wk prior to initiation of the study. The feed was tested for mycotoxin concentrations. Aflatoxin B₁, ochratoxin A, zearalenone, T₂ toxin, and deoxynivalenol were tested using a Veratox quantitative test kit. All other chemicals and reagents were of the highest grade available. They were purchased from Scharlau⁴ and Sigma Chemical Co.⁵ In all studies, distilled deionized water was used.

Biochemistry

Serum concentration of alanine aminotransferase EC 2.6.1.2, lactate dehydrogenase EC 1.1.1.27, alkaline phosphatase EC 3.1.3.1, aspartate aminotransferase EC 2.6.1.1, and γ-glutamyltransferase EC 2.3.2.2 were analyzed with a clinical chemistry analyzer⁶ according to international guidelines; values were expressed units per liter. Cholesterol was measured by enzymatic reaction, and protein was determined by using a Biuret modified method according to Vitros Chemistry recommendations.⁷

Sa/So Ratio Determination

Free sphinganine and free sphingosine were determined in bird serum, liver, and kidney by HPLC according to Riley et al. (1994b). Briefly, 0.2 nmol of C₂₀ sphinganine⁸ were added to 100 µL of serum or tissues homogenates. Lipids were extracted by alkaline methanol-chloroform and further hydrolyzed to liberate free sphinganine and sphingosine. The chloroform phase was then washed twice with alkaline water. Samples were dried and suspended in 20 µL of methanol. Extracts were derivatized with orthophtaldialdehyde and sonicated for 10 min before injection. Sphinganine, sphingosine, and C₂₀ sphinganine contents were determined by HPLC using an ICS M2200 solvent delivery module⁹ connected with a programmable fluorescence detector.¹⁰ Operating conditions were analytical Radial-Pak cartridge packed with Nova-Pak C18 and a C18 precolumn filter,¹¹ liquid phase: methanol-water (90:10), flow rate: 1.25 mL/min, excitation wavelength: 335 nm, emission wavelength: 440 nm. Every day a standard solution containing known amounts of sphingosine, sphinganine, and C₂₀ sphinganine mixture was run to verify column performance and stability of the orthophtaldialdehyde reagent. Mean retention times were 12, 17, and 29 min for sphingosine, sphinganine, and C₂₀ sphinganine, respectively.

Histopathology

Postmortem examinations were performed on each duck at the end of treatment. Ceca and liver samples were fixed in 10% neutral-buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin as described by Lillie et al. (1968). Tissues sections from all birds were then examined microscopically.

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⁴Scharlau Chemie S.A., Barcelona, Spain.
⁵Sigma Chemical Co., Saint Louis, MO.
⁶Aliso, Auch, France.
⁷Ortho-Clinical Diagnostics, Issy-Les-Moulineaux, France.
⁸BioValley S.A., Marne la Vallée, France.
⁹ICS, Toulouse, France.
¹⁰PD-500 Shimazu, Kyoto, Japan.
¹¹Waters Associates, Inc., Milford, MA.
Statistical Analysis

Data for all response variables were reported as means ± SE and subjected to 1-way ANOVA. When significant differences were obtained, differences between means were determined by the Tukey’s studentized range method.

RESULTS

Mortality and Performance

Neither mortality nor signs of mycotoxicosis were observed in all ducks during the 77 d of treatment. The chronic effect of feeding partially purified FB₁ on BW of ducks is presented in Figure 1. There was no significant difference in feed consumption (data not shown) or BW between the 4 treatment groups and control after 77 d of treatment. However, BW was significantly reduced from d 7 to 63 in ducks receiving 128 mg of FB₁/kg of feed (Figure 2). This effect was also observed from d 28 to 63 in ducks receiving 32 mg of FB₁/kg of feed. No effect on BW was observed in ducks receiving 2 and 8 mg of FB₁/kg of feed.

Relative Organ Weights and Histopathology

Whatever the diet, no macroscopic lesion was found during postmortem examination of tissues. The chronic effects of FB₁ on the relative weights of heart, breast muscle, gizzard, spleen, and liver in ducks after 77 d are shown in Table 1. There was no significant difference in the relative weights of heart and breast muscle between any of the treated groups and the controls (P > 0.05). By contrast, 128 mg of FB₁/kg of feed diet led to significant increase in the relative weights of gizzard, spleen, and liver. This effect was also observed for liver and spleen of ducks receiving 32 mg of FB₁/kg of feed. However, histopathological examinations indicated that these increases were not associated with microscopic lesions of these organs.

Serum Biochemistry

The effect of fumonisin B₁ on various serum biochemical parameters of ducks after 77 d of treatment are presented in Table 2. The total protein, cholesterol, alanine aminotransferase, and lactate dehydrogenase concentrations in the serum were significantly increased in ducks that consumed a diet containing 128 mg of FB₁/kg of feed. Serum alkaline phosphatase was also increased by the consumption of FB₁ at 32 and 128 mg/kg of feed. By contrast, serum aspartate aminotransferase and γ-glutamyltransferase were not affected by the presence of FB₁ in diets.
DISCUSSION

The results obtained in the present study indicate that the BW of ducks was strongly affected by FB1 from d 7 to 63 of treatment when this mycotoxin was administrated at 128 mg/kg of feed. This result is in agreement with previous studies that reported a decrease in BW of turkey and broiler chicks receiving FB1 greater than 50 and 75 mg/kg of feed for 21 d (Brown et al., 1992; Ledoux et al., 1992, 1996; Weibking et al., 1993a; Broomhead et al., 2002). The effect on BW cannot be explained by feed refusal because birds were force-fed the FB1 daily, and no difference on feed consumption was observed between control and treated ducks. By contrast, after 70 d of treatment, there was no significant difference between the BW of FB1-treated ducks and those of the control group. This observation was in agreement with results obtained in previous studies with laying hens fed diets containing 100 and 200 mg of FB1/kg of feed for 420 d (Kubena et al., 1999), with broiler chicks fed 25 and 50 mg of FB1/kg of feed for 49 d (Broomhead et al., 2002), and with male turkeys fed 50 and 75 mg of FB1/kg of feed for 98 and 126 d (Bermudez et al., 1996; Broomhead et al., 2002). This result confirmed that FB1 was less toxic in avian species than other mycotoxins. The lack of BW decrease observed after FB1 chronic exposure in comparison to subacute exposure is difficult to explain. It may be linked to an adaptation of animals to the toxin or to a lesser sensibility of adult in comparison to young animals. This last hypothesis is strengthened by previous data described with ducks, demonstrating that over a period of 14 to 21 d of treatment, ducks at 42 d of age are less sensitive to the toxin than ducklings at 1 d of age (Bermudez et al., 1995; Bailly et al., 2001).

The relative weights of the liver, spleen, and gizzard were affected by a 77-d treatment with 128 mg of FB1/kg of feed. This result agrees with previous reports in broiler chicks fed diets containing fumonisin B1 at greater than 100 mg/kg of feed for 21 d (Brown et al., 1992; Ledoux et al., 1992; Weibking et al., 1993a; Kubena et al., 1999) and in turkeys fed diets containing FB1 at concentration higher than 75 mg/kg of feed for 21 or 126 d (Weibking et al., 1994; Kubena et al., 1995a,b, 1997b; Bermudez et al., 1996; Ledoux et al., 1996). The increase of the relative weight of the liver might be linked to an

**Sa/So Ratio**

Free sphinganine and sphingosine were quantified in serum, liver, and kidney. The chronic effects of FB1 on Sa/So ratio in ducks after 77 d of treatment are shown in Figure 3. The increase in Sa/So ratio in tissues and serum of treated ducks was dependent on the dose used. This increase was observed in ducks receiving 8 to 128 mg of FB1/kg of feed. The greatest increase occurred in the kidney. Indeed, the Sa/So ratio increase of 74-fold in tissues and serum of treated ducks was compared with control ducks was measured in the kidney of ducks exposed to 128 mg of FB1/kg of feed, whereas this increase was 14- and 7-fold in the liver and serum, respectively. This increase was linear between 2 and 32 mg of FB1/kg of feed but appeared to reach saturation point for the highest dose used.

**TABLE 1. Effects of fumonisin B1 (FB1) on the relative weights of heart, breast muscle, gizzard, spleen, and liver in ducks after 77 d of treatment**

<table>
<thead>
<tr>
<th>Dietary FB1 (mg/kg)</th>
<th>Heart weight (g/kg)</th>
<th>Breast muscle weight (Pectoralis superficialis) (g/kg)</th>
<th>Gizzard weight (g/kg)</th>
<th>Spleen weight (g/kg)</th>
<th>Liver weight (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.27 ± 0.38</td>
<td>64.24 ± 2.28</td>
<td>24.05 ± 0.82</td>
<td>0.43 ± 0.04</td>
<td>13.53 ± 0.60</td>
</tr>
<tr>
<td>2</td>
<td>8.15 ± 0.18</td>
<td>62.06 ± 1.38</td>
<td>23.70 ± 0.99</td>
<td>0.44 ± 0.04</td>
<td>13.90 ± 0.47</td>
</tr>
<tr>
<td>8</td>
<td>8.35 ± 0.18</td>
<td>64.38 ± 0.75</td>
<td>24.85 ± 1.12</td>
<td>0.46 ± 0.03</td>
<td>14.02 ± 0.49</td>
</tr>
<tr>
<td>32</td>
<td>8.14 ± 0.11</td>
<td>61.14 ± 1.41</td>
<td>26.55 ± 1.36</td>
<td>0.61 ± 0.04</td>
<td>16.28 ± 1.01</td>
</tr>
<tr>
<td>128</td>
<td>8.26 ± 0.33</td>
<td>61.40 ± 2.12</td>
<td>29.16 ± 1.61</td>
<td>0.59 ± 0.05</td>
<td>16.87 ± 0.54</td>
</tr>
</tbody>
</table>

Values with different superscript letters are significantly different (ANOVA, followed by an individual comparison of means; P < 0.05).

**TABLE 2. Effects of fumonisin B1 (FB1) on various serum parameters of ducks after 77 d of treatment**

<table>
<thead>
<tr>
<th>Dietary FB1 (mg/kg)</th>
<th>Protein (g/L)</th>
<th>Cholesterol (g/L)</th>
<th>ALT (U/L)</th>
<th>LDH (U/L)</th>
<th>ALP (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>41 ± 1b</td>
<td>1.78 ± 0.06b</td>
<td>24 ± 1b</td>
<td>1,927 ± 153b</td>
<td>118 ± 6a</td>
<td>27 ± 2</td>
<td>11 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>39 ± 1b</td>
<td>1.59 ± 0.08b</td>
<td>25 ± 2b</td>
<td>2,352 ± 224b</td>
<td>109 ± 6b</td>
<td>32 ± 3</td>
<td>11 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>41 ± 1b</td>
<td>1.71 ± 0.07b</td>
<td>24 ± 1b</td>
<td>2,267 ± 278b</td>
<td>113 ± 5b</td>
<td>28 ± 4</td>
<td>11 ± 0.2</td>
</tr>
<tr>
<td>32</td>
<td>43 ± 2b</td>
<td>1.65 ± 0.09b</td>
<td>27 ± 1b</td>
<td>1,813 ± 155b</td>
<td>177 ± 23a</td>
<td>25 ± 2</td>
<td>11 ± 0.3</td>
</tr>
<tr>
<td>128</td>
<td>45 ± 1a</td>
<td>2.14 ± 0.14a</td>
<td>33 ± 4a</td>
<td>3,390 ± 303a</td>
<td>221 ± 19a</td>
<td>31 ± 5</td>
<td>12 ± 0.6</td>
</tr>
</tbody>
</table>

Values with different superscript letters are significantly different (ANOVA, followed by an individual comparison of means; P < 0.05).

Values are means ± SE of 8 ducks per group.

1 ALT = alanine aminotransferase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = γ-glutamyltransferase.
altered in ducks fed FB1 at 128 mg/kg of feed, as reported previously for ducks (Bailly et al., 2001; Tran et al., 2003) and broilers (Ledoux et al., 1992; Espada et al., 1997; Kubena et al., 1997a) but not in turkeys (Kubena et al., 1995a,b, 1997b). Similarly, the increased activities of serum alkaline phosphatase are in agreement with previous data obtained in ducks (Tardieu et al., 2004) and in opposition with data described for broilers, laying hens, and turkeys (Bermudez et al., 1997; Espada et al., 1997; Kubena et al., 1999; Henry et al., 2000). By contrast, activities of serum aspartate aminotransferase and γ-glutamyltransferase were not affected in this study, whereas these parameters increased in other studies conducted in birds (Ledoux et al., 1992, 1996; Espada et al., 1997; Kubena et al., 1999; Henry et al., 2000; Bailly et al., 2001).

Increased serum, liver, and kidney Sa/So ratios were observed in ducks fed diets with 8 to 128 mg of FB1/kg of feed. This finding indicated that an alteration of sphingolipid metabolism occurred after exposure to very low dose of FB1. This result is in agreement with studies conducted in rat, pig, and monkey demonstrating an increase of Sa/So ratio after exposure to a diet containing respectively 3.4, 5, and 10 mg of FB1/kg of feed (Voss et al., 1999; van der Westhuizen et al., 2001; Zomborszky-Kovacs et al., 2002). Changes in serum Sa/So ratio occur before other signs of intoxication in all species (Weibking et al., 1993a, 1994; Ledoux et al., 1996; Henry et al., 2000; Bailly et al., 2001; Tran et al., 2003). Moreover, this study demonstrates for the first time that Sa/So ratio was also increased in the kidney in ducks and that this organ is the most sensitive to FB1-induced disruption of sphingolipid metabolism. This result is in agreement with previous observations obtained in the rat (Riley et al., 1994a; Bondy et al., 1996) and rabbit (LaBorde et al., 1997).

In conclusion, FB1 administrated orally at different doses corresponding to ingestion of a diet containing 2, 8, 32, or 128 mg/kg of feed over 77 d of treatment had only weak effects on serum biochemistry. Surprisingly, the decrease in BW was more pronounced on d 7 to 63 than on d 70 to 77. After 77 d of treatment, only a weak increase of the relative weight of some organs was observed, without concomitant microscopic alteration, confirming that the kinetic of exposure to FB1 has to be carefully determined before to determine tolerable level of this mycotoxin in feeds. However, analyses of free sphinganine and free sphingosine indicate that FB1 at concentrations starting from 8 mg/kg of feed led to increased Sa/So ratios in serum, liver, and kidney. This study showed that changes in the Sa/So ratio are very sensitive biomarkers of fumonisin exposure.

**ACKNOWLEDGMENT**

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**FIGURE 3.** Chronic effects of fumonisin B1 (FB1) on serum (–o–), liver (– ▲ –), and kidney (– ○ –) sphinganine to sphingosine ratio in mule ducks after 77 d. Values were obtained from groups of 5 ducks receiving 0, 2, 8, or 128 mg of FB1/kg diet and are presented as means ± SE. *Significant difference between groups by using ANOVA (P < 0.05).
taining known levels of fumonisin B1 in ducks. Toxicology 163:11–22.


