IMMUNOLOGY, HEALTH, AND DISEASE

Chronic Effects of Fumonisin B_1 on Ducks

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ABSTRACT Partially purified fumonisin B_1 (FB_1) 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 FB_1/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, FB_1 ingested at 32 and 128 mg/kg led to decreased body weight from d 28 to 63 and from d 7 to 63, respectively. FB_1 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 FB_1/kg of feed without evidence of detectable microscopic modification of these organs. FB_1 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 FB_1/kg of feed was given. Serum, liver, and kidney sphinganine to sphingosine ratio was significantly increased in ducks fed 8 to 128 mg of FB_1/kg of feed. The biggest increase was observed in kidneys, suggesting that this organ is the most sensitive to detect FB_1-induced disruption of sphingolipid metabolism.

(*Key words*: fumonisin B_1, mycotoxin, duck, sphinganine, sphingosine)

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INTRODUCTION

Fumonisin B_1 (FB_1) 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 FB_1 are equine leukoencephalomalacia (Marasas et al., 1988) 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 FB_1 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 FB_1 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 FB_1-toxicity and FB_1-increased Sa/So ratio is not obvious at least in avian species.

Although subacute toxicity of FB_1 has been frequently investigated in avian species, the chronic effects of FB_1 are less understood. Previous studies conducted with turkeys for 14 and 18 wk demonstrated that 50 and 75 mg of FB_1/kg of feed, respectively, are detrimental to animal performance (Bermudez et al., 1996; Broomhead et al., 2002). By contrast, FB_1 does not have an effect on body weight gain of broilers fed 50 mg of FB_1/kg to market age (7 wk; Broomhead et al., 2002) or of laying hens fed 200 mg of FB_1/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 FB_1 to mallard ducks from 1 to 12 wk of age. Four levels of FB_1 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-

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Abbreviation Key: FB_1 = fumonisin B_1; Sa/So = sphinganine to sphingosine.

sumption, relative organ weights, serum chemistry, histopathology, and serum, liver, and kidney Sa/So ratios.

MATERIALS AND METHODS

Chemicals and Fumonisin Production

Fumonisin was produced as previously described by using a highly toxinogenic strain of *F. verticillioides* (NRRL-3428) isolated from corn associated with an acute case of equine leucoencephalomalacia (Bailly et al., 1996). Briefly, autoclaved rice was inoculated with 1 cm^2 of 1-wk 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 FB_1 was purchased.[2] The purity of the crude extract was of 54% FB_1, 8% FB_2, and 9% FB_3. 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, fusarin C). Aflatoxin B_1, ochratoxin A, zearalenone, T2 toxin, and deoxynivalenol were tested using a Veratox quantitative test kit.[3] All other chemicals and reagents were of the highest grade available. They were purchased from Scharlau[4] and Sigma Chemical Co.[5] 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[6] and water for 1 wk prior to initiation of the study. The feed was tested for mycotoxin concentrations. Aflatoxin B_1 and ochratoxin A were less than 10 μg/kg, zearalenone was less than 200 μg/kg, T2 toxin was less than 50 μg/kg, deoxynivalenol was less than 1,000 μg/kg, and FB_1 was less than 500 μg/kg.

At 7 d of age, ducks were wing-banded for identification and randomly placed into 5 groups of 8 birds each. Fumonisin B_1 was daily administered by gavage for 77 d at different doses. These doses were calculated according to feed consumption to obtain a treatment equivalent to the ingestion of a diet containing 0, 2, 8, 32, and 128 mg of FB_1/kg of feed. Every 7 d, ducks were individually weighed, and their average feed consumption was calculated. On d 77 of treatment, peripheral blood samples were collected into dry tubes for analysis of the Sa/So ratio and biochemistry. After blood sampling, all ducks were killed with carbon dioxide, and liver, heart, kidney, spleen, breast muscle, ceca, and gizzard were collected. These organs were weighed and fixed for histological analysis or stored at –80°C until use.

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 analyzer[7] 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 C_{20} sphinganine[8] were added to 100 μL of serum or tissues homogenates. Lipids were extracted by alkaline methanolic-chloroform and furtherly 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 C_{20} sphinganine contents were determined by HPLC using an ICS M2200 solvent delivery module[9] 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 C_{20} 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 C_{20} 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.

[2]Sigma, Saint Quentin Fallavier, France.
[3]Neogen Corporation, Villeneuve la Garenne, France.
[4]Scharlau Chemie S.A., Barcelona, Spain.
[5]Sigma Chemical Co., Saint Louis, MO.
[6]Aliso, Auch, France.
[7]Ortho-Clinical Diagnostics, Issy-Les-Moulineaux, France.
[8]BioValley S. A., Marne la Vallée, France.
[9]ICS, Toulouse, France.
[10]FD-500 Shimazu, Kyoto, Japan.
[11]Waters Associates, Inc., Milford, MA.

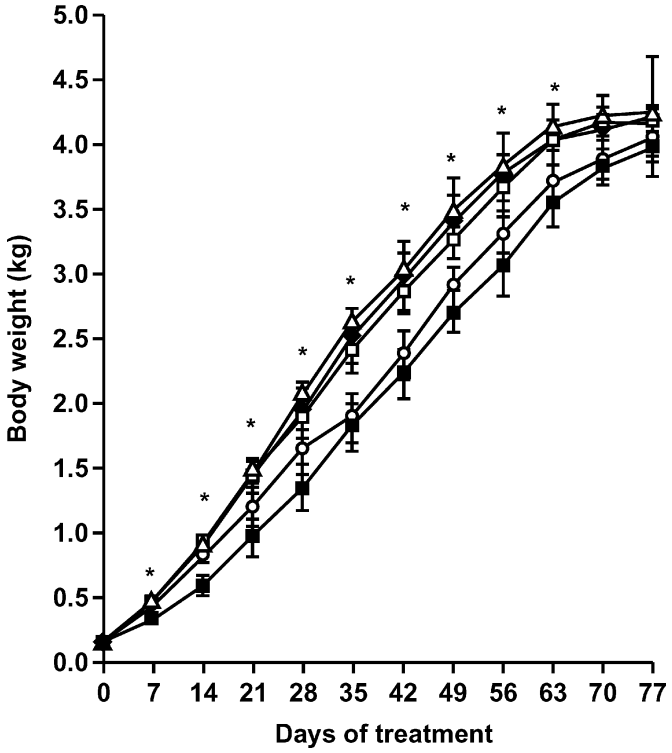

FIGURE 1. Chronic effects of fumonisin B_1 on BW in mule ducks. Values were obtained from groups of 8 ducks receiving 0 (-◆-), 2 (-□-), 8 (-△-), 32 (-o-), or 128 (-■-) mg of fumonisin B_1/kg of diet and are presented as means ± SE. *Significant difference between groups by using ANOVA ($P < 0.05$).

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 FB_1 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 FB_1/kg of feed (Figure 2). This effect was also observed from d 28 to 63 in ducks receiving 32 mg of FB_1/kg of feed. No effect on BW was observed in ducks receiving 2 and 8 mg of FB_1/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 FB_1 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 FB_1/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 B_1 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 FB_1/kg of feed. Serum alkaline phosphatase was also increased by the consumption of FB_1 at 32 and 128 mg/kg of feed. By contrast, serum aspartate aminotransferase and γ-glutamyltransferase were not affected by the presence of FB_1 in diets.

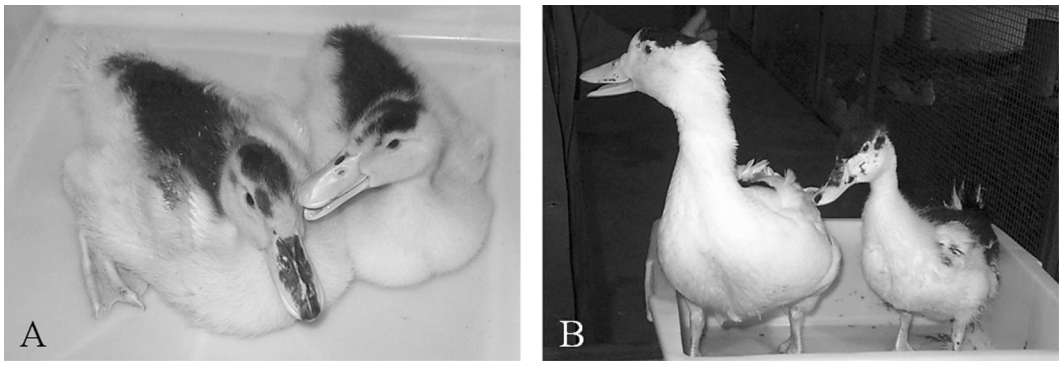

FIGURE 2. Effect of fumonisin B_1 (FB_1) on BW of mule ducks receiving 0 (on the left) or 128 mg of FB_1/kg diet (on the right). A) after 14 d of treatment, B) after 35 d of treatment.

TABLE 1. Effects of fumonisin B_1 (FB_1) on the relative weights of heart, breast muscle, gizzard, spleen, and liver in ducks after 77 d[1] of treatment

Dietary FB_1 (mg/kg)	Heart weight (g/kg)	Breast muscle weight (Pectoralis superficialis) (g/kg)	Gizzard weight (g/kg)	Spleen weight (g/kg)	Liver weight (g/kg)
0	8.27 ± 0.38	64.24 ± 2.28	24.05 ± 0.82^{b}	0.43 ± 0.04^{b}	13.53 ± 0.60^{c}
2	8.15 ± 0.18	62.06 ± 1.38	23.70 ± 0.99^{b}	0.44 ± 0.04^{ab}	13.90 ± 0.47^{bc}
8	8.35 ± 0 .18	64.38 ± 0.75	24.85 ± 1.12^{ab}	0.46 ± 0.03^{ab}	14.02 ± 0.49^{bc}
32	8.14 ± 0.11	61.14 ± 1.41	26.55 ± 1.36^{ab}	0.61 ± 0.04^{a}	16.28 ± 1.01^{ab}
128	8.26 ± 0.33	61.40 ± 2.12	29.16 ± 1.61^{a}	0.59 ± 0.05^{a}	16.87 ± 0.54^{a}

$^{a–c}$Values with different superscript letters are significantly different (ANOVA, followed by an individual comparison of means; $P < 0.05$).

[1]Values are means ± SE of 8 ducks per group.

Sa/So Ratio

Free sphinganine and sphingosine were quantified in serum, liver, and kidney. The chronic effects of FB_1 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 FB_1/kg of feed. The greatest increase occurred in the kidney. Indeed, the Sa/So ratio increase of 74-fold in comparison with control ducks was measured in the kidney of ducks exposed to 128 mg of FB_1/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 FB_1/kg of feed but appeared to reach saturation point for the highest dose used.

DISCUSSION

The results obtained in the present study indicate that the BW of ducks was strongly affected by FB_1 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 FB_1 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 FB_1 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 FB_1-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 FB_1/kg of feed for 420 d (Kubena et al., 1999), with broiler chicks fed 25 and 50 mg of FB_1/kg of feed for 49 d (Broomhead et al., 2002), and with male turkeys fed 50 and 75 mg of FB_1/kg of feed for 98 and 126 d (Bermudez et al., 1996; Broomhead et al., 2002). This result confirmed that FB_1 was less toxic in avian species than other mycotoxins. The lack of BW decrease observed after FB_1 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 FB_1/kg of feed. This result agrees with previous reports in broiler chicks fed diets containing fumonisin B_1 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., 1997a, 1999) and in turkeys fed diets containing FB_1 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

TABLE 2. Effects of fumonisin B_1 (FB_1) on various serum parameters of ducks after 77 d[1] of treatment[2]

Dietary FB_1 (mg/kg)	Protein (g/L)	Cholesterol (g/L)	ALT (U/L)	LDH (U/L)	ALP (U/L)	AST (U/L)	GGT (U/L)
0	41 ± 1^{b}	1.78 ± 0.06^{b}	24 ± 1^{b}	1,927 ± 153^{b}	118 ± 6^{b}	27 ± 2	11 ± 0.3
2	39 ± 1^{b}	1.59 ± 0.08^{b}	25 ± 2^{b}	2,352 ± 224^{b}	109 ± 8^{b}	32 ± 3	11 ± 0.3
8	41 ± 1^{b}	1.71 ± 0.07^{b}	24 ± 1^{b}	2,267 ± 276^{b}	113 ± 5^{b}	28 ± 4	11 ± 0.2
32	43 ± 2^{b}	1.65 ± 0.09^{b}	27 ± 1^{b}	1,813 ± 155^{b}	177 ± 23^{a}	25 ± 2	11 ± 0.3
128	45 ± 1^{a}	2.14 ± 0.14^{a}	33 ± 4^{a}	3,390 ± 303^{a}	221 ± 19^{a}	31 ± 5	12 ± 0.6

^{a,b}Values with different superscript letters are significantly different (ANOVA, followed by an individual comparison of means; $P < 0.05$).

[1]Values are means ± SE of 8 ducks per group.

[2]ALT = alanine aminotransferase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = γ-glutamyltransferase.

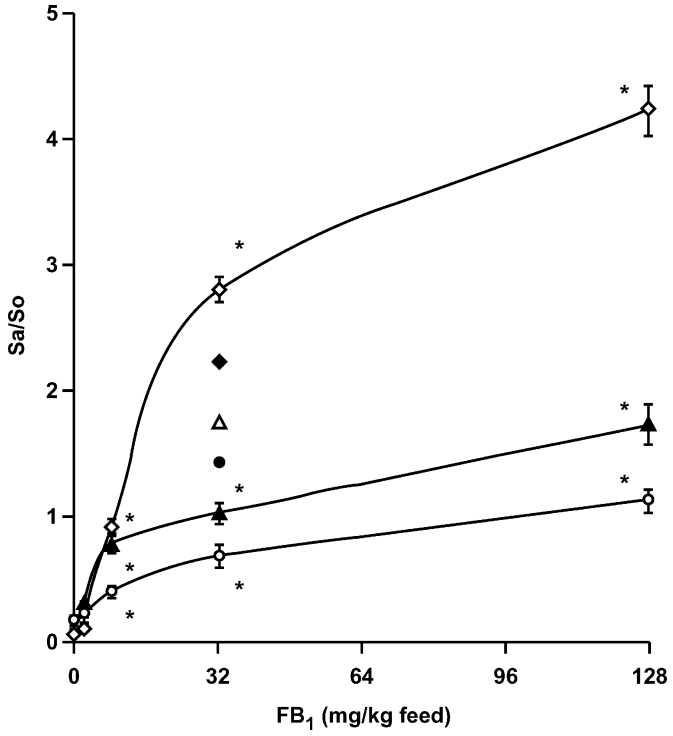

FIGURE 3. Chronic effects of fumonisin B_1 (FB_1) on serum (-o-), liver (-▲-), and kidney (-◇-) sphinganine to sphingosine ratio in mule ducks after 77 d. Values were obtained from groups of 5 ducks receiving 0, 2, 8, or 128 mg of FB_1/kg diet and are presented as means ± SE. *Significant difference between groups by using ANOVA ($P < 0.05$).

alteration of the lipid metabolism and steatosis or to a hyperplasia of the organ without steatosis. This last hypothesis is strengthened by the lack of steatosis observed with duck liver in this study and as previously described (Bailly et al., 2001). Moreover, when FB_1 was administrated during force-feeding, the mycotoxin decreased liver steatosis caused by feed excess (Tardieu et al., 2004). Increased relative weight of the gizzard has been previously reported for chicks and turkeys fed diets containing FB_1 (Ledoux et al., 1992; Kubena et al., 1995a,b, 1996). This effect has been linked to the overall irritative properties of feed containing mycotoxins (Hoerr et al., 1982). This explanation cannot be used in this study because FB_1 was administrated orally as partially purified extract of *F. verticillioides* culture material. Moreover, gizzard mucosa showed no sign of irritation when postmortem examination was done. This increase in gizzard weight could be linked to a slight increase in feed consumption in treated ducks compared with control ducks at the end of the treatment.

Concerning serum biochemical parameters, a significant increase in the concentration of total protein, alanine aminotransferase and lactate dehydrogenase was observed in ducks receiving a 128 mg of FB_1/kg of feed for 77 d, as described in previous studies with ducks receiving FB_1 ranging from 5 to 45 mg/kg of BW for 12 d (Bailly et al., 2001; Tran et al., 2003) and broiler chicks fed FB_1 at 300 mg/kg of feed for 16 d (Espada et al., 1997; Kubena et al., 1997a). Cholesterol levels were also significantly elevated in ducks fed FB_1 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., 1997a,b, 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 FB_1/kg of feed. This finding indicated that an alteration of sphingolipid metabolism occurred after exposure to very low dose of FB_1. 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 FB_1/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 FB_1-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, FB_1 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 FB_1 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 FB_1 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.

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REFERENCES

Bailly, J. D., G. Benard, J. Y. Jouglar, S. Durand, and P. Guerre. 2001. Toxicity of *Fusarium moniliforme* culture material con-

taining known levels of fumonisin B1 in ducks. Toxicology 163:11–22.
Bailly, J.-D., I. Raymond, P. Le Bars, Y. Guyomard, J. Abadie, J. Le Bars, P. Guerre, M. Delverdier, and V. Burgat. 1996. Leucoencéphalomalacie des Equidés. Cas rapportés au CNITV. Rev. Med. Vet. 147:787–796.
Bermudez, A. J., D. R. Ledoux, and G. E. Rottinghaus. 1995. Effects of Fusarium moniliforme culture material containing known levels of fumonisin B1 in ducklings. Avian Dis. 39:879–886.
Bermudez, A. J., D. R. Ledoux, G. E. Rottinghaus, and G. A. Bennett. 1997. The individual and combined effects of the Fusarium mycotoxins moniliformin and fumonisin B1 in turkeys. Avian Dis. 41:304–311.
Bermudez, A. J., D. R. Ledoux, J. R. Turk, and G. E. Rottinghaus. 1996. The chronic effects of *Fusarium moniliforme* culture material, containing known levels of fumonisin B1, in turkeys. Avian Dis. 40:231–235.
Bondy, G., M. Barker, R. Mueller, S. Fernie, J. D. Miller, C. Armstrong, S. L. Hierlihy, P. Rowsell, and C. Suzuki. 1996. Fumonisin B1 toxicity in male Sprague-Dawley rats. Adv. Exp. Med. Biol. 392:251–264.
Broomhead, J. N., D. R. Ledoux, A. J. Bermudez, and G. E. Rottinghaus. 2002. Chronic effects of fumonisin B1 in broilers and turkeys fed dietary treatments to market age. Poult. Sci. 81:56–61.
Brown, T. P., G. E. Rottinghaus, and M. E. Williams. 1992. Fumonisin mycotoxicosis in broilers: performance and pathology. Avian Dis. 36:450–454.
Espada, Y., R. Ruiz de Gopegui, C. Cuadradas, and F. J. Cabanes. 1997. Fumonisin mycotoxicosis in broilers: plasma proteins and coagulation modifications. Avian Dis. 41:73–79.
Espada, Y., R. Ruiz de Gopegui, C. Cuadradas, and F. J. Cabanes. 1994. Fumonisin mycotoxicosis in broilers. Weights and serum chemistry modifications. Avian Dis. 38:454–460.
Gelderblom, W. C., W. F. Marasas, R. Vleggaar, P. G. Thiel, and M. E. Cawood. 1992. Fumonisins: isolation, chemical characterization and biological effects. Mycopathologia 117:11–16.
Gelderblom, W. C., N. P. Kriek, W. F. Marasas, and P. G. Thiel. 1991. Toxicity and carcinogenicity of the Fusarium moniliforme metabolite, fumonisin B1, in rats. Carcinogenesis 12:1247–1251.
Groves, F. D., L. Zhang, Y. S. Chang, P. F. Ross, H. Casper, W. P. Norred, W. C. You, and J. F. Fraumeni, Jr. 1999. Fusarium mycotoxins in corn and corn products in a high-risk area for gastric cancer in Shandong Province, China. J. AOAC Int. 82:657–662.
Harrison, L. R., B. M. Colvin, J. T. Greene, L. E. Newman, and J. R. Cole, Jr. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of Fusarium moniliforme. J. Vet. Diagn. Invest. 2:217–221.
Henry, M. H., R. D. Wyatt, and O. J. Fletchert. 2000. The toxicity of purified fumonisin B1 in broiler chicks. Poult. Sci. 79:1378–1384.
Hoerr, F. J., W. W. Carlton, B. Yagen, and A. Z. Joffe. 1982. Mycotoxicosis produced in broiler chickens by multiple doses of either T-2 toxin or diacetoxyscirpenol. Avian Pathol. 11:369–383.
Kubena, L. F., T. S. Edrington, R. B. Harvey, S. A. Buckley, T. D. Phillips, G. E. Rottinghaus, and H. H. Casper. 1997a. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. Poult. Sci. 76:1239–1247.
Kubena, L. F., T. S. Edrington, R. B. Harvey, T. D. Phillips, A. B. Sarr, and G. E. Rottinghaus. 1996. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and diacetoxyscirpenol or ochratoxin A in turkey poults. Poult. Sci. 75:256–265.
Kubena, L. F., T. S. Edrington, R. B. Harvey, T. D. Phillips, A. B. Sarr, and G. E. Rottinghaus. 1997b. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and diacetoxyscirpenol or ochratoxin A in turkey poults. Poult. Sci. 76:256–264.
Kubena, L. F., T. S. Edrington, C. Kamps-Holtzapple, R. B. Harvey, M. H. Elissalde, and G. E. Rottinghaus. 1995a. Effects of feeding fumonisin B1 present in Fusarium moniliforme culture material and aflatoxin singly and in combination to turkey poults. Poult. Sci. 74:1295–1303.
Kubena, L. F., T. S. Edrington, C. Kamps-Holtzapple, R. B. Harvey, M. H. Elissalde, and G. E. Rottinghaus. 1995b. Influence of fumonisin B1, present in Fusarium moniliforme culture material, and T-2 toxin on turkey poults. Poult. Sci. 74:306–313.
Kubena, L. F., R. B. Harvey, S. A. Buckley, R. H. Bailey, and G. E. Rottinghaus. 1999. Effects of long-term feeding of diets containing moniliformin, supplied by Fusarium fujikuroi culture material, and fumonisin, supplied by *Fusarium moniliforme* culture material, to laying hens. Poult. Sci. 78:1499–1505.
LaBorde, J. B., K. K. Terry, P. C. Howard, J. J. Chen, T. F. Collins, M. E. Shackelford, and D. K. Hansen. 1997. Lack of embryotoxicity of fumonisin B1 in New Zealand white rabbits. Fundam. Appl. Toxicol. 40:120–128.
Ledoux, D. R., A. J. Bermudez, and G. E. Rottinghaus. 1996. Effects of feeding Fusarium moniliforme culture material, containing known levels of fumonisin B1, in the young turkey poult. Poult. Sci. 75:1472–1478.
Ledoux, D. R., T. P. Brown, T. S. Weibking, and G. E. Rottinghaus. 1992. Fumonisin toxicity in broiler chicks. J. Vet. Diagn. Invest. 4:330–333.
Lillie, R. D., J. G. Pasternack, and M. Gabe. 1968. Page 249 in Techniques Histologiques. Masson et Cie, Paris.
Marasas, W. F. 1995. Fumonisins: their implications for human and animal health. Nat. Toxins 3:193–198.
Marasas, W. F., T. S. Kellerman, W. C. Gelderblom, J. A. Coetzer, P. G. Thiel, and J. J. van der Lugt. 1988. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. 55:197–203.
Raynal, M., J. D. Bailly, G. Benard, and P. Guerre. 2001. Effects of fumonisin B1 present in Fusarium moniliforme culture material on drug metabolising enzyme activities in ducks. Toxicol. Lett. 121:179–190.
Rice, L. G., P. F. Ross, J. Dejong, R. D. Plattner, and J. R. Coats. 1995. Evaluation of a liquid chromatographic method for the determination of fumonisins in corn, poultry feed, and Fusarium culture material. J. AOAC Int. 78:1002–1009.
Riley, R. T., D. M. Hinton, W. J. Chamberlain, C. W. Bacon, E. Wang, A. H. Merrill, Jr., and K. A. Voss. 1994a. Dietary fumonisin B1 induces disruption of sphingolipid metabolism in Sprague-Dawley rats: a new mechanism of nephrotoxicity. J. Nutr. 124:594–603.
Riley, R. T., E. Wang, and A. H. Merrill. 1994b. Liquid chromatographic determination of sphinganine and sphingosine: use of the free sphinganine to sphingosine ratio as a biomarker for consumption of fumonisin. J. AOAC Int. 77:533–540.
Tardieu, D., J. D. Bailly, G. Benard, S. T. Tran, and P. Guerre. 2004. FB_1 toxicity in ducks during force-feeding. Poult. Sci. 83:1287–1293.
Tran, S. T., J. D. Bailly, D. Tardieu, S. Durand, G. Benard, and P. Guerre. 2003. Sphinganine to sphingosine ratio and predictive biochemical markers of fumonisin B1 exposure in ducks. Chem. Biol. Interact. 146:61–72.
Ueno, Y., K. Iijima, S. D. Wang, Y. Sugiura, M. Sekijima, T. Tanaka, C. Chen, and S. Z. Yu. 1997. Fumonisins as a possible contributory risk factor for primary liver cancer: a 3-year study of corn harvested in Haimen, China, by HPLC and ELISA. Food Chem. Toxicol. 35:1143–1150.
van der Westhuizen, L., G. S. Shephard, and D. J. van Schalkwyk. 2001. The effect of repeated gavage doses of fumonisin B1 on the sphinganine and sphingosine levels in vervet monkeys. Toxicon 39:969–972.

Voss, K. A., J. K. Porter, C. W. Bacon, F. I. Meredith, and W. P. Norred. 1999. Fusaric acid and modification of the subchronic toxicity to rats of fumonisins in *F. moniliforme* culture material. Food Chem. Toxicol. 37:853–861.

Weibking, T. S., D. R. Ledoux, A. J. Bermudez, and G. E. Rottinghaus. 1994. Individual and combined effects of feeding Fusarium moniliforme culture material, containing known levels of fumonisin B1, and aflatoxin B1 in the young turkey poult. Poult. Sci. 73:1517–1525.

Weibking, T., D. R. Ledoux, A. J. Bermudez, J. R. Turk, and G. E. Rottinghaus. 1995. Effects on turkey poults of feeding *Fusarium moniliforme* M-1325 culture material grown under different environmental conditions. Avian Dis. 39:32–38.

Weibking, T. S., D. R. Ledoux, A. J. Bermudez, J. R. Turk, G. E. Rottinghaus, E. Wang, and A. H. Merrill, Jr. 1993a. Effects of feeding Fusarium moniliforme culture material, containing known levels of fumonisin B1, on the young broiler chick. Poult. Sci. 72:456–466.

Weibking, T. S., D. R. Ledoux, T. P. Brown, and G. E. Rottinghaus. 1993b. Fumonisin toxicity in turkey poults. J. Vet. Diagn. Invest. 5:75–83.

Yoshizawa, T., A. Yamashita, and Y. Luo. 1994. Fumonisin occurrence in corn from high- and low-risk areas for human esophageal cancer in China. Appl. Environ. Microbiol. 60:1626–1629.

Zomborszky-Kovacs, M., F. Vetesi, P. Horn, I. Repa, and F. Kovacs. 2002. Effects of prolonged exposure to low-dose fumonisin B1 in pigs. J. Vet. Med. B Infect. Dis. Vet. Public Health 49:197–201.